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1 Introduction

The wheat pathogen *Zymoseptoria tritici* (Capnodiales; Dothideomycetes) (syn. *Mycosphaerella graminicola*; Quaedvlieg et al., 2011) is haploid and heterothallic (Mat1-1 and Mat1-2; Waalwijk et al., 2002). In addition, the *Zymoseptoria* genus also contains four other species. *Z. pseudotritici* and *Z. ardabiliae* were isolated from wild grasses in Iran and are pathogenic on wheat. *Z. passerinii* is a pathogen of barley, while Z. *brevis* was isolated from grasses. The closest genus to *Zymoseptoria* is *Ramularia* which includes many plant pathogens, including the barley pathogen *Ramularia collo-cygni* (Quaedvlieg et al., 2011; Stukenbrock et al., 2011, 2012a,b; Feurtey et al., 2020). *Z. tritici* is a major fungal pathogen of bread and durum wheat causing *Septoria tritici* blotch disease (STB; Fones and Gurr, 2015). STB is an important disease in most wheat-producing regions (Boukef et al., 2013; Pakholkova et al., 2016; Hartmann et al., 2018; Hassine et al., 2019), including Europe, Russia, Ukraine, Mediterranean countries, North America (USA, Canada, Mexico), South America (Argentina, Chile), Africa (Mekonnen et al., 2020) and Australia (McDonald et al., 2019).

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For unknown reasons, STB has not been reported in China, even though the environmental conditions in some parts of the country should be favorable for STB epidemics. Yield losses associated with STB in Europe range from 5% to 10%, despite the use of resistant varieties and fungicide treatments (Fones and Gurr, 2015). Yield losses higher than 50% have been reported in wheat fields with susceptible cultivars not treated with fungicides (Berraies et al., 2014; Tadesse et al., 2019; Hailemariam et al., 2020; Jalli et al., 2020).

Both sexual and asexual stages of *Z. tritici* are commonly observed in infected wheat fields during polycyclic STB epidemics (Suffert et al., 2011). Sexual reproduction takes place during the intercrop season on wheat debris, where pseudothecia release wind-dispersed ascospores (sexual spores) which act as the primary inoculum in autumn/winter on wheat seedlings (Zhan et al., 2001; Eriksen and Munk, 2003; Suffert et al., 2011; Morais et al., 2016). The density of leaf lesions is a crucial determinant of sexual reproduction at the plant and field scales (Suffert and Thompson, 2018) because physical contact between two compatible strains is required (Waalwijk et al., 2002). More severe epidemics are usually associated with higher levels of ascospore production on wheat debris from the previous year (Cowger et al., 2002; Suffert et al., 2011; Morais et al., 2016; Chaloner et al., 2019). Wheat residue management has long been known to be a factor controlling STB epidemics (Suffert et al., 2011), but it was shown that the amount of primary airborne inoculum available in a wheat crop is not actually a limiting factor for the onset of epidemics in the wheat production areas (Morais et al., 2016). The lifestyle of the fungus during the cropping season is characterized by a symptomless, relatively long, latent period (Suffert and Thompson, 2018; Chaloner et al., 2019). The rate of development of the epidemic, which progresses vertically in the canopy (Lovell et al., 2004), is determined by the number of asexual multiplication cycles completed by the pathogen (usually 5–7), which depends principally on temperature and rain events (Chaloner et al., 2019). Once a plant is infected, pycnidia release splash-dispersed pycnidiospore (asexual spores) that are responsible for secondary infections (Shaw, 1987; Karisto et al., 2021). Because of its tremendous impact on agriculture, *Z. tritici* biology, in particular asexual infection at the leaf scale, has been intensively studied (reviews: Suffert et al., 2011; Dean et al., 2012; Perez-Nadales et al., 2014; Rudd, 2015; McDonald et al., 2015a; Kettles and Kanyuka, 2016; Duba et al., 2018; Brennan et al., 2019). The description of the infection cycle at the cytological level has also been well-documented (see Section 4.1 for references). Infection begins with the deposition of spores on the leaf surface, their germination and the development of epiphytic hyphae on the leaf surface. These hyphae penetrate the leaf mostly through stomata. After penetration, *Z. tritici* infection is characterized by two distinct stages. An initial, relatively long, asymptomatic phase (10–12 days), is followed by a second phase characterized by the

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formation of large necrotic lesions associated with plant cell death (Keon et al., 2007). During this necrotrophic phase, *Z. tritici* produces a large number of pycnidia within necrotic lesions that release pycnidiospores expelled through stomata openings. Two types of pycnidiospores have been described. Macro-pycnidiospores (pycnidiospores *sensu stricto*) are composed of 4–8 cells and they are the main spores involved in disease dynamics during the crop season (reviewed in Suffert et al., 2011). Micro-pycnidiospores were sometimes reported in empty pycnidia present on wheat residues in dry conditions (Djerbi et al., 1974). These small spores were suspected to result from pycnidiospore germination inside the pycnidium, and they bear some resemblance to the yeast-like cells, called blastospores, produced in axenic cultures that divide by budding (Steinberg, 2015). Blastospores were also produced at an early stage of infection by germinating pycnidiospores (Francisco et al., 2019). In addition, chlamydospores, produced in response to nutritional and temperature stresses, were able to survive extreme cold, heat and drought treatments (Francisco et al., 2019).

2 *Z. tritici* genomics

2.1 Z. tritici *genome characteristics and annotation*

The first *Z. tritici* genome sequence was obtained from the bread wheat European isolate IPO323, using Sanger sequencing (Goodwin et al., 2011). This 39.7 Mb sequence is complete from telomere to telomere and includes centromeres (Goodwin et al., 2011; Schotanus et al., 2015). The *Z. tritici* genome is composed of 13 core chromosomes (CCs: 1.2–6.1 Mb), which are conserved across isolates, and up to 8 accessory chromosomes (ACs: 0.4–0.8 Mb), which vary in number and size among *Z. tritici* isolates (Goodwin et al., 2011; McDonald et al., 2015b; Badet et al., 2020). The first IPO323 genome annotation was obtained in 2011, using Fgenesh and Genewise *ab initio* gene predictors supported by EST and protein sequences (10 933 genes; Goodwin et al., 2011; available at NBCI, Mycocosm and Ensembl). A second IPO323 genome annotation was obtained in 2015, using GeneMark-ES, GeneMark-HMM and Augustus *ab initio* gene predictors supported by RNAseq and evolutionary evidences (11 839 genes; Grandaubert et al., 2015; available at FungiDB and Ensembl). Finally, a third IPO323 genome annotation was obtained in 2016, using the MAKER-HMM *ab initio* gene predictor with RNAseq evidence (13 583 genes; RRES available at Ensembl). These three annotations only share 4000 identical gene models, suggesting there are major differences in gene structures across predictions. A refined genome annotation with curated gene models is therefore needed. Specific annotations were performed for genes encoding plant cell wall-degrading enzymes (Brunner et al., 2013), secreted

proteins/effectors (Morais do Amaral et al., 2012; Mirzadi Gohari et al., 2015) and genes involved in secondary metabolism (Cairns and Meyer, 2017).

2.2 Z. tritici *pangenome*

The genomes of 18 additional *Z. tritici* isolates from different geographic origins were characterized using long-read sequencing and gene predictions with *ab initio* gene predictors and RNAseq evidence (Badet et al., 2020). These genomes varied in size from 37.1 Mb to 41.7 Mb, and they were organized in 13 CCs and 4–8 ACs. All ACs were similar to those identified in IPO323 (no new ACs were found). The individual gene repertoires of these 18 isolates and IPO323 varied from 11 657 to 12 787 genes. Comparison of these repertoires highlighted 15 474 orthologous gene families with 15 451 single gene orthogroups (pangenome). A sub-set of these orthogroups is conserved across isolates (9193 conserved genes, 60%) while accessory orthogroups (40%) were either confined to a subset of isolates (4690 genes, 30%) or isolate specific (1592 genes, 10%). This is the largest accessory genome reported in fungi to date. Conserved genes were enriched in genes encoding proteins with housekeeping functions and carbohydrate-active enzymes (92% of CAZymes). This enrichment might reflect the conservation of basic cellular functions needed for both growth/survival and plant infection. Secondary metabolism gene clusters were distributed between conserved (70%) and accessory (30%) genes, in similar proportions to the pangenome. However, accessory genes were enriched in genes encoding putative effectors (60%), defined as small secreted proteins, that could account for differences in the infection behavior among isolates.

2.3 Related Zymoseptoria *species genomics*

The genomes of four related *Zymoseptoria* species (*Z. ardibilae*, *Z. brevis*, *Z. passerinii*, *Z. pseudotritici*) were characterized using long-read sequencing and gene prediction with *ab initio* gene predictors and RNAseq evidence (Feurtey et al., 2020). These genomes varied in size from 38.1 Mb to 41.6 Mb, and they were organized in 13 CCs largely similar to those of *Z. tritici*. The number of ACs varied from 4 to 5. Large regions of these ACs were similar to ACs from *Z. tritici*. The gene repertoires varied from 10 528 (*Z. passerinii*) to 11 661 (*Z. pseudotritici*). Comparison of these gene repertoires highlighted 18 237 orthogroups within the *Zymoseptoria* genus including *Z. tritici*, among which 44 % were conserved (8310 core genes), and 56% were accessory either at the species (46%) or isolate level (10%). The number of core genes conserved across these five species (8310) is close to the number of core genes identified in *Z. tritici* (9193 genes, Badet et al., 2020). In addition, 25%

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of *Z. tritici* isolate-specific genes were identified in other species, suggesting a specific loss of these genes that are conserved at the genus level, in some *Z. tritici* isolates. Overall, a comparison of the genomes of these five species from the *Zymoseptoria* genus showed that they were highly related in both genome organization and gene repertoires. This similarity could result from a certain level of interspecific gene flow, as suggested by the detection of introgressions between these species (Feurtey et al., 2019).

2.4 Zymoseptoria *transposons*

A large proportion of the *Z. tritici* genome is composed of transposable elements (TEs). IPO323 genome TE content was estimated between 17% and 20% (Dhillon et al., 2014; Grandaubert et al., 2015, respectively), while the TE content of the global pangenome was estimated between 17% and 24% (19 isolates; 304 TE families; Badet et al., 2020). These variations in TE content across isolates explain 70% of their genome size variations. Related *Zymoseptoria* species have a similar TE content (13–25%), leading to 497 TE families in all *Zymoseptoria* species, among which 76 were conserved across species (Grandaubert et al., 2015; Feurtey et al., 2020). The most abundant TEs in *Z. tritici* are Class I retroelements (70%) from LTR-Gypsy and LTR-Copia super-families, followed by Class II DNA transposons (22%, Dhillon et al., 2014; Grandaubert et al., 2015; Badet et al., 2020). The distribution of TEs in the genome is not random. A small proportion of TEs were inserted in CDS (1-2%) or in promoter/terminator regions (4–6%, <1 Kb from CDS; Fouché et al., 2020; Badet et al., 2020). These insertions have a significant effect on the transcription of *Z. tritici* genes, including silencing of adjacent genes under specific conditions (Krishnan et al., 2018a; Fouché et al., 2020; Meile et al., 2020). The physical vicinity of TEs to genes might also influence their evolutionary dynamics by inducing mutations through the cell defense mechanism called repeat induced point mutations (RIP, Singh et al., 2021). In addition, a significant proportion of *Z. tritici* TEs (70%) was expressed, and specific TE families display a high level of expression during infection and under stress conditions (Fouché et al., 2020; Badet et al., 2020).

2.5 Zymoseptoria *core and accessory chromosomes*

All the information gathered on the *Z. tritici* genome allowed a detailed comparison of CCs and ACs. Compared to CCs, ACs displayed a lower gene density (2-fold, Goodwin et al., 2011; Kellner et al., 2014; Feurtey et al., 2020) and a highly reduced number of genes encoding secreted proteins (30-fold, Goodwin et al., 2011; Morais do Amaral et al., 2012). Compared to CC genes, AC genes displayed lower levels of expression *in vitro* (5–10 fold; Kellner et al.,

2014; Rudd et al., 2015; Palma-Guerrero et al., 2017; Feurtey et al., 2020). Only a small number of AC genes were differentially expressed during infection (79 genes; Rudd et al., 2015). ACs had a higher content of TEs than CCs (2-fold, Goodwin et al., 2011; Grandaubert et al., 2015; Badet et al., 2020). The role of ACs in the biology of *Z. tritici* is still a debate. Since there are no field isolates without ACs (McDonald et al., 2015b; Badet et al., 2020), and because many ACs genes are conserved in related *Zymoseptoria* species (Feurtey et al., 2020), they likely carry genes involved in functions that are important for the *Z. tritici* life cycle. Recently, forward genetics studies demonstrated that the experimental loss of some ACs from the IPO323 isolate leads to mutants with a quantitative increase in pathogenicity (2-fold) specifically on one of the four wheat cultivars tested (Habig et al., 2017). This result suggested that ACs could carry genes involved in adaptation to the host plant.

3 Molecular and biological tools available in *Z. tritici*

A number of molecular genetics tools are available for *Z. tritici* (Talbot, 2015). Genetic transformation is performed using either protoplast PEG-mediated transformation (Payne et al., 1998) or *Agrobacterium tumefaciens*-mediated-transformation (ATMT) (Zwiers and De Waard, 2001). ATMT is the method of choice since protoplast formation is now difficult to obtain due to changes in commercial fungal cell-wall degrading enzymes and possible differences among isolates in their sensitivity to these enzymes (Khan, 2017). ATMT vectors with different resistance markers (hygromycin, geneticin, sulfonylurea, basta) are available (Sidhu et al., 2015b). Targeted gene replacement is relatively easy in *Z. tritici* (5–40%; Bowler et al., 2010). *Ku70* deletion mutant strains deficient for the non-homologous end-Joining (NHEJ) DNA repair pathway displayed a strong increase in targeted gene replacement frequency (up to 100%; Bowler et al., 2010; Sidhu et al., 2015a,b). Vectors for GFP/RFP transcriptional and translational fusions are also available (Kilaru et al., 2015a; Schuster et al., 2015; Kilaru et al., 2017). Expression vectors with *Z. tritici* promoters were constructed either for over-expression (Sidhu et al., 2015a; Mehrabi et al., 2015) or for controlled expression (Marchegiani et al., 2015; Kilaru et al., 2015b; Sidhu et al., 2015a; Steinhauer et al., 2019; Fantozzi et al., 2020a), including vectors for infection-specific expression (Fantozzi et al., 2021a). Promoter replacement was achieved to identify essential genes (Marchegiani et al., 2015). Yeast-two-hybrid libraries (Ma et al., 2015) and an ORFeome library (Chaudhari et al., 2019) are available. All these molecular tools allow the genetic characterization of any gene of interest in *Z. tritici*. However, molecular tools such as *CAS9* gene editing, temperature-sensitive mutants, collections of genome-wide deletion and overexpression transformants and transposon-based insertional mutagenesis are still missing in *Z. tritici*. A first step toward

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genome-wide insertional mutagenesis was recently obtained by introducing the *Fusarium oxysporum TC1-mariner* transposable element *impala* (Dufresne and Daboussi, 2010) into *Z. tritici* (Pitarch, A., Delude, C., Petit, Y., Scalliet, G., and Lebrun, M. H., pers. com.). Transposon excision and re-insertion events were identified showing that *impala* is active in *Z. tritici* and that *impala* has an insertion preference for genes located on core chromosomes (84%), near transcriptional start sites (TSS, 50%). Collections of *impala* insertion mutants are currently screened to identify pathogenicity mutants. *Z. tritici* pathogenicity has been well-studied in laboratory conditions for decades, allowing the production of typical disease symptoms (chlorosis, necrosis), and the formation of pycnidia and pycnidiospores on experimentally infected leaves (Suffert et al., 2013; Rudd et al., 2015). Improved infection protocols have been developed to better quantify infection efficiency (Fones et al., 2015; Kay et al., 2019), as well as image analysis of infected leaves (Stewart and McDonald, 2014; Stewart et al., 2017). Controlled crosses between strains of opposite mating types have been obtained using infected plants left in outdoor conditions (Kema et al., 2008; Kema et al., 2018; Suffert et al., 2016; Suffert et al., 2019). Such crosses can also be performed under controlled greenhouse conditions (pers. comm. G. Scalliet and G. Kema). Protocols for isolation of *Z. tritici* from infected wheat leaves, *in vitro* culture, nucleic acid extraction, molecular characterization and pathogenicity assays in controlled conditions, were recently summarized by Fagundes et al. (2020).

4 Molecular biology of infection

4.1 Cell biology of wheat infection by Z. tritici

The *Z. tritici* infection process has been intensively studied using cytological methods (Cohen and Eyal, 1993; Kema, 1996; Duncan and Howard, 2000; El Chartouni et al., 2012; Yang et al., 2013; Steinberg, 2015; Fones et al., 2017; Haueisen et al., 2018; Francisco et al., 2020; Fantozzi et al., 2021; reviewed in Brennan et al., 2019). *Z. tritici* infection has been subdivided into six phases by Fantozzi et al. (2021): (1) spore deposition/adhesion on the leaf surface (surface resting), (2) spore germination and leaf surface colonization by epiphytic hyphae (surface exploration), (3) stomata penetration, (4) mesophyll/apoplast colonization, (5) initiation of pycnidia formation in the sub-stomatal cavity (fruiting body initiation), (6) pycnidia development and sporulation (fruiting body maturation). The infection of wheat by *Z. tritici* begins with the deposition and adhesion of spores on the leaf surface and the induction of their germination by an as-yet-unknown signal (Duncan and Howard, 2000). Germinating hyphae develop into epiphytic hyphae that grow abundantly on the leaf surface up to 18 dpi (Fones et al., 2017; Fantozzi et al., 2021). These

epiphytic hyphae penetrate leaves mostly through stomata from 2 dpi to 13 dpi (Fantozzi et al., 2021). However, penetration events mostly occur around 3–6 dpi depending on strains, cultivars and inoculation methods (reviewed in Brennan et al., 2019), but this estimate awaits more quantitative observations. Alternative penetration events through wounds or junctions between epidermal cells have been reported, but they have not been quantified accurately (Kema, 1996; Duncan and Howard, 2000; El Chartouni et al., 2012). *Z. tritici* does not form single-celled appressoria. Nevertheless, appressoria-like structures (hyphal swellings) were frequently described on the borders of stomatal openings during penetration (Kema, 1996; Duncan and Howard, 2000). The growth of epiphytic hyphae toward stomata occurs most likely through a stochastic process (Fones et al., 2017). However, it has been suggested that thigmotropic signals could direct hyphae toward stomata (Duncan and Howard, 2000). After penetration, infectious hyphae invade mesophyll intercellular spaces (apoplast) without causing visible symptoms (asymptomatic phase) up to 10–12 dpi. The duration of the asymptomatic phase varies according to strains, cultivars and leaf age (Suffert et al., 2013; Pnini-Cohen et al., 2000; Yang et al., 2013; Hehir et al., 2018; Haueisen et al., 2018; Rahman et al., 2020). Infectious hyphae were observed in the apoplast at a relatively long distance from the sub-stomatal cavity colonized by penetrating hyphae (Fantozzi et al., 2021). No specialized feeding structures such as haustoria were observed, nor intracellular hyphae. During this relatively long asymptomatic mesophyll/apoplast colonization, fungal biomass and transcripts are in low abundance (Kema, 1996; Pnini-Cohen et al., 2000; Yang et al., 2013; Rudd et al., 2015; Rahman et al., 2020), and plant defense responses are almost not induced (Yang et al., 2013; Orton et al., 2017; Rudd et al., 2015). Overall, during this asymptomatic phase, *Z. tritici* colonizes only slightly the sub-stomatal cavities, and instead, the fungus largely explores the mesophyll intercellular space, behaving almost like an endophyte. At 10–14 days after infection, *Z. tritici* switches to a necrotrophic behavior that starts by an exponential increase of fungal biomass and the development of chlorotic and necrotic leaf symptoms (Kema, 1996; Pnini-Cohen et al., 2000; Rudd et al., 2015; Rahman et al., 2020). During this infection phase, the sub-stomatal cavity is colonized by hyphae that initiate the formation of pycnidia (Kema, 1996; Duncan and Howard, 2000; Francisco et al., 2020; Fantozzi et al., 2021). During their maturation, pycnidia enlarge, melanize and produce a large number of pycnidiospores embedded in a hydrophilic jelly of unknown composition that is extruded through the stomatal opening (Duncan and Howard, 2000).

4.2 Transcriptomics of Z. tritici *infection*

After early transcriptomics experiments using microarrays (Keon et al., 2007; Shetty et al., 2007; Kema et al., 2008), several NGS RNAseq studies surveyed

the genome-wide expression patterns of *Z. tritici* during infection (Brunner et al., 2013; Yang et al., 2013; Kellner et al., 2014; Rudd et al., 2015; Palma-Guerrero et al., 2016, 2017; Haueisen et al., 2018; Ma et al., 2018). These high-quality transcriptomic analyses of the *Z. tritici* infection process have highlighted fungal and plant genes that are specifically expressed at different stages of infection, giving more insights into the *Z. tritici* infection process.

4.2.1 The fungal side

Using RNAseq, Brunner et al. (2013) analyzed three infection stages (7 dpi, 13 dpi and 56 dpi) of young wheat leaves by the Swiss isolate 3D7 (compatible interaction), for expression of genes encoding plant cell-wall degrading enzymes (PCWDEs). Yang et al. (2013) studied expression of genes from both the host and the pathogen (compatible and incompatible interactions) during the asymptomatic phase (4 dpi and 10 dpi) and the switch to necrotrophy (14 dpi), but this experiment suffered from a low read coverage (less than 10% of *Z. tritici* predicted genes), and a lack of comparison with the expression pattern in axenic culture. Kellner et al. (2014) compared expression of *Z. tritici* genes from isolate IPO323 in three different conditions: axenic culture, infected young wheat leaves at 4 dpi (compatible interaction) and infected young *Brachypodium* leaves at 4 dpi (incompatible non-host interaction), but this study did not include an analysis of infection kinetics. Haueisen et al. (2018) compared expression of genes from three *Z. tritici* isolates infecting young leaves from wheat (all compatible interactions) at four similar infection stages (stage A: 3-4-6 dpi, stage B: 8–11 dpi, stage C: 13 dpi, stage D: 20–24 dpi), but a comparison with expression patterns from axenic cultures was lacking. To date, the most comprehensive RNAseq transcriptomic studies of *Z. tritici* infection were performed by Rudd et al. (2015), Palma-Guerrero et al. (2016, 2017). Rudd et al. (2015) analyzed young wheat leaves infected by IPO323 at 1, 4, 9, 14 and 21 dpi and two axenic cultures. Metabolomics analyses of the same samples were also performed (Rudd et al., 2015). Palma-Guerrero et al. (2016, 2017) analyzed young wheat leaves infected by four Swiss *Z. tritici* isolates (3D7, 3D1, 1A5 and 1E4) at 3, 7, 11, 14, 21 and 56 dpi (all compatible interactions). Most results presented in Sections 4.2.1.1 to 4.2.1.3 are coming from these three transcriptomic experiments (Rudd et al., 2015; Palma-Guerrero et al. 2016, 2017).

4.2.1.1 Early infection

During the early stages of infection (1–4 dpi, surface resting and exploration stages, early penetration events) 700–1000 fungal genes were specifically expressed compared to axenic culture. They encode mostly proteins involved

in lipid degradation/metabolism and response to stress. A set of 155 genes encoding early effectors were up-regulated, including LysM proteins that protect the fungus from plant recognition of fungal chitin (Lee et al., 2014, Sánchez-Vallet et al., 2020). The most highly expressed genes at that early stage of infection, included chloroperoxidases, hydrophobins, lipases, fatty acid hydrolases and alcohol/aldehyde dehydrogenases. In addition, a strong expression of genes encoding multicopper oxidases, cytochrome P450s and oxygenases, was observed (4 dpi; Kellner et al., 2014), suggesting an active detoxification process. Overall, the transcriptomic signatures of early infection stages suggest that *Z. tritici* mostly relies on intracellular lipids from spores as a carbon source, circumvents plant defenses by detoxifying plant reactive oxygen and toxic compounds, and avoids its recognition by the plant by masking its cell wall using hydrophobins and LysM effectors.

4.2.1.2 Switch to necrotrophy

The switch to necrotrophy (9, 11, and 12 dpi, late mesophyll/apoplast colonization, fruiting body initiation stages), was characterized by the specific expression of 1000–2000 fungal genes compared to axenic cultures. These genes mostly encode proteins involved in nitrogen metabolism, nutrient uptake and growth. In addition, 235 secreted proteins without known functions, and known effectors (ECP2, Trp18, Trp21, LysM), were specifically expressed at this stage of infection. Some of these effectors were among the most highly expressed genes at that stage of infection (30% of the Top50). A strong up-regulation of genes encoding proteases, PCWDEs and lipases, was also observed, in contrast to earlier stages of infection when they were either silent or poorly expressed. In addition, genes encoding enzymes involved in secondary metabolite biosynthesis were up-regulated at this stage of infection. Overall, these transcriptomic signatures of the switch to necrotrophy suggest that the fungus is actively growing, using plant carbon and nitrogen sources, likely degraded by fungal proteases and PCWDEs, and manipulating the plant using both protein effectors and secondary metabolites. Similar to the early infection stage, the fungus continues to circumvent plant defenses by detoxifying plant reactive oxygen and toxic compounds, degrading plant defense proteins using proteases, and preventing its recognition by the plant by masking its cell wall using LysM effectors.

4.2.1.3 Necrotrophy

Early necrotrophy (14 dpi, fruiting body initiation and early maturation stages) was characterized by the over-expression of 1000 genes compared to axenic culture conditions. They mostly encode secreted proteins either with no known

functions (200 putative effectors) or with degradative activities (proteases, lipases, PCWDEs). PCWDEs were highly expressed at this stage of infection, despite their low number in the *Z. tritici* genome (Goodwin et al., 2011; Morais do Amaral et al., 2012). This necrotrophic-specific expression of proteases and PCWDEs was also observed by Haueisen et al. (2018). This suggests that *Z. tritici* utilize carbohydrates obtained by the degradation of plant cell walls as a carbon source during necrotrophy. Specific sets of genes encoding secreted effectors and enzymes involved in secondary metabolism (polyketide synthases, ABC-transporters, PKS-non-ribosomal peptide synthetase hybrid) were also expressed at this stage of infection. Overall, the transcriptomic signatures of early necrotrophy suggest that *Z. tritici* use plant carbon and nitrogen sources, likely released by fungal proteases and PCWDEs, and manipulates/damages the plant using both protein effectors and secondary metabolites that differ from those highly expressed during the transition to necrotrophy.

Overall, the *Z. tritici* infection transcriptome is characterized by waves of expression of genes encoding effectors (secreted proteins, secondary metabolite biosynthesis enzymes), proteases, lipases and PCWDEs that are specific to each stage of infection (early, mesophyll colonization, switch to necrotrophy, necrotrophy). This behavior is typical of hemibiotrophic fungal plant pathogens that display similar waves of expression of stage-specific effectors and a massive expression of genes encoding proteases and PCWDEs only during the switch to necrotrophy (O'Connell et al., 2012).

4.2.2 The plant side

To date, the most comprehensive RNAseq transcriptomic studies of the wheat side of *Z. tritici* infection were performed by Rudd et al. (2015), and Ma et al. (2018). Rudd et al. (2015) focused on wheat responses at 5-time points (1, 4, 9, 14 and 21 dpi) after infection of young wheat leaves with IPO323 (compatible), mirroring the fungal side RNAseq analysis performed by these authors. Ma et al. (2018) focused on wheat responses at 4-time points (7, 12, 14, 28 dpi) after infection of leaves with 3D7, 3D1, 1A5 and 1E4 *Z. tritici* isolates (compatible), corresponding to the fungal side RNAseq analyses of Palma-Guerrero et al. (2016, 2017). Rudd et al. (2015) showed that the asymptomatic phase of infection (4–11 dpi) is characterized by the absence or very weak defense response from the host plant, suggesting that *Z. tritici* is able to suppress its recognition by the plant in a compatible interaction such as in the highly susceptible cultivar Obelisk. However, when Ma et al., (2018) looked at transcriptional responses of the moderately susceptible wheat cultivar Drifter infected by different Swiss isolates at 7 dpi, they observed an up-regulation of wheat defense genes, including receptor-like kinases, pathogenesis-related proteins and the MLO gene. These apparent discrepancies could reflect differences in the abilities of

wheat cultivars to react to *Z. tritici* infection, although the moderate resistance of Drifter was not sufficient to stop infection. Transcriptomics analysis of the necrotrophic phase was characterized by a large transcriptional reprogramming of wheat plants. Rudd et al. (2015) observed that plant defense-related genes were up-regulated at this stage of infection culminating with hypersensitive response-like cell death as already suggested by Keon et al. (2007) for the same wheat cultivar Obelisk. Ma et al. (2018) observed that in infected leaves of the Drifter wheat cultivar, plant defense genes were greatly downregulated at the necrotrophic stage (14 dpi). However, at this stage of infection, a sharp decrease in wheat RNAseq reads was observed, suggesting a lack of transcriptional response due to extensive wheat cell collapse associated with the necrotrophic phase.

4.3 Z. tritici *secondary metabolism and infection*

Until now, there are no chemically characterized *Z. tritici* secondary metabolites apart from the melanin precursor di-hydroxy-napthalene (DHN; Sterigopoulos et al., 2013; Lendenmann et al., 2014; Muria-Gonzalez et al., 2015; Derbyshire et al., 2018). However, the genome of *Z. tritici* carries a significant number of genes involved in secondary metabolism (Goodwin et al., 2011; Ohm et al., 2012; Cairns and Meyer, 2017; Gluck-Thaler et al., 2020). Using Anti-Smash and SMURF, Cairns and Meyer (2017) predicted 32 putative biosynthetic gene clusters (BGCs) involved in secondary metabolism. These BGCs could possibly biosynthesize 11 polyketides (PKS and PKS-NRPS), 10 peptides (NRPS) and 5 terpenes (TSD). A significant number of these BCGs were located in subtelomeric regions (37%). All BCGs were located on core chromosomes. Most *Z. tritici* BCGs (80%) were expressed either in axenic culture or during infection with different stage-specific expression profiles (Rudd et al., 2015; Palma-Guerrero et al., 2017; Cairns and Meyer, 2017). In particular, two PKS and two NRPS BGCs were specifically expressed either at the onset or during the necrotrophic phase, suggesting that the corresponding secondary metabolites are involved in necrotic symptoms development. Even though BGCs were expressed, their corresponding secondary metabolites have not been characterized so far. Nevertheless, some *Z. tritici* BGCs are sufficiently related to known fungal BGCs to deduce which metabolite is likely biosynthesized. For example, the gene encoding the NPRS involved in the biosynthesis of the siderophore fusarine-C (NRPS1) was deleted by targeted gene replacement (Derbyshire et al., 2018). The corresponding null mutant was hypersensitive to iron and pathogenic on wheat, showing that *Z. tritici* does not require such an iron acquisition pathway to infect wheat. Similarly, the gene encoding the PKS involved in the biosynthesis of melanin precursor DHN (PKS1) was deleted by targeted gene replacement (Derbyshire et al., 2018). The corresponding null mutant was still pathogenic on

wheat, showing that *Z. tritici* does not require DHN melanin to infect wheat, even though pycnidia from this mutant were completely unpigmented. In addition, deletion of phosphopantetheinyl transferase encoding gene (PPT1) leads to a strong defect in pathogenicity. PPT1 provides an essential co-factor for all PKS and NRPS, including the NRPS involved lysine biosynthesis, the NRPS involved in siderophore biosynthesis (NRPS1) and the PKS involved in the biosynthesis of the melanin precursor DHN (PKS1, Derbyshire et al., 2018). Siderophore and DHN melanin are not essential for pathogenicity, while lysine biosynthesis is. Consequently, these authors concluded that the pathogenicity defect of their PPT1 deletion mutant is caused by its lysine auxotrophy. However, adding lysine to spores did not rescue the pathogenicity defect of PPT1 mutants and the lysine auxotroph mutant AAR1. This is in contrast with similar experiments performed on other plant pathogenic fungi, in which the pathogenicity defects of PPT1 null mutants and lysine auxotrophs were partially rescued by exogenous lysine or wounding (Horbach et al., 2009; Zainudin et al., 2015). These first results await additional experiments, but it is tempting to speculate that, as in other fungi, the pathogenicity defect of the *Z. tritici PPT1* mutant is due to a lack of production of yet unknown secondary metabolites, independently of its lysine auxotrophy, and DHN melanin/siderophore deficiencies. Overall, *Z. tritici* has the potential to produce at least 20 secondary metabolites that are currently unknown, some of which are likely produced during wheat leaf infection, since the genes encoding the corresponding biosynthetic enzymes are expressed during infection (Rudd et al., 2015; Palma-Guerrero et al., 2017; Cairns and Meyer, 2017).

4.4 Z. tritici *secretome and infection*

Z. tritici genome has about 970 genes encoding putative secreted proteins according to bioinformatic criteria (N-terminal signal peptide), among which 492 belong to the manually curated (refined) secretome published by Goodwin et al. (2011) and Morais do Amaral et al. (2012). One striking feature of the *Z. tritici* genome is that none of the genes encoding proteins from the refined secretome were located on dispensable chromosomes. Among the refined secretome, 321 proteins (65%) have a known functional domain, corresponding mostly to enzymes active on plant polymers (proteases, lipases and PCWDEs) and to fungal cell wall-associated proteins (LysM, hydrophobins). Another set of 171 secreted proteins encode putative effectors, including cysteine-rich small proteins without known function (n: 94). Only a small number of these secreted proteins have been functionally characterized. The most studied effectors of *Z. tritici* are the LysM effectors, homologs of the *Cladosporium fulvum* Ecp6 effector involved in protecting fungal cell walls from plant chitinases. Three LysM effector-encoding genes are present in the genome of *Z. tritici* (*Mg1LysM*, *Mg3LysM* and *MgxLysM*; Marshall et al., 2011). Mg1LysM and Mg3LysM both

interact with chitin. The crystal structure or Mg1LysM in complex with chitin was obtained showing that chitin-dependent dimerization of Mg1LysM is crucial to mask chitin from the fungal cell wall (Sánchez-Vallet et al., 2020). Only the *ΔMg3LysM* mutant is unable to colonize wheat leaves and is impaired in sporulation (Marshall et al., 2011). Silencing of the wheat chitin receptors CERK1- and CEBiP-encoding genes fully restored the pathogenicity defect of the *ΔMg3LysM* mutant (Lee et al., 2014), demonstrating that the main role of fungal LysM effector is to prevent the recognition of the fungus by these plant immunity receptors. Other well-studied *Z. tritici* effectors are the necrosis-inducing proteins expressed during the switch to necrotrophy, namely ZtNIP1 and ZtNIP2. These effectors were identified using a proteomics strategy and were able to induce chlorotic and/or necrotic lesions on several wheat cultivars (Ben M'Barek et al., 2015). *Z. tritici* has a single necrosis-inducing like protein (NLP1), expressed during the switch to necrotrophy that induces necrosis in *Arabidopsis thaliana,* but not wheat (Motteram et al., 2009). The screen for *Z. tritici* effectors inducing necrosis in *Nicotiana benthamiana* has revealed 14 proteins out of 63 candidates that likely induce resistance in this non-host plant (Kettles et al., 2017). However, these effectors were not toxic to wheat. Few other *Z. tritici* secreted proteins have been characterized for their role in pathogenicity. A secreted ribonuclease (Zt6) acting on rDNA from both plants and microbes, but not from *Z. tritici*, is essential for pathogenicity (Kettles et al., 2018). A secreted glycosyltransferase (ZtGT2) is involved in hyphal growth and pathogenicity (King et al., 2017). A secreted cysteine-rich protein interacting with a wheat E3 ubiquitin ligase is involved in infection (Karki et al., 2021). The secretion of proteins by *Z. tritici* has also been studied. Fungal extracellular vesicles (EVs) containing secreted proteins have been isolated from *Z. tritici* axenic culture filtrates. Proteomics analysis identified 240 proteins similar to those of known fungal EVs (Hill and Solomon, 2020). Whether those EVs and their secreted proteins content play a role in *Z. tritici* pathogenicity remains to be determined. The deletion of the *ZtALG2* gene encoding a protein involved in protein glycosylation led to a mutant defective in the secretion of many proteins (Motteram et al., 2011). This mutant also displayed a strong defect in pathogenicity suggesting,that the glycosylation of secreted proteins is indeed important for infection, as observed for LysM effectors.

4.5 Other mechanisms involved in Z. tritici *pathogenicity*

Fungal small RNAs (sRNAs) have been hypothesized to play a role in infection, but their suppression by the deletion of genes encoding enzymes involved in their production had no effect on pathogenicity (Kettles et al., 2019; Ma et al., 2020). *Z. tritici* is able to switch between a filamentous and a yeast-like morphology (Steinberg, 2015), and several signaling pathways are involved

in this dimorphic switch. ZtWOR1 encodes a general transcription factor (TF) required for the formation of yeast-like cells that is critical for pathogenicity (Mirzadi Gohari et al., 2014). Zt107320, a TF homologous to *M. oryzae* COD1, is quantitatively involved in the switch from yeast to hyphae and in pycnidia formation (Habig et al., 2020). The mitogen-activated protein-kinases (MAPK) such as ZtHog1 and ZtSlt2 (Mehrabi et al., 2006a,b), some key components of the cAMP pathway (Mehrabi et al., 2009) and cyclin (Choi and Goodwin, 2011) are involved in the switch from yeast to hyphae and in infection. The ZtALG2 gene required for protein glycosylation was unable to switch from yeast to hyphae and was non-pathogenic (Motteram et al., 2011). More recently, a T-DNA insertion mutagenesis identified 11 genes involved in the dimorphic switch, 2 of which encode a two-component response regulator SSK1p and a phosphoribosylamine-glycine ligase (Yemelin et al., 2017). However, these mutants were not affected in pathogenicity. Still, these results suggest that some of the genes involved in the dimorphic switch are necessary for pathogenicity. Vegetative hyphal fusions are also important during the late stages of infection, since the deletion of the ZtSof1 gene abolishes anastomosis and leads to an absence of pycnidia (Francisco et al., 2020). Few other genes involved in infection have been characterized. Deletion of the ABC transporter ZtATR4 leads to a quantitative defect in pathogenicity (Sterigopoulos et al., 2003). Deletion of the TF-encoding gene ZtVf1 leads to a quantitative defect in pathogenicity due to a reduced penetration frequency and pycnidia formation (Mohammadi et al., 2017). ZtRlm1, a TF of the MADS-box family that is critical for aerial mycelium formation, hyphal growth and melanization, is also quantitatively involved in pycnidia formation (Mohammadi et al., 2020). Finally, homologs of *Aspergillus nidulans* conidiation genes were studied in *Z. tritici* (Tiley et al., 2018). Out of five genes encoding TFs known to be involved in sporulation in fungi, *ZtFlbC* and *ZtBrlA2* were shown to be involved in pycnidiospore formation, whereas *ZtStuA* deletion resulted in non-pigmented mutants unable to differentiate pycnidia and pycnidiospores (Tiley et al., 2018; Derbyshire et al., 2018).

5 *S. tritici* blotch disease management

Fungicide applications are currently the main strategy used to control STB. This is particularly true in Europe, where most fungicide applications on cereals are dedicated to control STB. However, a major problem for chemical control is the emergence of fungicide-resistant *Z. tritici* isolates that rapidly become dominant in populations. The deployment of cultivars resistant to STB is the most economical method for managing STB. However, almost all highly resistant cultivars have not remained effective for a long time, due to the emergence of

virulent *Z. tritici* isolates. Cultural practices and biological control can reduce the incidence and severity of STB, but these methods are not very efficient.

5.1 S. tritici *blotch control using fungicides*

STB epidemics are mainly controlled by fungicide applications. In Europe, about 70% of the wheat fungicide market is targeting the control of *Z. tritici* (Fones and Gurr, 2015; Torriani et al., 2015). In practice, up to four fungicide applications are performed in the United Kingdom and Ireland, where the climate is strongly conducive for STB (Bouma, 2005; Chaloner et al., 2019). The earliest foliar treatments are performed at GS30, at the start of stem elongation (see https://www.far.org.nz/assets/files/uploads/Iss_02_Cereal_Growth_Stages_June_09.pdf) in order to limit primary STB infections of young leaves. In most cases, the first foliar treatment is performed at the first node stage (GS31-32). Predictive tools that consider meteorological events and wheat varietal resistance help growers in deciding the timing of the first foliar application (http://www.barometre-maladies.arvalis-infos.fr/bletendre/). Additional treatments are focused on protecting the upper leaves from STB between the start of flag leaf emergence and ear emergence (GS39-49; Carmona et al., 2020). Since the introduction of synthetic fungicides to protect cereals from fungal diseases in the 1940s, four groups of single-site fungicides were used for STB control (Morton and Staub, 2008). The benzimidazoles, targeting fungal tubulin, were introduced in the late-1970s. This fungicide class is currently rarely used for STB control since the resistance-conferring beta-tubulin E198A-target mutation is widespread in *Z. tritici* populations (Lucas et al., 2015; Garnault et al., 2019). The DMI or azole fungicides, inhibitors of lanosterol 14α-demethylase in the fungal ergosterol biosynthesis pathway, were introduced in the 1970s and are still widely used for STB control (Clarke, 2006). Resistance toward DMIs occurs either through mutations of the *CYP51* gene encoding the target and/or by mutations of its promoter leading to its overexpression (Leroux and Walker, 2011; Cools et al., 2012; Cools and Fraaije, 2013). A third resistance mechanism is based on an increased efflux through the overexpression of the MgMfs1 transporter (Omrane et al., 2015; Omrane et al., 2017; Maë et al. 2020). The increased occurrence of less sensitive phenotypes from year to year correlates with a gradual erosion of DMI fungicide activity in the field (Blake et al., 2018), due to accumulation of non-synonymous substitutions in *CYP51* (Brunner et al., 2008). Azole-resistant populations also emerged in Australia sometime after 2011 (Milgate et al., 2016) with similar alleles to those found in European populations (McDonald et al., 2019). At a global scale, fungicide applications (mostly DMIs) have been a major driver of *Z. tritici* evolution, both through *de novo* mutations and gene flow (Hartmann et al., 2020). Nevertheless, the increase in DMI resistance frequency in populations is slow and azole fungicides are still effective, despite the

presence of resistant alleles (Garnault et al., 2019). This could be due to the spectrum of resistance of each allele that confers resistance only to specific azole fungicides (incomplete cross-resistance, Fraaije et al., 2007; Leroux et al., 2007). In addition, primary CYP51 azole-resistant alleles display quantitative enzymatic defects, suggesting that *Z. tritici* populations slowly evolved complex CYP51 alleles (multiple mutations) to compensate for these defects (Cools et al., 2011; Cools and Fraaije, 2013). The quinone outside inhibitors (QoIs or strobilurins fungicides), targeting respiration complex III and displaying a high activity against STB, were introduced in the 1990s (Morton and Staub, 2008). However, resistance, caused by a mutation of the mitochondrial *cytb* gene, resulting in a G143A protein substitution, led to a major decrease of efficacy shortly after their introduction (Grasso et al., 2006; Sierotzki et al., 2007; Gisi et al., 2002; Fraaije et al., 2005; Torriani et al., 2009). Transmission of the mitochondrial *cytb* G148A resistant allele was facilitated during sexual reproduction on treated wheat plants (Kema et al., 2018). QoIs resistance is now fixed within most *Z. tritici* populations (Cheval et al., 2017). The succinate dehydrogenase inhibitors (SDHis), targeting complex II of the mitochondrial respiratory chain, were the last class of single-site inhibitors introduced for STB control in the late 2000s (Sierotzki and Scalliet, 2013). Resistance to SDHIs has arisen through mutations in genes encoding subunits of the ubiquinone binding pocket of the enzyme (Fraaije et al., 2012; Scalliet et al., 2012; Dooley et al., 2016), conferring different resistance levels and cross-resistance profiles across the SDHIs (Fraaije et al., 2012; Scalliet et al., 2012). Numerous field mutations were observed since 2012 (Garnault et al., 2020; https://www.frac.info/frac-teams/working-groups/sdhi-fungicides/), while the H152R mutation conferring a high level of resistance, was only reported after 2015 (Dooley et al., 2016; Rehfus et al., 2018). Subsequently, this H152R mutation is only present at a low frequency, likely as a result of a fitness penalty (Scalliet et al., 2012). Interestingly, a dispensable paralog of the C-subunit encoding gene was discovered that is affecting a particular subclass of SDHIs (Yamashita and Fraaije, 2018; Steinhauer et al., 2019). This is the first example of a pre-existing resistance in *Z. tritici* populations toward agrochemicals. The last group of inhibitors used for STB control is multisite inhibitors (e.g. folpet, chlorothalonil and mancozeb). These are thiol-reactive molecules acting on multiple targets (Medina-Cleghorn et al., 2015) and no resistance has been reported in *Z. tritici* so far (Birr et al., 2021). Recently, two novel complex III inhibitors, fenpicoxamid and metyltetraprole, have been reported. Fenpicoxamid is a semi-synthetic derivative of UK-2A, which is a natural molecule produced by *Streptomyces* sp. (Owen et al., 2017; Shibata et al., 1998). Fenpicoxamid is converted to UK-2A by *Z. tritici* and plant cells enabling the UK-2A molecule, structurally related to antimycin A, to specifically bind and inhibit the cytochrome bc1 complex at the quinone inside (Qi) site (Machida et al., 1999; Owen et al., 2017; Young et al., 2018). UK2A

binds to a different target site of the cytochrome bc1 complex, and it does not display cross-resistance with QoI-resistant isolates. Metyltetraprole is a member of a new generation of QoI fungicides compatible with existing QoI resistance mutations that inhibit mitochondrial electron transport at the Qo site of the cytochrome bc1complex (Arakawa et al., 2018; Suemoto et al., 2019; Matsuzaki et al., 2020). Qi mutations, causing fenpicoxamid/UK-2A resistance, were generated in *Z. tritici* (Fouché et al., 2021) and *Saccharomyces cerevisiae* (Young et al., 2018). Novel molecules for the control of STB were recently reported. A novel C_{18} mono-alkyl lipophilic cation, displaying a high efficacy against STB, acts on mitochondrial respiration by inhibiting oxidative phosphorylation and inducing reactive oxygen species production at the level of respiratory complex I (Steinberg et al., 2020). Semi-synthetic rhamnolipids (RLs) were active on *Z. tritici* (Platel et al., 2020). Semi-synthetic sesamol esters displayed high activity against *Z. tritici* (Damiens et al., 2021). Effusol, a 9,10-dehydrophenanthrene natural product from rhizomes of the plant *Juncus maritimus*, is active against *Z. tritici* (Sahli et al., 2018). Cyclic lipopeptides from *Bacillus subtilis* such as mycosubtilin was active on *Z. tritici in vitro* and *in planta* (Mejri et al., 2018). In addition, Kerdraon et al. (2019) showed that *Z. tritici* alters microbial community networks during its crop residue pathogen stage. However, no significant direct interactions between microbes and *Z. tritici* were found. A deeper understanding of microbial networks will support the discovery of species which might be developed as biocontrol agents. Overall, because of the rapid emergence and spread of *Z. tritici* isolates resistant to most fungicides, there is a need for the discovery of the novel mode of actions and molecules (Torriani et al., 2015), and the development of application methods that lower the negative impact of fungicide resistance (timing of application, application rate, fungicides mixtures or alternation; van den Bosch et al., 2014).

5.2 S. tritici *blotch control using wheat resistant cultivars*

STB epidemics are also controlled by resistant wheat cultivars, carrying major resistance genes (*Stb*) and/or quantitative polygenic resistance genes. To date, 22 *Stb* genes have been identified in cultivated, landraces, wild and synthetic hexaploid wheat (Brown et al., 2015; Arraiano and Brown, 2017; Yang et al., 2018). All *Stb* genes have been overcome by virulent *Z. tritici* isolates (Cowger et al., 2000; Krenz et al., 2008; Brown et al., 2015; McDonald and Mundt, 2016). For example, *Stb1* and *Stb4* were efficient for over a decade, but they became ineffective due to the emergence of virulent isolates in *Z. tritici* populations which became widespread (Ponomarenko et al., 2011). To date, two wheat *Stb* resistance genes have been characterized. *Stb6* encodes a wheat receptor-like kinase conferring resistance against *Z. tritici* isolates carrying the *AvrStb6* avirulence gene (Saintenac et al., 2018). *Stb16q* encodes a wheat cysteine-rich

receptor-like kinase conferring resistance against avirulent *Z. tritici* isolates (Saintenac et al., 2021). *AvrStb6* encodes a secreted protein with an unknown function that is expressed during infection (Zhong et al., 2017; Kema et al., 2018). Virulent isolates carry alleles of *AvrStb6* that are highly divergent from those of avirulent isolates (Zhong et al., 2017; Brunner and McDonald, 2018; Stephens et al., 2021). Another avirulence gene of *Z. tritici*, *Avr3D1*, also encodes a secreted protein with unknown function that is recognized by resistant wheat cultivars likely carrying the *Stb7* resistance gene (Meile et al., 2018). However, this recognition only induces partial resistance. Several sources of partial resistances have been identified as QTLs, although few turned out to be monogenic. For example, *Stb17* is involved in a quantitative resistance trait expressed at the adult plant stage (Tabib Ghaffary et al., 2011, 2012). To date, 167 QTLs of resistance have been detected in bi-parental mapping populations (Brown et al., 2015). A total of 105 resistance QTLs have been integrated into 27 meta-QTLs (Goudemand et al., 2013). These resistance QTLs were associated with either a reduction of leaf surface covered with necrosis or pycnidia or a reduction of disease progression (area under the disease progress curve, AUDPC). Two minor QTLs increasing the latent period have also been detected (Tabib Ghaffary et al., 2011; Hehir et al., 2018). A recent GWAS study of 175 winter wheat landraces and cultivars, identified 10 novel resistance QTLs (Odilbekov et al., 2019). Other sources of quantitative resistances have been discovered using molecular strategies, such as wheat small secreted proteins (SSPs) interacting with *Z. tritici* SSPs (Zhou et al., 2020), and taxonomically restricted wheat genes encoding proteins interacting with *Z. tritici* SSPs (Brennan et al., 2020). Unexpected responses to STB were identified in the wheat cultivar Stigg, displaying a partial resistance associated with a long asymptomatic phase (Benbow et al., 2020), and a weak defense response likely delaying the switch to necrotrophy (Benbow et al., 2020). In addition, the impact of STB can be reduced by traits contributing to disease escape, which limits the spread of fungal inoculum within crops (Arraiano et al., 2009). For example, wheat cultivars which are taller and later-heading will reduce the spread of spores to the upper leaves, and slow down the epidemic. However, disease escape traits can be undesirable in terms of agronomic properties and yield. Wheat genes suspected to enhance susceptibility to STB are thought to have been introduced inadvertently into UK wheat cultivars between the 1950s and 1980s, as a consequence of breeding programs aimed at increasing yield, rust and eyespot resistance (Arraiano and Brown, 2017). Indeed, in the United Kingdom, STB was not a major wheat disease from the 1900s to 1980s (Bearchell et al., 2005; Shaw et al., 2008). It re-occurred in the 1980s on newly released cultivars such as Norman and Longbow that turned out to be highly susceptible to STB (Arraiano and Brown, 2017). Overall, breeding for STB-resistance over the last 30 years in Europe presumably happened by phenotypic

selection of field resistance rather than marker-assisted breeding, leading to the introduction of some *Stb* genes and the accumulation of resistance QTLs (Miedaner et al., 2013; Torriani et al., 2015; Mikaberidze and McDonald, 2020). Even if defeated, *Stb* resistance genes could be used either for pyramiding or for mixture/alternation deployment strategies (Kristoffersen et al., 2020). QTLs involved in partial resistance still needs to be better characterized at the genetic/molecular level, but their accumulation using marker-assisted breeding is feasible. Improvement of *Stb* genes by molecular techniques is also a promising area, as it may lead to novel resistance alleles recognizing virulent isolates, as proposed for other plant–microbe interactions (Bisht et al., 2019).

6 Conclusion

Z. tritici is an important fungal wheat pathogen both at the economic level and as a model to study plant–fungal interactions, their evolutionary dynamics and the molecular mechanisms involved. As such, it is considered among the top 10 fungal plant pathogens (Dean et al., 2012). *Z. tritici* has been the subject of intense genetics, molecular and genomics analyses (see Sections 2– 4). In particular, it has the largest number of ACs reported so far in fungi. These ACs have specific features compared to other chromosomes, such as a lower number of genes and a higher content of TEs. In addition, genes from ACs are less expressed than those from CCs including during infection. The role of ACs in the biology of *Z. tritici* is still open to debate, but there are no field isolates without ACs. In addition, many ACs genes are conserved in related *Zymoseptoria* species (Feurtey et al., 2020). Therefore, they likely carry non-essential genes important for the *Z. tritici* life cycle, including genes involved in adaptation to the host plant (Habig et al., 2017).

The molecular mechanisms underlying *Z. tritici* infection process are still poorly understood. Nevertheless, some important features of *Z. tritici* infection have been uncovered. Massive transcriptomic analyses have laid the foundations for studying the molecular mechanisms involved in this particular infection process. The early stages of *Z. tritici* infection are now better described. Spores germinate on the leaf surface and develop immediately into epiphytic hyphae that grow abundantly on the leaf surface up to 18 dpi (Fones et al., 2017; Fantozzi et al., 2021). This epiphytic behavior may have important consequences for the infection process and STB control. Indeed, it allows the penetration into wheat leaves through stomata over a long period (2 dpi to 13 dpi, Fantozzi et al., 2021). Since most penetration events are relatively late (4–6 dpi), the destruction of epiphytic hyphae that are easily accessible to fungicides could hamper infection. The penetration of *Z. tritici* through stomata is well-documented, but the mechanisms involved are poorly understood. *Z. tritici* does not differentiate single-cell appressoria. However, hyphal swellings

were frequently described on the borders of stomata openings during penetration (Kema, 1996; Duncan and Howard, 2000). These swellings could help the fungus to redirect hyphal growth from the leaf surface plane to the interior of the stomata, as most fungal appressoria-like structures do (Demoor et al., 2019). These structures that have so far received little attention could be essential for the success of penetration. Transcriptomics of early infection stages (1–4 dpi, before penetration) are characterized by expression of fungal genes encoding proteins involved in detoxifying processes and stage-specific effectors and proteases, suggesting that *Z. tritici* is actively modifying its environment and manipulating its host. After penetration, infectious hyphae invade the mesophyll intercellular space (apoplast) without causing visible symptoms (asymptomatic phase) nor inducing plant defenses responses, up to 10 dpi. These infectious hyphae grow a long distance away from the sub-stomatal cavity colonized by penetrating hyphae (Fantozzi et al., 2021). This large exploration of the apoplast by *Z. tritici* hyphae creates a web of fungal cells in close contact with plant cells, as observed for cereal endophytes. This stage of infection is also not yet sufficiently documented, although transcriptomics identified stage-specific effectors and enzymes from secondary metabolism, suggesting that *Z. tritici* is actively manipulating its host plant. The switch to the necrotrophic stage is associated with an exponential fungal growth, expression of a large number of stage-specific effectors and enzymes involved in plant cell wall degradation, as well as stage-specific enzymes involved in secondary metabolism, although the triggers of this switch are not known. The role of these late effectors and secondary metabolites is still unknown, but some are likely involved in the development of chlorotic and necrotic symptoms. After this switch, *Z. tritici* recolonizes sub-stomatal cavities to differentiate pycnidia. Understanding this fungal development process (Tiley et al., 2018; Francisco et al., 2020) will be key for controlling this disease. Overall, *Z. tritici* infection is characterized by waves of expression of stage-specific effectors (proteins, secondary metabolites) and degradative enzymes (proteases, lipases and PCWDEs). These waves of fungal gene expression were also observed in hemibiotrophic fungal plant pathogens, including the massive expression of genes encoding proteases and PCWDEs during the switch to necrotrophy (O'Connell et al., 2012). These advances in understanding *Z. tritici* infection strongly suggest that it behaves as a hemibiotrophic fungus, acting as an endophyte during its asymptomatic phase. Even though the trigger that induces the switch to necrotrophy is unknown, the behavior of *Z. tritici* during this stage of infection relies on the massive expression of effectors and degradative enzymes, as observed in hemibiotrophic fungi. The regulatory networks involved in the control of these infection stage-specific waves of expression are unknown, but their study will likely bring important information on this infection process.

Z. tritici has not been always a major pathogen of wheat across the last century in Europe. Long-term trials in the United Kingdom (Broadbalk experiment from 1843 to 2003 in the United Kingdom in Bearchell et al., 2005; Shaw et al., 2008, and from 2015 to 2017 at other UK sites in Justesen et al., 2021) have shown that *Parastagonospora nodorum* and *Z. tritici* had shared and distinct occurrences on wheat over a long period of time. Both are leaf wheat pathogens from the Dothideomycetes, producing pycnidia on infected wheat leaves, although using very different infection strategies. Nevertheless, they frequently occur on the same infected wheat leaves (Justesen et al., 2021). PCR quantification of each fungal species in samples from these long-term trials have clearly shown that both fungi were detected in similar abundance in wheat leaves from 1840 to 1860, followed by a near absence of both pathogens until 1910. From 1910 to 1980, *P. nodorum* was the dominant species on wheat leaves, although *Z. tritici* was detected in low abundance. Since the 1980s, *P. nodorum* incidence has rapidly decreased, and it is now completely absent from most bread wheat fields in the United Kingdom and most European countries. Still, it is responsible for significant epidemics on *durum* wheat in Southern Europe (Iori et al., 2015), and on bread wheat in Northern Europe (Blixt et al., 2010; Justesen et al., 2021), Northern America and Australia (Downie et al., 2020). Since the 1980s, *Z. tritici* has emerged as the major pathogen of wheat in the United Kingdom and Europe, replacing *P. nodorum*. The biological causes underlying this intriguing switch in dominance between two fungal species having the same ecological niche (wheat) are still open to debate. Originally, it was proposed that the decrease in *P. nodorum* observed in the 1980s was due to the increase in SO_2 pollution, suggesting that *Z. tritici* was more resistant to this pollution. Subsequently, other parameters have been suggested to be involved, including the use of wheat cultivars that are highly susceptible to *Z. tritici* in the 1980s (Arraiano and Brown, 2017), the massive use of fungicides since the 1970s, and the development of resistance to these chemicals in *Z. tritici* (Lucas et al., 2015), but not in *P. nodorum*, which is still sensitive to many fungicides (Blixt et al., 2009). Despite being responsible for epidemics on wheat worldwide, *Z. tritici* is absent from some regions such as China, in which *P. nodorum* epidemics are frequent, and it is not the main pathogen of wheat in some regions such as Australia and North America, where *P. nodorum* dominates (Downie et al., 2020). These fluctuations in *Z. tritici* occurrence over time and space, are puzzling questions to tackle, but the answers could help to control this disease. In regions where *Z. tritici* is responsible for sustained and massive epidemics, this disease is still difficult to control. Fungicides have been the main control method in Europe since the 1980s, but the rapid emergence and spread of *Z. tritici* fungicide-resistant isolates have dramatically reduced the efficiency of some fungicides (Lucas et al., 2015). For example, strobilurins resistant *Z. tritici* isolates carrying a mutation of the mitochondrial *Cytb* gene have emerged

rapidly (in a few years) after the release of these fungicides in the 1990s (Morton and Staub, 2008; Lucas et al., 2015). Because this resistance was rapidly fixed in most *Z. tritici* populations, the use of these fungicides was discontinued for controlling *Z. tritici*, but not other wheat fungal diseases. As a consequence, there is strong demand for the discovery of novel fungicides controlling *Z. tritici* populations resistant to previously developed molecules (Torriani et al., 2015). In addition, methods that counteract/lower the emergence of fungicide resistance are more than ever needed (van den Bosch et al., 2014). The same scenario has been observed for *Stb*-resistant wheat cultivars, and all *Stb* resistance genes have been defeated by virulent isolates (Brown et al., 2015), likely carrying a mutation in a single gene (avirulence gene, Zhong et al., 2017; Brunner and McDonald, 2018). As a consequence, there is a need for identifying new major resistance genes in wheat. Even if defeated, *Stb* resistance genes could be used either for pyramiding or for mixture/alternation deployment strategies, to counteract/lower the incidence of virulent isolates (Pilet-Nayel et al., 2017; Kristoffersen et al., 2020). QTLs involved in partial resistance are thought to be more durable, and their use in breeding programs is now more feasible, but their erosion due to constantly evolving *Z. tritici* populations is still possible, as it has been observed in other fungal plant interactions (Mundt, 2014).

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8 Where to look for further information

A large community of scientists is working on *Z. tritici*. A list of the laboratories involved is available as well as introductions to the different aspects of *Z. tritici* research, on the website: http://www.septoria-tritici-blotch.net/.

The *Z. tritici* research community is very active and can be followed up on Twitter: https://twitter.com/Zymoseptoria.

Regular meetings are organized (ISCLB). The last ISCLB was organized in Dublin in 2019. The program and abstracts are available at: https://researchrepository.ucd.ie/handle/10197/11177.

Many protocols for *Z. tritici* are available, and the best introductions are:

Fungal Genetics and Biology special issue on *Z. tritici* (2015) vol. 79.

Fagundes, W. C., Haueisen, J. and Stukenbrock, E. H. (2020), Dissecting the Biology of the Fungal Wheat Pathogen *Zymoseptoria tritici*: A Laboratory Workflow, *Current Protocols in Microbiology*, 59, e128.

https://www.protocols.io/workspaces/zymoseptoria-community-protocols.

Z. tritici genomics websites displaying different annotations of IPO323 genomic sequence.

https://mycocosm.jgi.doe.gov/Mycgr3/Mycgr3.home.html.

https://fungi.ensembl.org/Zymoseptoria_tritici/Info/Index.

https://fungidb.org/fungidb/app/record/dataset/DS_36dcafc6dd.

Genomic websites for the other *Z. tritici* stains and other *Zymoseptoria* species are available at Ensembl and Mycocosm.

Updates on fungicide resistance surveys of *Z. tritici* population and recommendations on using fungicides for its control are displayed at: FRAC: https://www.frac.info/frac-teams/working-groups/.

Impact of STB, disease management practices and cultivar resistance are displayed at:

ARVALIS (France): https://www.arvalis-infos.fr/septoriose-observer-pour-decider-d-une-intervention-@/view-18536-arvarticle.html.

AHDB (UK): https://ahdb.org.uk/knowledge-library/septoria-tritici-in-winter-wheat.

TEAGASC (Ireland): https://www.teagasc.ie/crops/crops/cereal-crops/spring-cereals/disease/.

GRDC (Australia): https://grdc.com.au/resources-and-publications/grdc-update-papers/tab-content/grdc-update-papers/2021/08/cereal-disease-update-2021.

FAR (New Zealand): https://www.far.org.nz/articles/tags/2/cereal/15/wheat.

International research centers working on wheat and *Z. tritici:*

CIMMYT: https://www.cimmyt.org/ and https://repository.cimmyt.org/.

ICARDA: https://www.icarda.org/.

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