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Advances in genetic mapping of Septoria nodorum blotch resistance in wheat and applications in resistance breeding

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1 Introduction

As one of the most important staple food sources, wheat (*Triticum* spp.) is a key component of global food security, with allohexaploid bread wheat (*Triticum aestivum* L., AABBDD) dominating wheat production as the most widely grown cereal species. However, wheat cultivation is limited by many stresses, including fungal diseases. Septoria nodorum blotch (SNB), caused by the fungal pathogen *Parastagonospora nodorum*, is notably a problem in wheat-growing areas with high rainfall such as North-Western, Central and Eastern Europe, Canada, the United States, South America and western Australia. For a general review of the disease we refer to Ruud and Lillemo (2018) for details. In this chapter, we aim to provide an in-depth overview of recent findings with a focus on the genetics of adult plant SNB resistance in wheat and its implications for resistance breeding.

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2 Pathogen

Parastagonospora nodorum (syn. *Phaeosphaeria nodorum* (E. Müll.), syn. *Leptosphaeria nodorum* (E. Müll.), syn. *Stagonospora nodorum* (Berk.), syn. *Septoria nodorum* (Berk.)) is a typical necrotrophic fungal pathogen. It belongs to the Dothideomycetes class of the Ascomycota (Quaedvlieg et al. 2013). *P. nodorum* is mainly known as a pathogen of wheat, but can occasionally also infect barley (*Hordeum vulgare*) with less damage, reviewed by Cunfer (2000), as well as wild grasses (Williams and Jones 1973). The disease caused by *P. nodorum* is most commonly called Septoria nodorum blotch (SNB), but is also known as Stagonospora nodorum blotch. When *P. nodorum* infects glumes, the resulting disease is called wheat glume blotch (Oliver et al. 2016).

2.1 Symptoms

Symptoms of *P. nodorum* infection on wheat leaves start as oval brown necrotic lesions that are typically surrounded by chlorosis. As they develop, these lesions turn into irregular dark brown areas of necrotic tissue (Fig. 1a). In the field, the symptoms can easily be confused with those caused by two other important wheat leaf blotch fungal diseases: tan spot (TS) (caused by *Pyrenophora tritici-repentis*) and Septoria tritici blotch (STB) (caused by *Zymoseptoria tritici*) (Ficke et al. 2018a). Coinfection of these three diseases is common in field conditions (Blixt et al. 2010; Justesen et al. submitted). However, as both STB and TS are well-known as leaf diseases which seldom cause symptoms on wheat heads,

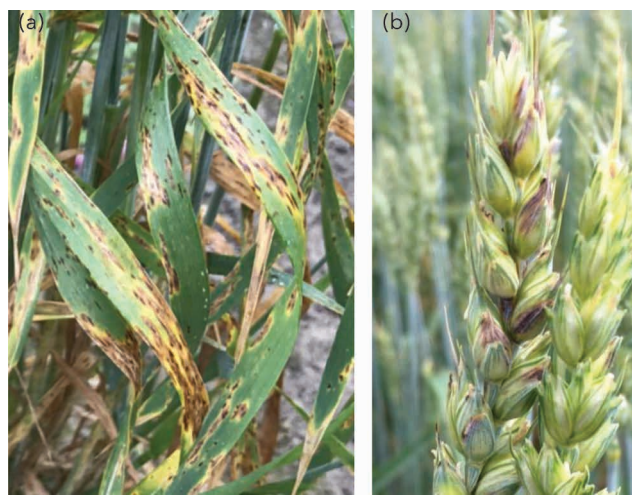

Figure 1 Symptoms of Septoria nodorum blotch in wheat. (a) Symptoms on leaves, photographed by Morten Lillemo. (b) Symptoms on head, photographed by Andrea Ficke.

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glume infection is an important indicator of SNB infection. Figure 1b shows the SNB symptoms on wheat heads, which start from brown to dark brown spots on the glumes.

2.2 Infection cycle and epidemics

P. nodorum is a heterothallic fungus which requires two mating-type idiomorphs in the population for sexual reproduction (Bennett et al. 2003). Wind-spread ascospores released from wheat debris serve as the major primary source of inoculum early in the season (Bathgate and Loughman 2001). As shown in Fig. 2, *P. nodorum* is also seed-transmitted (Sommerhalder et al. 2006). Infected seeds can also be a source of primary inoculum when seed treatments are poorly applied. After the establishment of the initial infection, the pathogen starts producing massive pycnidiospores which can be spread through rain splash to neighboring plants. The pathogen is polycyclic and can complete multiple cycles in one growing season, producing a considerable amount of pycnidiospores as secondary inoculum (Eyal et al. 1987; Sommerhalder et al. 2011). Epidemics of *P. nodorum* used to be common in all wheat-growing areas with suitable climatic condition for disease development across all six

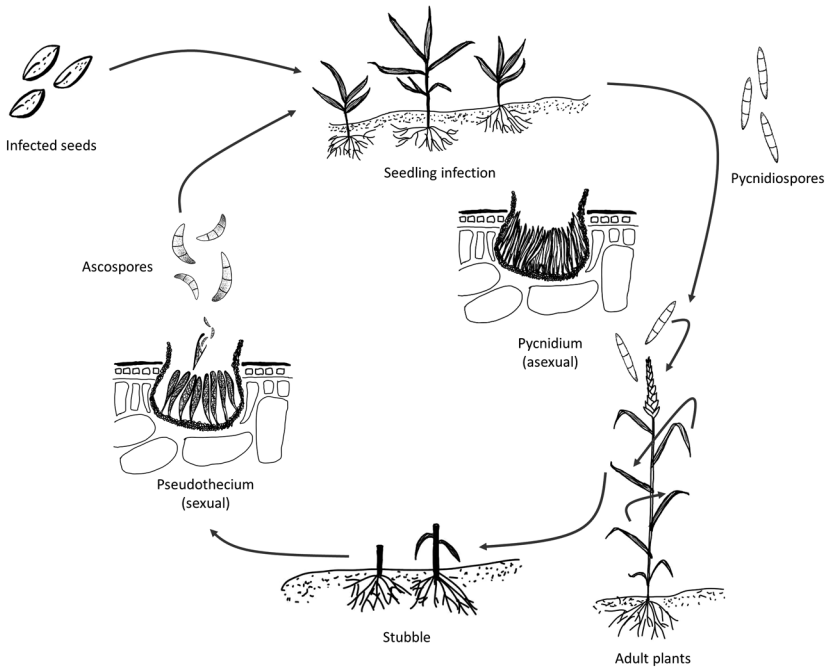

Figure 2 Infection cycle of *P. nodorum*. Drawing by Ling Su, adapted from Sommerhalder et al. (2011).

wheat-growing continents (Ficke et al. 2018a; Leath et al. 1993). The pathogen shares the same center of origin as its wheat host in the Fertile Crescent and probably spread during wheat germplasm exchange (McDonald et al. 2012; Ghaderi et al. 2020). As mentioned previously, *P. nodorum* and *Z. tritici* often cause coinfection on the host in the same field, because the asexual spores of both pathogens are spread by rain splash and prefer to grow in similar warm and humid conditions (Bearchell et al. 2005). *P. nodorum* was the dominating leaf blotch pathogen in Europe until the 1980s (Bearchell et al. 2005). Since then, it has in most of Europe been replaced by *Z. tritici*, and STB management is now responsible for large proportions of the fungicide applications in wheat (Bearchell et al. 2005; Shaw et al. 2008; Torriani et al. 2015). The reduction of *P. nodorum* epidemics in the UK has been correlated with the decrease in sulfur pollution (Shaw et al. 2008). However, the same sulfur theory could not explain the dominance of *P. nodorum* in western Australia (Oliver et al. 2012), or Norway, which is one of the remaining *P. nodorum* hot spots in Europe.

2.3 Population genetics studies of P. nodorum

P. nodorum undergoes frequent sexual reproduction, and high frequency of recombination results in pathogen populations with high genetic diversity (McDonald and Linde 2002). Studies on *P. nodorum* population structure have been carried out based on different molecular markers or sequence variations of selected genes (McDonald et al. 2012; Murphy et al. 2000; Stukenbrock et al. 2006). McDonald et al. (1994) investigated the genetic variability of two *P. nodorum* populations in the United States with eight restriction fragment length polymorphism (RFLP) markers and high level of genetic diversity was found even among isolates collected from the same lesion. A similar study investigated genetic variations of *P. nodorum* collections from Europe and the United States, while only small genetic differentiations were observed between populations (Keller et al. 1997). A western Australian *P. nodorum* population genetic study was undertaken using the same RFLP markers described above, and no evidence of population subdivision was observed due to high genetic variability within the population (Murphy et al. 2000). Similar results of a highly diverse pathogen population with no signs of population structure were recently also shown for the Norwegian *P. nodorum* population (Lin et al. 2020b); see Section 5 of this chapter. Stukenbrock et al. (2006) used 12 simple sequence repeat (SSR) markers to characterize the population structure of *P. nodorum* at a global scale, based on nine populations from five continents. As expected, high genetic diversity was observed within each population, but only moderate differentiation was detected between genetically divided populations (Stukenbrock et al. 2006). McDonald et al. (2013) compared the genetic diversity of three *P. nodorum* necrotrophic effector (NE) genes (*SnToxA*,

SnTox1 and *SnTox3*) and found significant differences in allele frequencies of the three genes among populations. However, *P. nodorum* populations with high *SnTox* gene sequence diversities were not correlated with high diversity at neutral loci, suggesting that the *SnTox* genes were under selection by local host cultivars (McDonald et al. 2013). A high frequency of the *SnToxA*, *SnTox1* and *SnTox3* genes and higher genetic diversity at neutral SSR loci of isolates collected in Iran compared to other geographic origins provided further evidence to the hypothesis that *P. nodorum* originated as a pathogen on wheat in the Fertile Crescent (Ghaderi et al. 2020). A recent study of 197 *P. nodorum* isolates originating from the United States found evidence of two populations that corresponded to the Upper Midwest and South-Eastern United States (Richards et al. 2019). Interestingly, isolates belonging to the South-Eastern populations lacked the *SnToxA* effector gene, which fitted well with the absence of the ToxA-sensitivity gene, *Tsn1*, in the widely grown winter wheat cultivars in the region. Overall, this suggests that effector genes in pathogen populations are under selection pressure for fitness on host genotypes.

3 Agricultural importance and management of Septoria nodorum blotch (SNB)

In modern agroecosystems, where high density of crops are grown, favorable environments are provided for multi-infections where different strains of the same pathogen can infect the same host, especially for pathogens with large population sizes combined with high genetic diversity (McDonald and Stukenbrock 2016; van Baalen and Sabelis 1995). It also promotes the development of virulence due to competition between strains from the same pathogen species and/or among pathogen species under coinfection conditions (McDonald and Stukenbrock 2016; van Baalen and Sabelis 1995). Under favorable climate conditions, *P. nodorum* can cause severe grain shriveling and reduce yield up to 30% (Bhathal et al. 2003) by destroying the photosynthetic capacity of the leaves and glumes.

Ascospores originating from wheat debris serve as the primary inoculum of SNB (Bathgate and Loughman 2001). Reduced tillage, which is advocated to reduce soil erosion, can promote SNB as it leaves higher amounts of infected wheat straw on the soil surface, which can then serve as primary inoculum (Ficke et al. 2018a). In addition, the pycnidiospores are spread by rain-splash. The high density of plants within the wheat fields makes it easier for pycnidiospores to spread to neighboring plants.

Disease management of SNB includes cultivar resistance, fungicide treatment and stubble management. Both seed treatment and fungicide application in the field are widely used chemical control methods for SNB management (Solomon et al. 2006). However, Blixt et al. (2009) reported that

the majority of tested Swedish *P. nodorum* isolates showed reduced sensitivity to strobilurins, which was caused by amino acid substitutions in the *cytochrome b* gene. Pereira et al. (2017) reported variations in sensitivities to sterol demethylation inhibitors (DMIs) within a global *P. nodorum* collection, and the reduced sensitivity was due to non-synonymous mutations in the *CYP51* gene. Neither study included *P. nodorum* isolates collected in recent years (2011–present) for fungicide resistance testing. Therefore, higher frequency of fungicide insensitive isolates would be expected in the natural *P. nodorum* population due to the high selection pressure. However, field resistance to azoles or DMIs has not been reported so far.

As mentioned previously, reduced tillage increases the amount of crop residues that can serve as inoculum for residue-borne leaf blotch diseases (Ficke et al. 2018a; Shaner 1995). SNB disease severity in the field has shown significant correlations with the amount of residues, as can be expected (Mehra et al. 2015). Residue management (e.g. burial of residues and crop rotation) can effectively reduce the disease severity by decreasing the amount of primary inoculum when healthy seeds are used (Mehra et al. 2015).

4 Methods used in genetic studies of Septoria nodorum blotch (SNB)

Due to the large genome size of hexaploid bread wheat (approx. 16 Gb), genetic studies of wheat were among the most complicated of all cultivated plants (International Wheat Genome Sequencing 2014). Previous studies show that the inheritance of SNB resistance is quantitative (Bostwick et al. 1993; Wicki et al. 1999). However, neither the genetic location nor the molecular basis of SNB resistance genes was clear before the usage of molecular markers in the mapping of quantitative trait loci (QTL) (Czembor et al. 2003; Friesen and Faris 2010). Thanks to the rapid development of high-throughput genotyping technologies, genotyping by single nucleotide polymorphism (SNP) chips are nowadays widely used in wheat (Allen et al. 2017; Wang et al. 2014a), and have largely replaced the use of SSR or DArT (diversity arrays technology) markers which were the most commonly used marker types for wheat genetic studies less than a decade ago (Akbari et al. 2006; Langridge et al. 2001), resulting in an increasing numbers of published QTL mapping studies. In addition to QTL mapping using biparental populations, genome wide association studies (GWAS) serve as an alternative approach to genetic mapping of SNB resistance (Adhikari et al. 2011; Gurung et al. 2014; Jighly et al. 2016; Liu et al. 2015; Ruud et al. 2019). GWAS has two main advantages. First, the association mapping (AM) panel saves the cost and time to construct mapping populations (Bernardo 2016; Gupta et al. 2014). Second, the genetic diversity and map resolution are higher in an AM panel compared to

biparental populations, since multiple historical recombination and multiple alleles per locus are available in diverse germplasm collections (Bernardo 2016; Gupta et al. 2014). However, genetic subpopulation structure in AM panels may result in false positive associations (Breseghello and Sorrells 2006; Gupta et al. 2014). In addition, GWAS analysis has its limitation for detecting QTL associated with rare alleles or rare variants; therefore, it can only be used for detecting QTL controlled by alleles with relatively high allele frequency in the panel (Bernardo 2016; Breseghello and Sorrells 2006; Gupta et al. 2014). Recently, multiparent advanced generation intercross (MAGIC) populations have also been used for SNB resistance studies (Cockram et al. 2015; Downie et al. 2018; Lin et al. 2020a; Lin et al. 2021). The design of MAGIC populations involves intercrossing of multiple parental lines (2^n), followed by several rounds of selfing to achieve a RIL population, which allows for increased allelic diversity and genetic recombination relative to comparatively sized biparental populations, while reducing the risk of false positive associations caused by the population structure commonly present in AM panels (Cavanagh et al. 2008; Mackay et al. 2014).

4.1 Genetics of SNB resistance and the inverse gene-for-gene model

Resistance to SNB is truly quantitative in the sense that no cultivar has been identified with complete resistance (Aguilar et al. 2005). In addition, due to the complexity of the pathogen population, seedling resistance to single isolates does not guarantee adult plant resistance in the field. Moreover, the fact that leaf blotch and glume blotch might be controlled by different genetic mechanisms (Aguilar et al. 2005; Wicki et al. 1999) adds further to the difficulties of SNB resistance breeding.

Most known SNB resistance mechanisms are due to lack of sensitivity genes (*Snn*) in wheat genotypes to corresponding *P. nodorum* necrotrophic effectors (inverse gene-for-gene model) (reviewed by Ruud and Lillemo 2018). The gene-for-gene model is well known for interactions between biotrophic pathogens, such as those causing powdery mildew and rust diseases, and their host plants. As biotrophic pathogens require living host tissues, when the host plant contains the resistance (R) gene able to recognize the product of a pathogen's avirulence (*Avr*) gene, a hypersensitive reaction (HR) will be induced resulting in programmed cell death (PCD), thus limiting the infection of the biotrophic pathogens (Fig. 3a). On the contrary, necrotrophic pathogens, such as *P. nodorum*, utilize nutrients from dead or dying host tissues and interact with host plants via an inverse gene-for gene model (Friesen et al. 2007). Via the production of NEs, which interact with host susceptibility genes (*Snn*), necrotrophic pathogens can trigger PCD to accelerate their infection (Fig. 3b).

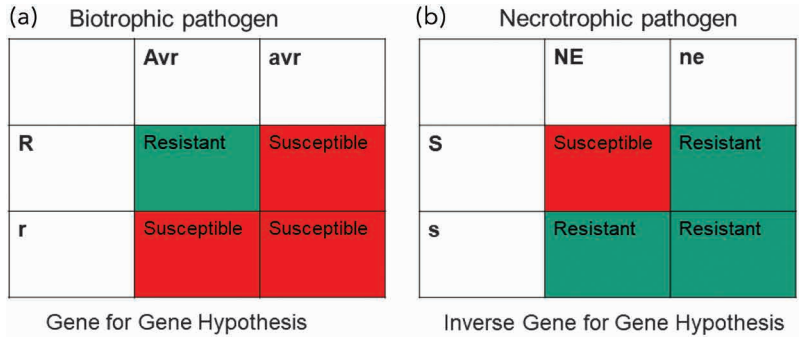

Figure 3 Different plant–pathogen interaction mechanisms. (a) Gene-for-gene model, adapted from Flor (1971). (b) Inverse gene-for-gene model, adapted from Friesen et al. (2007). R: resistant gene, r: absence of resistant gene; Avr: avirulence gene, avr: absence of avirulence gene; NE: necrotrophic effector, ne: absence of necrotrophic effector; S: susceptibility gene, s: absence of susceptibility gene.

Up to now, nine NE-*Snn* interactions have been described; however, only three *P. nodorum* NE coding genes (*SnToxA*, *SnTox1* and *SnTox3*) and two host NE sensitivity genes (*Tsn1* and *Snn1*) have been cloned (reviewed by Peters Haugrud et al. (2019); Ruud and Lillemo (2018)).

ToxA was characterized as a 13-kDa polypeptide host selective toxin (now referred to as NE) produced by the wheat tan spot pathogen, *Pyrenophora tritici-repentis*, and which interacts with the sensitivity gene *Tsn1* on the long arm of wheat chromosome 5B (Faris et al. 1996; Tomas et al. 1990). Later, Liu et al. (2006) reported that *P. nodorum* also contains a ToxA coding gene, the product of which targets the same wheat *Tsn1* locus as tan spot. Through gene diversity analysis, it was shown that the ToxA coding gene *SnToxA* in *P. tritici-repentis* likely originated from *P. nodorum* through a recent horizontal gene transfer event (Friesen et al. 2006). The cloned ToxA sensitivity gene, *Tsn1*, has a typical R gene structure containing nucleotide binding site (NBS) and a leucine-rich repeat (LRR) domain, as well as a serine/threonine protein kinase (S/TPK) domain (Faris et al. 2010). However, the Tsn1 protein does not directly interact with ToxA, suggesting that *Tsn1* may mediate the signaling pathway of effector-triggered immunity (ETI) but is not the ToxA receptor (Faris et al. 2010). Recently, it was shown that another wheat and barley pathogen, *Bipolaris sorokiniana*, the cause of spot blotch, also possesses a *ToxA* gene that likely originated from *P. nodorum*, pointing to a selective advantage of carrying the virulence factor ToxA (Friesen et al. 2018; McDonald et al. 2018).

Tox1-Snn1 was the first reported NE-Snn interaction in the wheat–*P. nodorum* pathosystem, where Tox1 was characterized as a NE produced in *P. nodorum* culture filtrates interacting with the wheat sensitivity locus *Snn1* on chromosome 1B (Liu et al. 2004a). However, the cloning of *SnTox1* was not

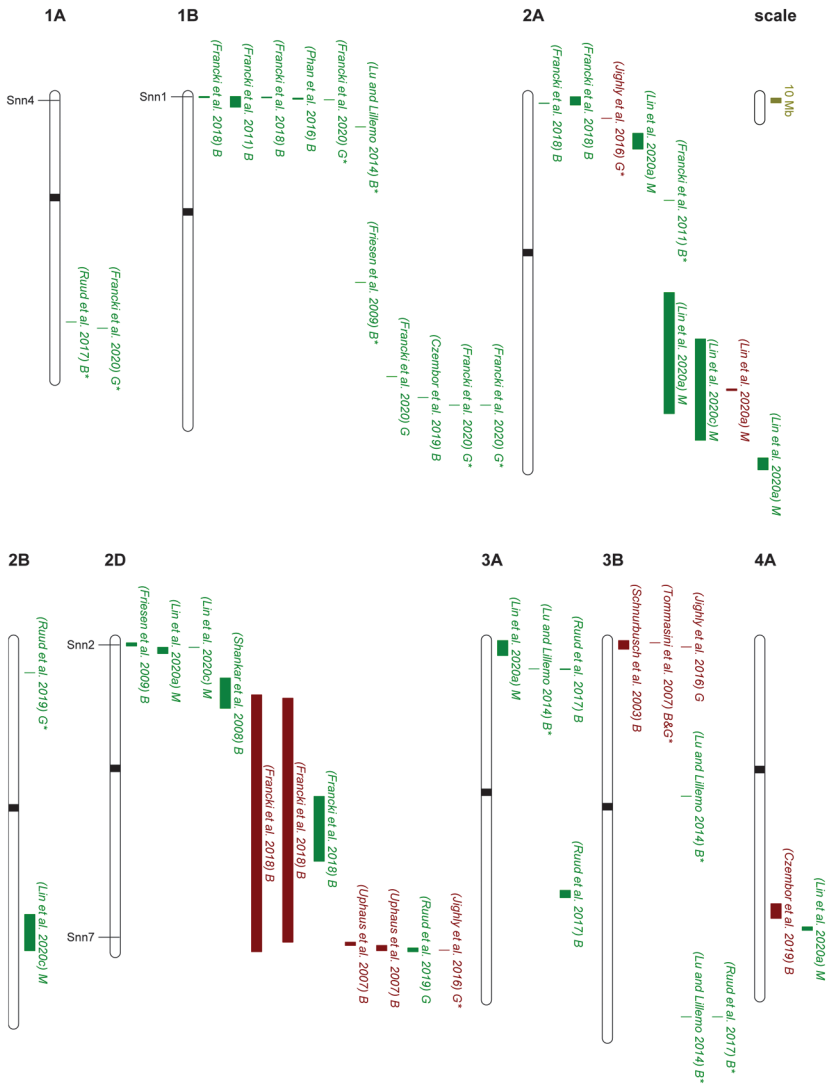

Figure 4 Projection of published field resistance QTL for SNB leaf blotch (green), glume blotch (dark red) onto the wheat reference genome assembly (RefSeq v1.0; IWGSC 2018). B: biparental population, G: GWAS panel, M: MAGIC population. If sequence of only single marker was available for the QTL, QTL were marked with a superscript asterisk.

achieved until eight years after the discovery of the Tox1-Snn1 interaction (Liu et al. 2012). *SnTox1* encodes a cysteine-rich protein with 117 amino acids which is light dependent and critical for fungal penetration (Liu et al. 2012). Further research on Tox1 showed that it serves as a dual function protein, which can bind the host chitinases to protect fungal infection as well as behaving like

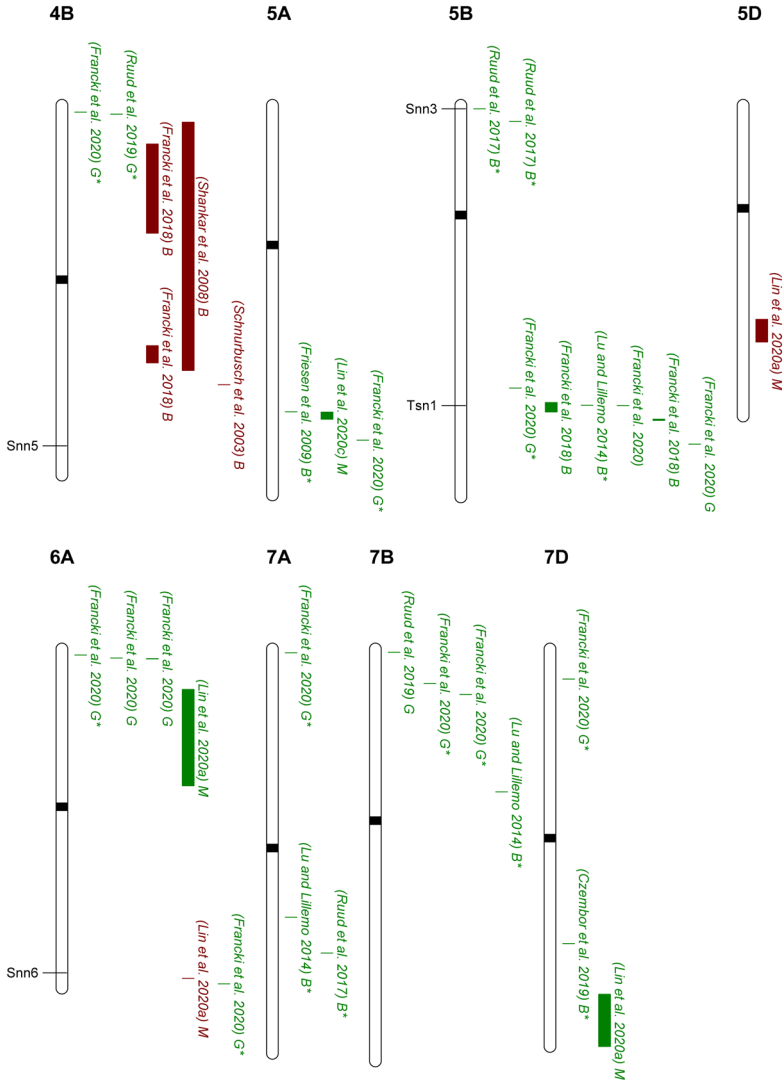

Figure 4 (*Continued*).

a virulent NE (Liu et al. 2016). In the same year, Shi et al. (2016) cloned the Tox1 wheat sensitivity gene *Snn1*, which encodes a wall-associated kinase (WAK). WAK proteins are pattern recognition receptors (PRRs) which can interact directly with pathogen-associated molecular patterns (PAMPs), such as oligogalacturonides (OGs), which trigger PCD and are involved in plant defense mechanisms against biotrophic pathogens (Brutus et al. 2010). In

contrast to ToxA-Tsn1, which interacts in the ETI pathway, the cloning of *Snn1* revealed that *P. nodorum* could also hijack the PAMP-triggered immunity (PTI) pathway against biotrophic pathogens and lead to disease (Shi et al. 2016).

Tox3 was characterized after the discovery of ToxA, Tox1 and Tox2 by Friesen et al. (2008) as a novel *P. nodorum* NE, interacting with the wheat sensitivity locus designated as *Snn3* on the short arm of chromosome 5B. The Tox3 protein is around 26 kDa in size and the coding gene, which was cloned by Liu et al. (2009), showed little homology to the previously cloned *P. nodorum* NE gene *SnToxA*. In addition, sensitivity to Tox3 was also shown in the diploid wheat progenitor species *Aegilops tauschii* and the sensitivity locus was mapped to the short arm of chromosome 5D and is probably homoeologous to the *Snn3* locus on chromosome 5B (Zhang et al. 2011). Accordingly, the *Snn3* loci present in bread wheat and *Ae. tauschii* are now denoted as *Snn3-B1* and *Snn3-D1*, respectively. Though the remaining six NE–*Snn* interactions have been characterized and the sensitivity loci were mapped to wheat chromosomes (Fig. 4) (Abeysekara et al. 2012; Abeysekara et al. 2009; Friesen et al. 2012; Friesen et al. 2007; Gao et al. 2015; Shi et al. 2015), further studies are required to fine-map and elucidate the molecular mechanisms of these sensitivity loci.

4.2 Relationship between NE–Snn interactions and field SNB severity

All the described NE–*Snn* interactions were firstly characterized by seedling inoculations and infiltrations in greenhouse conditions, but there is evidence that some NE-*Snn* interactions also contribute to field SNB susceptibilities (Ruud and Lillemo 2018). Friesen et al. (2009) studied adult plant resistance in the field by spray inoculating a mapping population segregating for *Tsn1*, *Snn2* and *Snn3-B1* with an isolate producing both ToxA and Tox2. They found the *Tsn1* and *Snn2* loci to explain 18% and 15% of the phenotypic variation, respectively. In another study, a mapping population segregating for *Snn1* and *Snn3* was inoculated in the field with an isolate producing all three known NEs, showing that the *Snn1* locus could explain 19% of the phenotypic variation for adult plant disease severity (Phan et al. 2016). Furthermore, a field study conducted with natural *P. nodorum* inoculum showed that *Snn3-B1* was the major determinant of SNB susceptibility in the 'SHA3/CBRD × Naxos' population, explaining up to 24% of the phenotypic variation (Ruud et al. 2017). It is still unclear whether there are more NE-*Snn* interactions influencing the adult plant susceptibilities. A recent study by Lin et al. (2020a) reported that one robust adult plant susceptibility QTL co-located with a seedling sensitivity QTL to an unknown NE, which will be discussed in detail in the case study.

4.3 Overview of reported QTL for adult plant leaf blotch resistance

Since Aguilar et al. (2005) published the QTL study for both adult plant leaf blotch and glume blotch resistance, more than 60 QTL have been characterized for adult plant leaf blotch resistance on 15 different wheat chromosomes (1A, 1B, 2A, 2B, 2D, 3A, 3B, 4A, 4B, 5A, 5B, 6A, 7A, 7B and 7D) (Fig. 4). As shown in Fig. 4, some of the adult plant leaf blotch resistance/susceptibility QTL colocate with the wheat NE sensitivity loci, such as *Tsn1*, *Snn1*, *Snn2* and *Snn3* (Francki et al. 2018; Friesen et al. 2009; Lin et al. 2020a; Lu and Lillemo 2014; Phan et al. 2016; Ruud et al. 2017). By basic local alignment search tool (BLAST) analysis of the marker sequences of published QTL against the reference genome (International Wheat Genome Sequencing et al. 2018), more adult plant leaf blotch resistance QTL, which were detected using different mapping populations and different genetic markers, were projected into the similar physical map region and could potentially be the same QTL (Fig. 4). For example, overlapping QTL interval was found for the QTL reported by Friesen et al. (2009) and Lin et al. (2021) on the long arm of chromosome 5A (Fig. 4). However, most of the adult plant leaf blotch resistance QTL have not been fine-mapped and the molecular mechanisms behind these QTL are still unclear.

4.4 Relationship between seedling and adult plant resistance

Previous studies showed that SNB seedling resistance and adult plant resistance were not highly correlated (Francki 2013; Ruud and Lillemo 2018; Shankar et al. 2008b; Uphaus et al. 2007), which could partly be due to the fact that many of these studies used different isolates in the greenhouse experiments and the field testing (Ruud and Lillemo 2018; Ruud et al. 2017). It has also been shown that the correlations between seedling and adult plant leaf blotch resistance can be relatively high when the same isolate is used for both seedling tests in the greenhouse and adult plant tests in the field (Jönsson 1985). However, in general, the correlations between SNB resistance at these two growth stages are low. Since the natural *P. nodorum* populations are genetically diverse, it is difficult to identify representative isolates for greenhouse assays. In addition, even though Shankar et al. (2008b) used the same isolate mixture for inoculation in the greenhouse and in the field in the year 2003, they found only a weak correlation between seedling and flag leaf disease scores ($r = 0.31$), and a non-significant relationship between seedling and glume blotch severity ($r = 0.09$). However, results of field testing might still be influenced by the natural *P. nodorum* population even though specific isolate or isolates mixture are used as inoculum.

4.5 The genetics of glume blotch resistance

The first QTL mapping study for SNB glume blotch resistance was done by Schnurbusch et al. (2003) using a Swiss biparental winter wheat mapping population 'Arina × Forno', where two significant QTL were detected on chromosomes 3B and 4B, respectively. Up to date, 17 SNB glume blotch QTL have been characterized on chromosomes 2A, 2B, 2D, 3B, 4A, 4B and 5D (Fig. 4) (Czembor et al. 2019; Francki et al. 2018; Jighly et al. 2016; Lin et al. 2020a; Schnurbusch et al. 2003; Shankar et al. 2008a; Tommasini et al. 2007; Uphaus et al. 2007). Although leaf blotch and glume blotch are symptoms caused by the same pathogen on the same host, previous studies showed that the genetic mechanisms controlling resistance to SNB leaf blotch and glume blotch are different (Aguilar et al. 2005; Fried and Meister 1987; Wicki et al. 1999). Aguilar et al. (2005) carried out the first study to investigate leaf blotch and glume blotch resistance by assessing the disease on the same mapping population in the same field. They identified one common QTL for both leaf blotch and glume blotch on chromosome 2B; however, that QTL was associated to confounding morphological traits such as heading date and ear length (Aguilar et al. 2005). Therefore, they concluded that the resistance of leaf blotch and glume blotch were controlled by genetically independent mechanisms. A recent study showed that one glume blotch resistance QTL on chromosome 2A colocated with a leaf blotch resistance QTL, which was detected in the same study using a MAGIC population tested in the field (Lin et al. 2020a). However, the haplotype analysis revealed that the haplotype effects for the leaf and glume blotch QTL were different, which was congruent with previous studies that different mechanisms controlled the resistance to leaf and glume blotch (Lin et al. 2020a). In addition, to our knowledge, no glume blotch resistance QTL has been characterized at the sequence level nor being applied in resistance breeding by MAS.

5 Case study: Septoria nodorum blotch (SNB) resistance in wheat in Norway

5.1 Status of SNB knowledge in Norwegian wheat

SNB is the dominating leaf blotch disease on wheat in Norway, and there is a need to improve resistance in current cultivars. As reviewed by Ruud and Lillemo (2018), the first genetic studies of SNB resistance in Norwegian germplasm started in 2010. Based on the 'SHA3/CBRD × Naxos' population, SNB-resistant QTL were detected on wheat chromosomes 1B, 3A, 3B, 5B, 7A and 7B (Lu and Lillemo 2014). Ruud et al. (2017) used the same population for both greenhouse and field testing, and confirmed that the QTL on the short arm of chromosome

5B detected in the previous study (Lu and Lillemo 2014) was the Tox3 sensitivity locus *Snn3-B1*, which showed a major effect on wheat susceptibility at both seedling and adult plant stages. In addition, through NE infiltrations, Ruud et al. (2018) found that large proportions of lines in the Norwegian spring wheat collection were sensitive to ToxA and Tox3. Interestingly, *SnToxA* and *SnTox3* frequencies in their Norwegian *P. nodorum* isolate collections were also quite high, with *SnToxA* and *SnTox3* frequencies of 69% and 76%, respectively (Ruud et al. 2018). A recent study by Ruud et al. (2019) identified many adult plant resistant QTL by GWAS using a collection of 121 Nordic spring wheat cultivars and breeding lines. Among those, one QTL on chromosome 2D was robust in most of the tested years and significant correlations were found between field disease severity and sensitivity to ToxA (Ruud et al. 2019). However, although ToxA sensitivity is common in Norwegian spring wheat cultivars and showed positive correlation with SNB severity in the field (Ruud et al. 2018), the *Tsn1* locus was not significantly detected by this association study (Ruud et al. 2019).

With this as a background, we set out to do a more in-depth study of the pathogen population in Norwegian wheat fields and conduct genetic mapping of more germplasm using two winter wheat MAGIC populations and an expanded GWAS panel of both spring and winter wheat lines.

5.2 Background and main objectives of recent SNB studies in Norway

Cultivar resistance is usually considered as an effective and environmentally friendly disease management method. However, the durability of cultivar resistance is always being challenged due to the long time frame required for resistance breeding and the fast evolution of virulent pathogens. The pathogen population can adapt to new sources of host resistance quickly once a new cultivar is released to the market. Therefore, knowledge of the pathogen population is vital to optimize resistance breeding strategies and to help exploit the limited resistance resources in an effective way. In addition, apart from improving the usage of cultivar resistance, knowledge of the local pathogen population would also be beneficial to improve other disease management approaches such as chemical application and agronomic control methods. For example, a pathogen population that undergoes regular sexual reproduction and has a high mutation rate, high gene flow and a large population size is considered to have high evolutionary potential (McDonald and Linde 2002). Typically, the risk of breaking qualitative cultivar resistance is high for such populations. Additionally, fungicide resistance alleles may also spread quickly when pathogen populations are under high selection pressure. Moreover,

as such diverse populations evolve rapidly, they will also adapt quickly to changing environments. Therefore, disease management should be adjusted accordingly. For instance, stacking different qualitative resistance genes or using quantitative resistance to breed resistant cultivar can make it more difficult for a highly adaptive pathogen population to overcome the host resistance.

In order to gain knowledge of the local Norwegian *P. nodorum* pathogen population, we collected and genotyped single spore isolates from spring and winter wheat fields across three seasons in the main wheat-growing regions in Norway. Different methods were used to analyze potential subdivision of the pathogen population due to sampling location, time or the cultivars the isolates were collected from. Since coinfection of *P. nodorum* and *Z. tritici* was more common on winter wheat but less on spring wheat (Justesen et al., submitted), we hypothesized to find differences between pathogen populations on different wheat types due to different competition pressure. Moreover, we analyzed the allele frequencies of *SnTox* genes and investigated whether local adaptation was evident due to these virulence factors.

Cultivar resistance is still insufficient and currently no cultivar shows complete resistance to SNB. One of the main objectives of our genetic studies has been to identify loci associated with SNB resistance in breeder-relevant germplasm in order to improve resistance breeding. Up to now, nine NE–*Snn* interactions have been characterized, with only a few of them showing important role in SNB susceptibility in the field as reviewed in section 4.2. However, since the *P. nodorum* populations are characterized by high genetic diversity, we hypothesized that additional NE-*Snn* interactions might be present, but not discovered yet. Three genetic studies of SNB resistance will be described here: (1) using one winter wheat MAGIC population (NIAB Elite) from the UK (Lin et al. 2020a), (2) a winter wheat MAGIC population (BMWpop) from Germany (Lin et al. 2021) and (3) a GWAS of field resistance using two Norwegian association mapping panels: one winter wheat panel and one spring wheat panel (Lin et al. in prep.).

The main objectives were to:

- investigate the genetic structure of the Norwegian *P. nodorum* pathogen population;
- evaluate NE–*Snn* interaction-related QTL at both seedling and adult plant stages using a MAGIC population of UK origin;
- assess the genetics of SNB disease severity of another MAGIC population of German origin and compare the QTL detected from the two MAGIC populations; and
- discover robust SNB resistance QTL in the field using two association mapping panels.

5.3 Main results and discussions of case study

5.3.1 Case study 1: Investigating the genetic structure of Norwegian *P. nodorum* populations

In the pathogen population study (Lin et al. 2020b), wheat leaf samples were collected from three major Norwegian wheat-growing areas including five counties, from 2015 to 2017. A total of 165 single spore isolates of *P. nodorum* were isolated from the leaf samples and recorded with information on sampling location, year and source cultivar. We genotyped the isolate collection together with nine foreign isolates using 20 SSR markers, three known *SnTox* genes (*SnToxA*, *SnTox1* and *SnTox3*) and two mating-type idiomorphs (Lin et al. 2020b). We used different methods to analyze whether the pathogen population could be subdivided by location, time or the cultivars the isolates were collected from. McDonald and Linde (2002) hypothesized that pathogen populations with combined sexual and asexual reproduction, high gene flow, high mutation rate and large population size have relatively high evolutionary potential. The *P. nodorum* population in Norway was found to perfectly fit this model. Both mating types (MAT1-1 and MAT1-2) were present in all tested locations and the ratio between the two mating types did not significantly ($p < 0.05$) deviate from 1:1 in any location, although the nationwide mating type ratio showed a slight deviation from 1:1 ($p < 0.05$). Overall, the Norwegian *P. nodorum* population exhibited the signature of sexual reproduction. Results from principal component analysis (PCA), STRUCTURE and 'snapclust' analyses revealed that there was no genetic population structure in the collected *P. nodorum* isolates. The analysis of molecular variance (AMOVA) confirmed that the genetic variations were larger within location, year, cultivar or wheat type rather than between these classifications, and no population subdivisions could be observed by location, year, cultivar or wheat types. Interestingly, we did not even find any substantial genetic differentiation between the Norwegian *P. nodorum* population and the nine foreign isolates included in the PCA analysis. This finding was supported by the observation that genetic distances estimated between individual Norwegian isolates were as large as, or sometimes even larger than, those between Norwegian isolates and foreign isolates. Consistent with previous *P. nodorum* population genetic studies (Keller et al. 1997; McDonald et al. 2012; Murphy et al. 2000; Stukenbrock et al. 2006), high genetic variability was found in the Norwegian *P. nodorum* population. Even though we found two isolates with the same SSR multilocus genotype, no clonal isolates were found in a single sampling location. This finding is also an indicator of large effective population size. As the mutation rate for a pathogen is usually fixed and generally low (McDonald and Linde 2002), large population size also means that a large number of mutants exist in the population.

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Pathogens with high evolutionary potential usually have specific characteristics, such as a high potential of adaptation, high risk of breaking down qualitative host resistance, substantial advantage in competition with other pathogens and a high risk of developing fungicide resistance (McDonald and Linde 2002). The management control for *P. nodorum* should take all these characteristics into account. The results of the pathogen population study (Lin et al. 2020b) showed that Norwegian *P. nodorum* isolates have significantly higher frequency of the virulence gene, *SnToxA* (67.9 %), compared to a previously reported European population study (12%) (McDonald et al. 2013). We hypothesized that this large difference in *SnToxA* allele frequency was due to the local adaptation to the high frequency of the ToxA sensitivity allele *Tsn1* in Norwegian spring wheat cultivars; in Norway more spring wheat is grown than winter wheat, and ToxA sensitivity is common in Norwegian spring wheat cultivars (Ruud et al. 2018). Our 165 *P. nodorum* isolates were collected from 13 cultivars which covered 95% of the wheat market share from 2015 to 2017. Among these cultivars, only four are sensitive to ToxA and three of those are spring wheat cultivars. The only ToxA-sensitive winter wheat cultivar was the relatively old cultivar 'Magnifik' which had only 6–7% of the market share, while the widely cultivated recent spring wheat cultivars 'Mirakel', 'Krabat' and 'Demonstrant' are all sensitive to ToxA (Ruud et al. 2018), implying a high frequency of ToxA-sensitive alleles at *Tsn1* in current Norwegian spring wheat cultivars. The main results from the GWAS (Lin et al. in prep.) also supported this, showing that *Tsn1* was significantly detected in the field using the full set of the Norwegian spring wheat association panel. As mentioned in Section 4.1, ToxA is not only a virulence factor for *P. nodorum*, but also for the tan spot (*D. tritici-repentis*) and spot blotch (*B. sorokiniana*) pathogens. Removing the *Tsn1* susceptibility allele from current wheat cultivars may, therefore, be beneficial for multiple diseases.

However, eliminating a single susceptibility gene will not manage the disease completely. As discussed earlier, qualitative resistance is easy to break by a pathogen with high evolutionary potential. *P. nodorum* isolates likely carry more than one NE gene, and could regulate the expression level of effector genes based on the host sensitivity (Peters Haugrud et al. 2019). For example, *Tsn1* was not significantly associated with SNB in the winter wheat panel (Lin et al. in prep.), as most winter wheat lines did not carry this susceptibility gene. However, two other NE sensitivity loci, *Snn1* and *Snn3*, which were not significant in the Norwegian spring wheat association panel, were significantly associated with SNB resistance/susceptibility in the winter wheat panel. One could also hypothesize that high genetic diversity and frequent sexual recombination leads to more complicated effector profiles in the natural *P. nodorum* population. Therefore, stacking more resistance QTL is needed to decrease the SNB disease severity (Lin et al. in prep.).

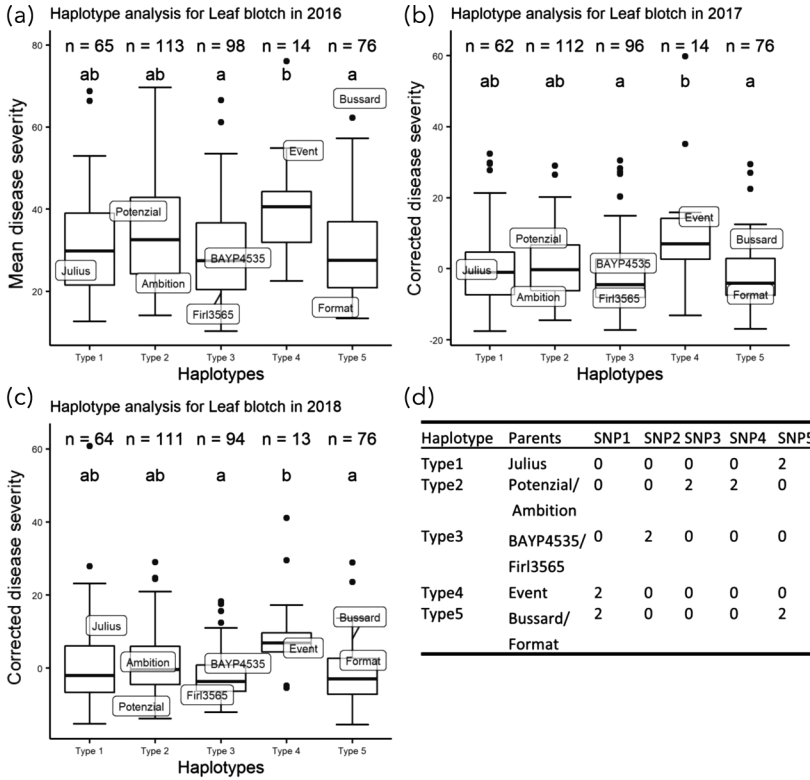
As mentioned in Section 2.2, *Z. tritici* is the dominant pathogen in the leaf blotch disease complex in many other European countries, while *P. nodorum* is still the major leaf blotch pathogen in Norway (Ficke et al. 2018b; Justesen et al., submitted). One possible explanation could be that, since more spring wheat is grown in Norway, the limited growing season of spring wheat is too short for the longer latent period in *Z. tritici* development, which limits the expansion of its population size. In the meantime, *P. nodorum* could successfully maintain large natural populations on spring wheat due to its shorter latent period (Cunfer 1999) and rapid adaptation, which makes it more competitive in comparison to *Z. tritici* on the same host.

Resistances to different groups of fungicides have been reported in *P. nodorum* populations by different studies, as described in Section 3. As Norwegian *P. nodorum* has a large effective population size and has been treated with fungicides for decades, we would expect a large amount of fungicide-resistant mutations to exist in the population. When being consistently exposed to the same fungicide, the mutant allele may spread quickly in the population due to rapid sexual recombination and massive production of asexual pycnidiospores. Therefore, the large population size, frequent sexual reproduction and high genetic variability in Norwegian *P. nodorum* indicated a potentially high risk of fungicide resistance.

Thus, integrated pest management (IPM) is recommended to control SNB. Firstly, wheat debris should be removed before the next growing season in order to reduce the primary inoculum source, and consequently the pathogen population size. Alternatively, a two-year crop rotation appears to effectively reduce the risk of leaf blotch epidemics even under conducive environmental conditions (Pedersen and Hughes 1992). Secondly, cultivars insensitive to known effectors and possessing other resistance mechanisms should be preferred. Thirdly, use of healthy or fungicide-treated seeds should be recommended to decrease the spread of disease. Lastly, combining or rotating fungicides with different modes of action may effectively decrease the selection pressure of fungicide-resistant mutants in the pathogen population.

5.3.2 Case study 2: QTL mapping using NIAB MAGIC population

The NIAB Elite MAGIC population consists of more than 1000 RILs and was genotyped using an Illumina iSelect 90K SNP array (Mackay et al. 2014; Wang et al. 2014a). A subset of around 500 RILs were tested in Norway for four years using infected straw as inoculum and in the UK for two years of SNB resistance testing, inoculated by spore suspension. We also conducted seedling infiltration and inoculation assays using three *P. nodorum* isolates in order to compare QTL identified in the field with those identified under controlled conditions at the seedling stage (Lin et al. 2020a). The population segregates for sensitivity to



(d)

Haplotype	Parents	SNP1	SNP2	SNP3	SNP4	SNP5
Type1	Julius	0	0	0	0	2
Type2	Potenzial/ Ambition	0	0	2	2	0
Type3	BAYP4535/ Firl3565	0	2	0	0	0
Type4	Event	2	0	0	0	0
Type5	Bussard/ Format	2	0	0	0	2

Figure 5 Haplotype analysis for BMWpop leaf blotch QTL *QSnb.nmbu-2A.1/2018*. (a) Haplotype analysis of mean disease severity in field season 2016. (b–c) Haplotype analysis of corrected disease severity in field season 2017 and 2018, respectively, and the mean disease ratings for the eight founders are indicated. Haplotypes labeled with same letter represented no significant differences between haplotype disease severities as detected by Kruskalmc test ($p < 0.05$). (d) Genotype of each haplotype based on five SNP markers. SNP marker names are listed in order as below: *wsnp_CAP8_c2677_1394934*, *AX-95661975*, *RAC875_c38018_278*, *AX-94508462* and *BS00090569_51* (Lin et al. 2021).

all three well-characterized NEs (SnToxA, SnTox1 and SnTox3), and showed a broad genetic variation for both seedling and adult plant resistance. However, the correlation in resistance between seedling and adult plant resistance was low.

As described above, disease severities of both adult plant leaf blotch and glume blotch were scored in field trials in Norway, while only leaf blotch was scored in the UK. By extracting residuals of disease scores from a linear regression using plant height and days to heading as covariates, we tried to reduce the influences caused by these confounding traits in order to detect true SNB resistance QTL (Lin et al. 2020a). When assessing the disease

severities of the MAGIC founders, rankings of disease severities were different for leaf blotch and glume blotch. Notably, the most susceptible founder Xi19 for leaf blotch showed moderate resistance to glume blotch, suggesting that the resistance mechanisms to leaf blotch and glume blotch might be different. Eight QTL on chromosomes 2A, 2D, 3A, 4A, 6A and 7D were identified for leaf blotch, while three QTL on chromosomes 2A, 5D and 6A were identified for glume blotch, and most of the QTL were different. Interestingly, we identified an overlapping QTL, *QSnb.niab-2A.3*, on chromosome 2A for both leaf blotch and glume blotch. Therefore, we considered that they represented a common QTL associated with both leaf blotch and glume blotch. Results of haplotype analysis showed that haplotype effects were significant for leaf blotch in many years and one year for glume blotch. However, the susceptible haplotype for leaf blotch showed the opposite effect for glume blotch, implying that leaf blotch and glume blotch resistance might indeed be controlled by different mechanisms and that the QTL identified might represent two closely located but independent genes or gene clusters, which is in agreement with the results from other studies (Aguilar et al. 2005; Francki 2013; Shankar et al. 2008b). Nevertheless, when comparing published QTL with QTL identified in our study, we found colocation of the glume blotch QTL with an NE sensitivity locus. *QSnb.niab-6A.2* detected for glume blotch in 2016 might colocate with the Tox6 sensitivity locus *Snn6* (Gao et al. 2015) indicating that NE–*Snn* interactions might potentially also play a role in glume blotch susceptibility. NE–*Snn* interactions were deeply investigated for leaf blotch at the seedling stage with only a few cases where seedling resistance QTL showed an effect on adult plant leaf resistance. However, whether NE–*Snn* interactions play a role in glume blotch resistance is still unknown. More research on glume blotch is needed to clarify the genetic mechanism of glume blotch resistance and whether NE–*Snn* interactions are involved in glume susceptibility.

5.3.3 Case study 3: QTL mapping using BMWpop MAGIC population

We validated the most robust QTL, *QSnb.niab-2A.3*, identified using the NIAB MAGIC population on an independent multi-founder population with different genetic background, the BMWpop MAGIC (Lin et al. 2021). The BMWpop consists of 394 $F_{6:8}$ RILs and was genotyped using the 20K Infinium iSelect array (Stadlmeier et al. 2018). Field testing of SNB resistance using BMWpop was conducted in Norway from 2016 to 2018 using the same methodology as for the NIAB MAGIC population (Lin et al. 2020a).

The QTL (*QSnb.nmbu-2A.1*) was significantly detected in two years out of a three-year study, and the haplotype effect of the QTL (Fig. 5, (Lin et al. 2021)) was significantly associated with field SNB susceptibility across all years tested,

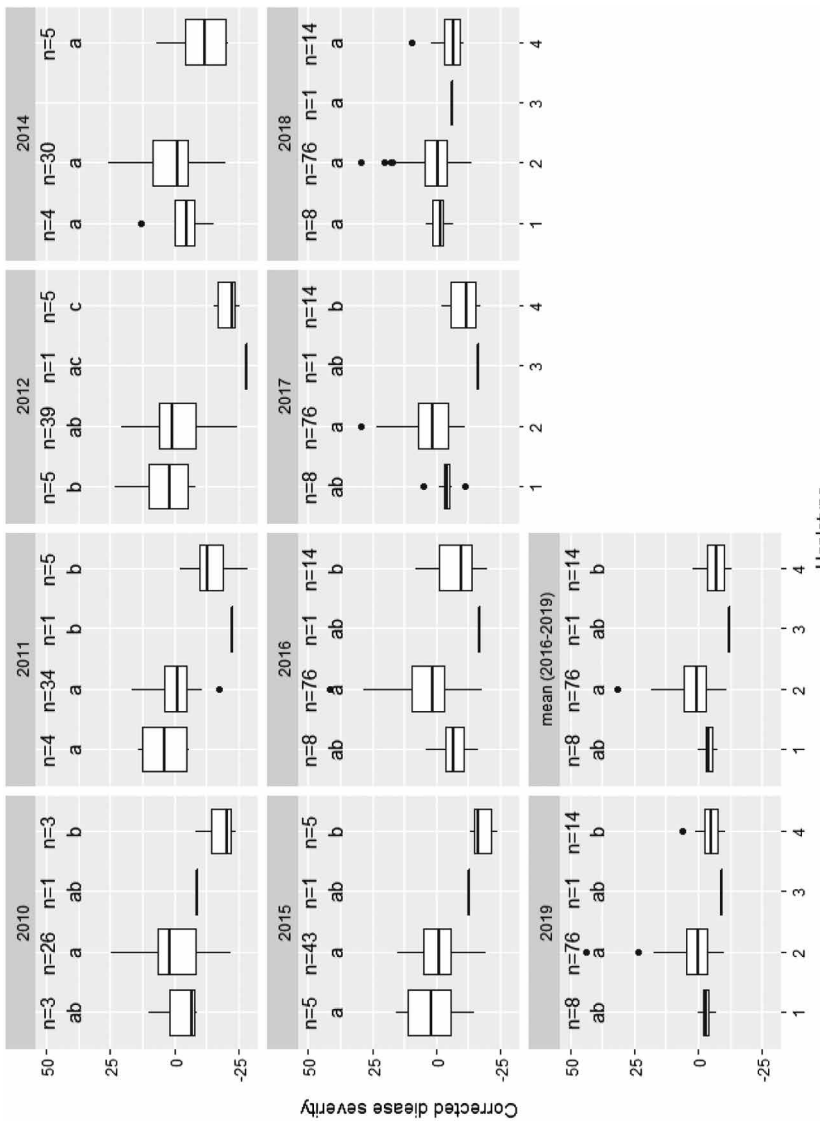


Figure 6 Haplotype analysis of the 2AS QTL in the winter wheat panel of nine years' field trial and mean of four years from 2016 to 2019 (Bottom right). Same letter on boxplots indicate no significant difference between haplotypes determined by Tukey's HSD test ($p < 0.05$).

indicating the robustness of the QTL and the potential value of applying it in MAS. In addition, another robust adult plant resistance QTL, *QSnb.nmbu-5A.1*, which was only found in this BMWpop MAGIC population, showed additive effect to *QSnb.nmbu-2A.1*. Therefore, it was concluded that using MAS to combine resistance alleles at these two loci could be a promising strategy for improving SNB resistance in wheat breeding. Moreover, the multi-locus haplotypes determined in the study provided markers for efficient tracking of these beneficial alleles in future wheat genetics and breeding activities (Lin et al. 2021).

5.3.4 Case study 4: QTL detected using GWAS

The GWAS was conducted using a winter wheat panel consisting of 103 lines and a spring wheat panel of 296 lines. Both panels consisted of cultivars, breeding lines and frequently used crossing parents representing the relevant genetic diversity for Norwegian wheat breeding, and were genotyped using the 35K Axiom array (Allen et al. 2017). The winter wheat panel was tested for SNB resistance in field trials from 2016 to 2019, while the spring wheat panel was tested from 2016 to 2018 (Lin et al. in prep.). The most consistent QTL across years were detected on chromosomes 2A and 5A using the winter wheat association panel, while on chromosomes 1A, 2B and 5B using the spring wheat panel. Interestingly, the significant QTL on 5B detected by the spring wheat panel is likely the ToxA sensitivity gene *Tsn1*, which was not significantly detected when the subset of this panel was used for analysis (Ruud et al. 2019). In addition, one robust QTL on the short arm of chromosome 2A detected by the winter wheat panel was of interest for further haplotype analysis, as it explained a relatively large proportion of the phenotypic variation (R^2 = 14%). By combining historical data, the haplotype effect was shown to be significant in seven out of nine tested years (Fig. 6). The resistant allele on chromosome 2A was carried by many lines with German origin while most Norwegian and Swedish lines carried the susceptible allele. Moreover, we found that the resistant haplotype was rare in the Norwegian spring wheat panel and that all lines with the resistant haplotype originated from CIMMYT. It was, therefore, concluded that integrating this resistant allele into local germplasm may improve the SNB resistance in Norwegian wheat (Lin et al., in prep.).

5.3.5 Can sensitivity to NEs explain differences in host SNB resistance in the field?

Since the discovery of the first NE–*Snn* interaction in the wheat *P. nodorum* pathogen system by Liu et al. (2004b), many studies have focused on these interactions and more NEs have been characterized (described in Section 4.1).

However, debates as to how much of the host resistance/susceptibility can be explained by NE–*Snn* interactions are still ongoing (Francki 2013; Ruud and Lillemo 2018). We also found interesting results from our own studies. In the GWAS (Lin et al., in prep.), we identified SNB field QTL to colocate with numerous NE sensitivity loci, such as *Tsn1*, *Snn1*, *Snn2* and *Snn3-B1*, even though some of them were not consistently detected across multiple years. However, our results highlighted the potential usefulness of screening NE sensitivities in breeding lines to reduce field SNB susceptibility. For the QTL mapping using the NIAB Elite MAGIC population, except for QTL *QSnb.niab-2A.3* and *QSnb.niab-3A*, which were detected by both crude CF infiltration and in the field, no other QTL were detected as significant in both greenhouse (i.e. culture filtrate, NE infiltration and seedling *P. nodorum* resistance) and field conditions (Lin et al. 2020a). Furthermore, the disease severities of seedling testing and field testing were not highly correlated. One possible explanation could be that the isolates selected in our greenhouse testing were not representative for the Norwegian *P. nodorum* population. Probably some uncharacterized NEs played an important role in the field but were not expressed or possessed by the selected isolates in our greenhouse study. Since naturally infected straw was used as inoculum for field experiments in Norway, multiple infections by different isolates were expected in the field. From the pathogen population study (Lin et al. 2020b) we knew that all of the three well-characterized NE genes (*SnToxA*, *SnTox1* and *SnTox3*) were common in the Norwegian *P. nodorum* population. NIAB Elite MAGIC segregates for all related sensitivity loci *Tsn1*, *Snn1* and *Snn3-B1*, but these QTL were not detected in the field in any of the tested years neither in Norway nor in the UK, while only the *Snn2* locus was identified in one year from the UK trial as a 'weak QTL' (Lin et al. 2020a). Similar results were shown for the BMWpop, which segregated for both *Snn1* and *Snn3-B1*; however, neither of these QTL were identified in the field using the BMWpop (Lin et al. 2021). Therefore, collectively our observations supported the hypothesis proposed by Peters Haugrud et al. (2019) that *P. nodorum* isolates may not simultaneously express all of the NE genes they harbor. Instead, depending on the host genetic background, the pathogen exploits a 'cost-effective' way to choose which NE to express when the host possesses many sensitivity loci (Peters Haugrud et al. 2019). Epistatic effects caused by host *Snn* genes may result in this phenomenon as well; however, more gene expression analyses on host *Snn* genes are required to disentangle this issue. As resistance/susceptibility to SNB is a quantitative trait, plant defense mechanisms other than NE–*Snn* interactions are also likely to be involved in the *P. nodorum*–wheat interaction in the field. For example, some resistant wheat cultivars minimize fungal penetration by producing lignified papillae (Bird and Ride 1981). Recently, Zhang et al. (2019) reported the genetic introgression of novel resistance genes to both tan spot and SNB from the diploid wheat species *Aegilops speltoides* to bread wheat

by chromosome engineering, which provided new resistance resources and opportunities to investigate SNB resistance mechanisms other than the NE–*Snn* interactions. Other general resistance mechanisms are still unexplored.

5.3.6 Field inoculation methods

From an SNB resistance breeding point of view, our results illustrated the importance of field testing using natural pathogen populations as inoculum instead of arbitrarily selecting isolates for resistance screening. Spraying spore suspensions for SNB field inoculation is a standard method used by many studies (Fried and Meister 1987; Laubscher et al. 1966; Uphaus et al. 2007; Wicki et al. 1999), and it has its own specific advantages. For instance, the same isolates could be used in both greenhouse and field studies. Therefore, higher correlations between field and controlled environments would be expected compared to using natural inoculum. In addition, as inoculum is applied directly to either wheat leaves or heads, this method could reduce the influence of confounding traits such as plant height and days to heading, which was observed in the UK trial of the NIAB Elite MAGIC (Lin et al. 2020a). However, as discussed in Section 5.3.1, the high genetic diversity of the *P. nodorum* natural population made it difficult to select representative isolates. Such diversity is expected when the natural population has been long established and commonly undergoes sexual reproduction, and the resulting large effective population size increased the difficulty of choosing representative isolates for screening. Moreover, large proportions of field resistance could not be explained by known NE–*Snn* interactions alone. Therefore, NE screening under controlled greenhouse conditions and field testing with natural *P. nodorum* populations should be combined in order to breed for better resistance.

5.3.7 Influences of plant height and days to heading on SNB disease severity in the field

Under natural conditions, SNB develops from the lower leaves to the upper leaves. Thus, tall and late lines may avoid heavy infection due to morphological and phenological avoidance. Therefore, as previously discussed, confounding traits such as plant height and days to heading would interfere with the detection of the true SNB QTL. Correcting the effects caused by such traits were achieved in this study by extracting the residuals of disease scores from a linear regression using plant height and days to heading as covariates. Additionally, we compared QTL detected by the corrected disease data and uncorrected disease data for the NIAB Elite MAGIC population to further investigate the influences caused by these traits (Lin et al. 2020a). In general, all 'strong QTL'

identified by uncorrected leaf blotch data were detected by corrected data, indicating that the methodology used was robust. In addition, less QTL were identified by the uncorrected dataset and common significant QTL detected by both datasets became less significant using uncorrected data, highlighting the influence of the confounding traits on the reliable detection of true SNB QTL. In addition, QTL analysis of uncorrected glume blotch data showed that only two 'strong QTL' were detected and both were common with plant height QTL on chromosomes 4B and 4D. 'Weak QTL' detected by uncorrected glume blotch data were all detected using corrected glume blotch data. And similar to the analysis of leaf blotch, those true SNB QTL detected using uncorrected glume blotch phenotypes were less significant than the ones detected using the corrected glume blotch phenotypes (Lin et al. 2020a).

5.4 Case study conclusions

- The Norwegian *P. nodorum* population has high evolutionary potential, high genetic diversity and no detectable population subdivision.
- Due to its ability of rapid local adaptation and risk of evolving fungicide resistance, the principles of integrated disease management should be used in order to control SNB in Norway. The overall disease pressure should be reduced by the joint efforts of good agronomic practice (stubble management, crop rotation), choice of resistant cultivars and application of fungicides when needed.
- ToxA is the major virulence factor in the Norwegian *P. nodorum* population, probably due to the local adaptation to Norwegian spring wheat cultivars. Eliminating the ToxA sensitivity allele *Tsn1* in Norwegian spring wheat cultivars may reduce future SNB infection.
- Three robust QTL were detected: two by QTL mapping in MAGIC (on the long arm of chromosome 2A and on 5A) and one by GWAS (on the short arm of 2A). All effects were confirmed by haplotype analysis and the corresponding markers can be used in MAS.
- Correlations between SNB seedling resistance and adult plant resistance are generally low and not all seedling-stage NE–*Snn* interactions detected in the greenhouse are of field relevance.
- In order to improve SNB resistance, field testing should be carried out using natural *P. nodorum* populations as inoculum and cannot be completely replaced by greenhouse assays.
- Resistance to SNB leaf blotch and glume blotch are controlled by different mechanisms.
- SNB resistance is quantitative and most of the SNB resistance-associated QTL each explain a low proportion of the phenotypic variations (<10%) under field conditions. However, stacking of resistant alleles in MAGIC

RILs and the GWAS panels showed significant effect on reducing disease severity.

6 Future trends

6.1 Applying CRISPR/Cas9 technology to improve SNB resistance

Classic plant breeding is mainly based on crossing and selection, which requires genetic recombination and allelic variability. However, sometimes allelic diversity is limited in domesticated crops or it is only some specific traits, such as disease resistance, that is the intended breeding target. In order to introduce beneficial traits from wild relatives to domesticated crops, it often takes 10–15 years to break the linkage drag with undesired traits (Steuernagel et al. 2016). While mutation breeding is a fast way to create genetic variation, mutations caused by either physical or chemical mutagens are usually unpredictable. Genome editing can precisely edit the plant genome and create predictable mutations in elite cultivars, thereby saving the time for backcrossing and overcoming linkage drag. Simultaneously, it also accelerates the selection process by decreasing the effect of random mutations (Li et al. 2012). Genome editing based on the recent CRISPR (clustered, regularly interspaced, short palindromic repeats)/Cas9 system (Zhang et al. 2014) has already been applied to multiple crops. CRISPR/Cas9 can also target multiple homoeologues simultaneously in polyploid crops, such as hexaploid bread wheat and tetraploid potato (Andersson et al. 2017; Feng et al. 2016; Liang et al. 2017; Wang et al. 2014b; Zong et al. 2017). In addition, CRISPR/Cas9 has been successfully used for editing susceptibility genes to key pathogens, with the knock-out mutants all showing enhanced resistance (Berg et al. 2017; Peng et al. 2017; Wang et al. 2014b). As discussed in Section 4.1 (this chapter), two NE sensitivity loci, *Tsn1* and *Snn1*, have been characterized at the gene level, and no yield penalty associated with NE insensitivity has been found so far (Oliver et al. 2014). Eliminating such NE sensitivity alleles by CRISPR/Cas9 from elite cultivars would reduce susceptibility to SNB, and at the same time keep all other desired traits. However, applying this technology requires sequence knowledge of the candidate gene, and is in many countries also subject to the same regulations as genetically modified organisms (Turnbull et al. 2021). At the moment, the other seven known *Snn* loci remain uncloned, therefore eliminating susceptibility at these loci currently still relies on MAS or NE screening assays.

6.2 Marker-assisted selection (MAS)

MAS has been widely used in wheat breeding for selecting major agronomic and quality traits, as well as resistances to different diseases (Bernardo 2008;

Dreisigacker et al. 2016; Miedaner and Korzun 2012; Toth et al. 2019). In comparison to traditional phenotypic selection, which usually requires selection in many generations, selections based on molecular markers can fix the allele of the desired trait in the early generations with high accuracy in the absence of phenotypic data (Dreisigacker et al. 2016; Toth et al. 2019). It is practical especially for breeding disease resistance traits, as MAS can be applied for stacking multiple resistance genes even when some of the diseases have not been present in the field environment (Toth et al. 2019). The selection accuracy of MAS also largely depends on the markers being used. Functional markers which locate within the target genes are diagnostic; however, such markers are not always available. Alternatively, markers linked to the target gene/QTL that to a large degree co-segregate with the desired allele can also be used in MAS. But the selection accuracy depends on the genetic distance between the marker and the gene/QTL, and sometimes may result in false positives in a different population. Therefore, linked markers should be validated in populations with different genetic background before being applied in MAS. As described in the previous sections, MAS can be used to eliminate the NE sensitivity loci in the breeding germplasm. For example, Zhang et al. (2009) developed SSR markers linked to *Tsn1* and *Snn2* which could be used for MAS. In recent years, mapping populations for SNB resistance are genotyped by SNP chips (Cockram et al. 2015; Francki et al. 2020; Lin et al. 2020a; Lin et al. 2021). Significant SNP markers can be simply converted into KASP genotyping system for selection (Cockram et al. 2015; Downie et al. 2018). Moreover, as *Tsn1* and *Snn1* genes have been cloned, diagnostic markers can be easily developed based on the gene sequence. Nevertheless, the resistance/susceptibility to SNB is a quantitative trait. There will be limitation to how much of the genetic variation can be captured in MAS even if diagnostic markers would become available for all the important sensitivity loci.

6.3 Genomic selection (GS)

Genomic prediction/selection uses large amounts of genetic markers covering all chromosomes and prior phenotypic and genotypic data of a training population to estimate the breeding values or predict the phenotypic performance of genotypes with unknown phenotypes (Meuwissen et al. 2001). Unlike conventional MAS which selects breeding germplasm using a relatively small number of genetic markers linked to previously detected major QTL/genes, GS includes genetic information of the whole genome which might give a more accurate prediction of an individual's breeding value (Bernardo and Yu 2007). As reviewed by Bernardo (2016), with the increase in marker density, size of the training population and heritability of the trait, the accuracy of genomic prediction will also increase. In addition, compared to treating all markers with

equal random effects as is normally used in GS, fixing marker effects of major genes can also improve the prediction accuracy (Bernardo 2014). Genomic selection may well represent an efficient solution toward SNB resistance breeding as the SNB resistance is a complicated quantitative trait and only few diagnostic markers are available for MAS. Indeed, in our GWAS study only a small part (≤ 17%) of the genetic variation for the trait was explained by the QTL detected (Lin et al. in prep.). Besides, many markers were detected, which would make MAS unfeasible. Genomic selection using genome-wide markers will likely capture much more of the genetic variances. Juliana et al. (2017) conducted the first genomic prediction study on wheat leaf blotch resistances including SNB. However, the study was based on seedling SNB resistance which might not highly correlate with field resistance, as discussed previously in this chapter. Odilbekov et al. (2019) conducted a genomic prediction study on another wheat leaf blotch disease – STB – using a Nordic winter wheat panel, and found that by setting significant markers from GWAS as fixed effects, the prediction accuracy improved from 0.47 to 0.62. With the prior knowledge of the significant markers obtained from our GWAS study, genomic prediction based on field SNB resistance will probably be more relevant for breeders to use in practice.

7 Conclusion

Wheat resistance to *P. nodorum* is quantitatively inherited and is usually a result of complicated interactions. Interactions between NEs produced by *P. nodorum* and wheat sensitivity loci have been well characterized since Liu et al. (2004a). In addition to that, additive and epistatic effects were found between different NE–*Snn* interactions (Peters Haugrud et al. 2019; Phan et al. 2016; Shi et al. 2016). Moreover, due to the coevolutionary nature of the host and pathogens, *P. nodorum* populations may also shift virulence factors, for example, which NE to produce, by adapting to the NE sensitivities of the host cultivars (Lin et al. 2020b; Phan et al. 2019; Richards et al. 2019). Though leaf blotch and glume blotch are just different symptoms caused by the same pathogen on the same host, resistance to leaf blotch and glume are likely controlled by different genetic mechanisms (Aguilar et al. 2005; Francki 2013; Lin et al. 2020a; Shankar et al. 2008b), which makes it more difficult to breed for SNB resistance. It has been proved that some of the NE sensitivity loci play important roles in field susceptibility (Friesen et al. 2009; Phan et al. 2016; Ruud et al. 2017), for which either MAS or NE infiltration can be applied on germplasm screening to eliminate the sensitivity alleles. Despite the fact that many NE sensitivity loci have been characterized, functional markers are in most cases not available. Thanks to the rapid development of wheat genome studies, such as the Chinese spring reference genome (International Wheat Genome

Sequencing et al. 2018) and the recently published pan-genome of 15 diverse cultivars representing the global wheat diversity (Walkowiak et al. 2020), it is expected that the new bioinformatic tools will assist the cloning of more and more wheat resistance/susceptibility genes in the upcoming years. Thus, gene editing technology might also be applied to knock out the susceptibility genes in elite cultivars to reduce their SNB severity. Nevertheless, most of the NE–*Snn* interactions were based on seedling testing with selected isolates, which were quite different from the field conditions where plants were usually exposed to the highly diverse natural pathogen population. Therefore, MAS based on *Snn* genes might still not be able to significantly reduce the field susceptibility. Genomic selection might provide higher selection accuracy than MAS for SNB adult plant resistance, as it is suitable for predicting quantitative traits and taking genotype-by-environment interactions into account.

8 Where to look for further information

Below are some selected papers which are recommended for further reading. Friesen and Faris (2012) provide detailed descriptions of the methodologies for investigating the NE-*Snn* interactions. The short review by Oliver et al. (2016) studied the most common necrotrophic wheat diseases in Australia and wheat resistance mechanisms against those diseases. Francki (2013) reviewed the SNB resistance from a breeding point of view and proposed strategies for improving the SNB resistance in breeding programs. The study by Ruud and Lillemo (2018) generally reviewed SNB and provided details of published SNB-related QTL. Insights into breeding strategies based on the current knowledge of resistance mechanisms are also provided by Downie et al. (2020). A recent published research article by Peters Haugrud et al. (2019) investigated the interactions of the wheat–*P. nodorum* pathosystem, which illustrated the complicated nature of SNB resistance.

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