Chapter 6

Diseases affecting wheat: Septoria nodorum blotch

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Abstract

Septoria nodorum blotch (SNB) is an important wheat disease in many wheat production areas around the world, and is caused by the ascomycete fungal pathogen *Parastagonospora nodorum*. Breeding for SNB resistance has been hampered by the polygenic and quantitative nature of the resistance, and the farmers often have to rely on fungicides to control the disease. In recent years, substantial progress has been made in understanding the *P.nodorum*-wheat pathosystem. Several host-specific interactions between necrotrophic effectors (NEs) and host sensitivity (*Snn*) genes have been identified and play major roles in SNB seedling resistance. Some of the NEs have been cloned and can be used to screen breeding material at the seedling stage. This book chapter provides an overview of the molecular understanding of host-pathogen interactions, progress in understanding the genetics of host resistance and use of molecular markers and effector screening as promising tools in resistance breeding for this challenging wheat disease.

Contents

1. Introduction

1.1. Wheat

The global production of wheat was approximately 729 million tons in 2014 and around 70% is used for human consumption (FAO, 2017), making it one of the largest food crops in the world. Due to its adaptability, it is grown in a wide range of climates. Bread wheat (*Triticum aestivum*, L.) accounts for roughly 95% of the wheat production, durum (*T. durum*, L.) for the remaining 5%.

Plant diseases constitute a major threat to productivity, and the Green Revolution breeders were early advocates of incorporating general resistance against diseases (Borlaug, 1966; Caldwell, 1968), as a more durable strategy than race specific resistance. Broad spectrum and durable resistance has been deployed for the biotrophic diseases stripe, leaf and stem rusts and powdery mildew (Krattinger et al., 2009; Lillemo et al., 2008; Moore et al., 2015; Singh et al., 2011).

1.2. The Septoria leaf blotch disease complex

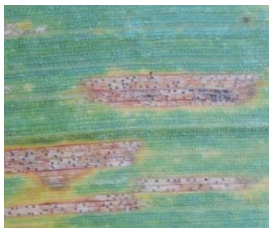
The "*Septoria* leaf blotch disease complex" includes Septoria nodorum leaf and glume blotch (SNB) caused by *Parastagonospora nodorum*, Septoria tritici leaf blotch (STB) caused by *Zymoseptoria tritici*, tan spot caused by *Pyrenophora tritici-repentis* and, of less importance, Septoria avenae blotch caused by *Parastagonospora avenae*. Due to symptom similarity in the field, and that the different pathogens often occur together, even on the same leaf (Blixt et al., 2010) they are often referred to as "Septoria leaf blotch" or simply "leaf blotch". One should, however, be aware that different pathogens are involved, and that resistance to one pathogen of the leaf blotch disease complex does not necessarily imply anything about resistance to any of the other pathogens. Microscopic evaluation of spores from leaf samples, or DNA-based identification, is necessary to reliably identify the causal agents.

1.3. Nomenclature

The classification and nomenclature of the leaf blotch pathogens have changed many times – an overview of distinctive features and synonyms is provided in Table 1.

Table 1 Nomenclature, causal agents, synonyms and main features of leaf blotch in wheat

Disease	**Pathogen**	**Main distinctive features (symptoms, spores)**
Septoria nodorum (leaf and glume) blotch	***Parastagonospora nodorum***	**Lesions:** Lens-shaped lesions, with or without pycnidia. Pycnidia also on stem, leaf sheats, glumes and nodes

Stagonospora nodorum blotch SNB	*Phaeosphaeria* (Hedjar.) syn. *Leptosphaeria nodorum* (Müll.), syn. *Septoria nodorum* (Berk.), syn. *Stagonospora nodorum* (Berk.)	**Conidiospores:** cylindrical, 0-3 septa, 15-32 x 2-4 µm  (Photo: A. Ruud)
Septoria tritici blotch STB	***Zymoseptoria tritici*** syn. *Mycosphaerella graminicola*, syn *Septoria tritici*	**Lesions:** Rectangular lesions with or without pycnidia **Conidiospores:** (two forms) – macropycnidiospores 35-98 x 1-3 µm, 3-5 septa micropycnidiospores: 8-10.5 x 0.8-1 µm, without septa  (Photo: M. Lillemo)
Tan spot DTR, yellow (leaf) spot	***Pyrenophora tritici-repentis*** (Died) Shoem, syn. *Drechslera tritici-repentis* (Died.) Shoem. ***Helminthosporium tritici-vulgaris, H. tritici-repentis***	**Lesions:** Oval to diamond shaped lesions, with tan necrosis and extensive chlorosis. "Eyespot" – chlorotic lesion with small dark brown spot. Reddish discoloration of kernels (red smudge) **Conidiospores:** cylindrical, 12-21 x 45-200 µm with 4-7 septa  (Photo: NDSU photo, https://www.ag.ndsu.edu/publications/crops/fungal-leaf-spot-diseases-of-wheat-tan-spot-septoria-stagonospora-nodorum-blotch-and-septoria-tritici-blotch , need to ask for permission to use, or replace)

1.4. Impacts of leaf blotch diseases

The Septoria leaf blotch diseases have only been recognized as major diseases since the introduction of high yielding, semi-dwarf wheat cultivars (King et al., 1983; Scharen, 1999). The relative importance of the individual pathogen species in the complex varies between geographic regions and has also changed over time.

In Europe, particularly in Mediterranean climate and the "Maritime Zone" including Northern France, Germany and the UK, *Z. tritici* is the most important wheat leaf blotch pathogen (Eyal, 1999; Fones and Gurr, 2015), while *P. nodorum* is associated to the Northern regions (Eyal, 1999). Approximately 70% of the share of fungicides used on wheat in Europe ($1.2bn, ~€1bn) is primarily targeted towards the management of *Z. tritici* (Torriani et al., 2015).

From the 1970s-80s, the prevalence and importance shifted from *P. nodorum* to *Z. tritici* in the UK and Germany (Jones, 1985; Polley and Thomas, 1991; Royle et al., 1986; Shaw, 1999). Possible reasons for the shift are increased nitrogen fertilization, susceptible cultivars, early sowing, climate changes (higher summer rainfalls), altered atmospheric SO_2 concentration, and differential response and resistance development towards fungicides (Bayles, 1991; Bearchell et al., 2005; Cools et al., 2011; Lovell et al., 1997; Meien-Vogeler et al., 1994; Pereira et al., 2017; Polley and Thomas, 1991; Shaw, 1999)

Tan spot caused by *P. tritici-repentis* first emerged as a disease on wheat in 1934 to 1941 in Canada, USA and India (Barrus, 1942; Hosford, 1982; Johnson, 1942; Mitra, 1934; Oliver et al., 2008). The emergence of *P. tritici-repentis* as a wheat pathogen has been attributed to the horizontal gene transfer of the necrotrophic effector-gene *ToxA* from *P. nodorum* (Friesen et al., 2006). Tan spot has since become an important leaf blotch disease in major wheat growing countries, especially in the US northern Great Plains and Australia, and can be an important component of the leaf blotch disease complex in Europe, Canada and USA (Bhathal et al., 2003; Carmona et al., 2006; Hosford, 1982; Lamari et al., 2005; Murray and Brennan, 2009).

P. nodorum, the causal agent of SNB, occurs in all regions where wheat is grown, and can cause significant losses (Eyal, 1981; King et al., 1983). Although STB has become more important in some European countries, the incidence has increased in other regions (DePauw, 1995), and SNB is still a major disease in many areas (Solomon et al., 2006). In Western Australia SNB can be responsible for yield losses up to 31 % (Bhathal et al., 2003). In Norwegian trials, the estimated yield loss due to SNB in the susceptible cultivar Bjarne was calculated to be on average almost 25% based on data from 2009 to 2012 and a mean SNB

severity of 20% (Abrahamsen, 2013). Quality measures like thousand kernel weight, hectoliter weight and grain filling were also well correlated with fungicide treatment.

2. *Parastagonospora nodorum* – the causal agent of Septoria nodorum blotch

Parastagonospora nodorum is a filamentous Ascomycete and member of the Dothideomycetes class, which includes several phytopathogens (Murray and Brennan 2009; Crook et al. 2012; Quaedvlieg et al. 2013; Stergiopoulos et al. 2013).

2.1. Life cycle and epidemiology

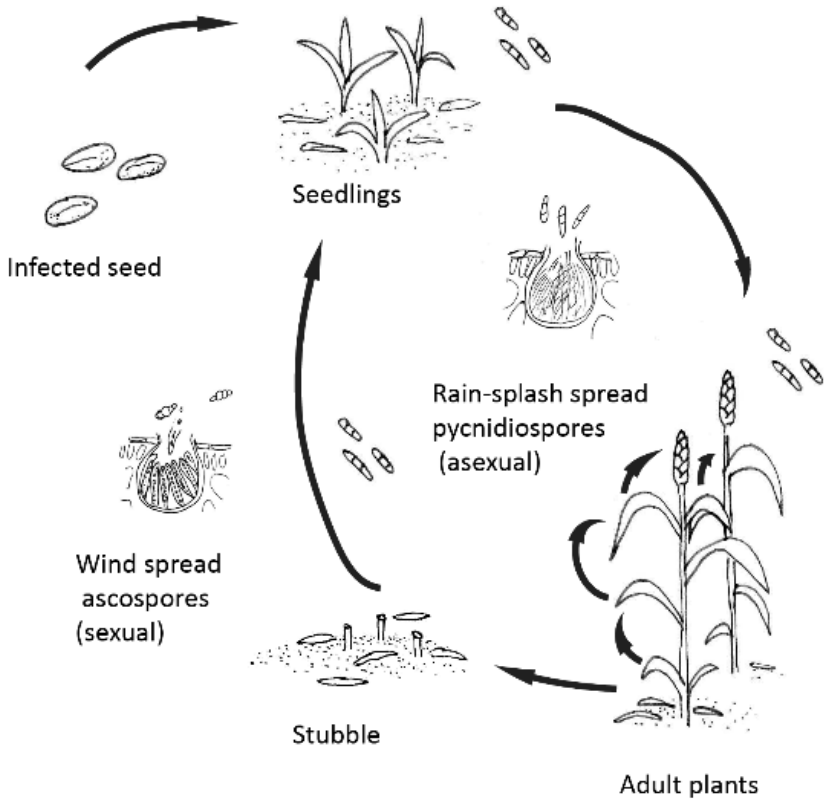


Figure 1. The life cycle of *P. nodorum.* Adapted from Sommerhalder et al. (2011).

P. nodorum has a mixed reproduction system. The fungus is heterothallic with two mating types. Both mating types have to be present for sexual recombination to occur (Halama and Lacoste, 1991). The sexual fruiting bodies, pseudothecia (Figure 1), contain numerous asci which release ascospores. These ascospores are wind borne over short and long distances

(Bathgate and Loughman, 2001). The sexual stage is known from most areas where SNB is significant, including Norway (Bathgate and Loughman, 2001; Blixt et al., 2008; Cowger and Silva-Rojas, 2006; Ficke et al., 2011b). Asexual fruiting bodies, pycnidia, produce pycnidiospores which are splash dispersed within the canopy during rain events (Figure 1) (Eyal et al., 1987; Solomon et al., 2006; Sommerhalder et al., 2011).

The mixed reproduction system provides both increased diversity due to the potential for variation through genetic recombination and fast clonal reproduction of favorable genotypes. Selection in different environments has likely given rise to high levels of variation in aggressiveness and as far as it has been investigated, no single *P. nodorum* genotype dominates in any environment (Ali and Adhikari, 2008; Blixt et al., 2008; Engle et al., 2006; Francki, 2013; Stukenbrock et al., 2006).

The pathogen survives on infected seeds and wheat stubble, which serve as primary inoculum sources (Figure 1). Wind-borne ascospores are released from stubble and sexual reproduction and formation of pseudothecia occur the whole growth season (Blixt et al., 2008; Sommerhalder et al., 2010). However, ascospore release also seems to be seasonal and often assumed to be most important during fall and spring, coinciding with the emergence of wheat seedlings (Bathgate and Loughman, 2001; Bennett et al., 2007; Mittelstädt and Fehrmann, 1987). Rain-splash dispersed asexual pycnidiospores produced on the infected plants serve as primary and secondary inoculum (Eyal et al., 1987). In order for efficient splash dispersal to occur, an intense rain shower of at least 5 mm rainfall and temperature >10 ° C, followed by at least 10 mm rainfall within the next 48 hours is necessary (Eyal et al., 1987) although dew and mist is sufficient to promote spore release (Bathgate and Loughman, 2001).

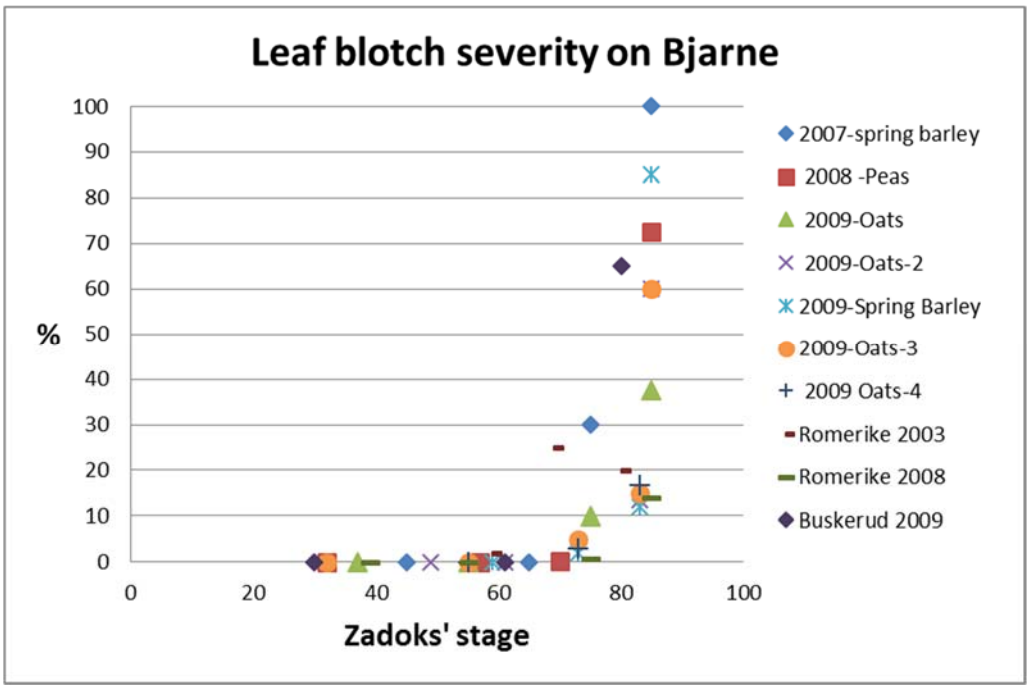


Figure 2. Percentage of SNB on the spring wheat cultivar Bjarne after various pre-crops. The disease develops exponentially from growth stage 70-75. Adapted from Ficke et al. (2011b).

The disease progresses exponentially after Zadoks (Zadoks et al., 1974) growth stage 70-75 when the plant approaches maturity (Figure 2), but with different slope depending on factors like pre-crop, inoculum pressure, cultivar resistance and environmental conditions.

2.2. Symptoms

The *P. nodorum* germ tubes penetrate the leaf either directly through the cuticle or through open or closed stomata. Chlorosis at the infection site expands into oval lesions, often accompanied by necrosis. Pycnidia (Figure 3, middle, top) can form in the infected tissue within a week under optimal conditions (Solomon et al., 2006).

Figure 3. Left: Septoria nodorum leaf blotch symptoms in the field. Middle, top: Pycnidia developing in the necrotic lesion. Middle, bottom: Necrotic lesions and chlorosis on a flag leaf. Right: Septoria nodorum glume blotch. Photos: A. K. Ruud (left, middle), M. Lillemo (right).

In the field, symptoms of SNB first develop on the lower leaves and progress to the upper leaves through rain splash dispersal. Under sufficiently long growth season and favorable weather conditions the pathogen will eventually reach the glumes and cause glume blotch (Figure 3, right) (Eyal et al., 1987; McMullen and Adhikari, 2009; Solomon et al., 2006).

3. Disease management

SNB can be controlled through appropriate agricultural practices like crop rotation and tillage, fungicides and by using resistant cultivars. In later years, the recommended agricultural practice of reduced tillage to prevent soil erosion leads to increased disease pressure. The plant residues serve as primary inoculum in the subsequent growth season (Lillemo and Dieseth, 2011; McMullen and Adhikari, 2009).

3.1. Agricultural practice

Cultural practices have always been used to control disease pressure and optimize growth conditions for the crop. Rotation with crops that are non-hosts to *P. nodorum* is advised, since

the pathogen survives on plant residues from the previous wheat crop. Rotations with resistant cultivars may also reduce the disease pressure since less inoculum is carried over from these (Krupinsky, 1999). Crop rotation is most effective to control diseases disseminated over short distances, like *P. nodorum* (Cunfer, 1998). However, political and economic incentives affect whether crop rotation is feasible for the farmer. In Norway, for example, farmers often grow wheat after wheat due to the larger interest in and price for this crop than for the alternatives (Lillemo and Dieseth, 2011).

P. nodorum is further promoted if direct seeding or minimum tillage practices are applied (Krupinsky, 1999; Sutton and Vyn, 1990). This has become common practice in modern agriculture in many parts of the world, in order to save costs, reduce soil erosion and limit water evaporation.

3.2. Fungicide control

Leaf blotch diseases are mainly controlled by application of fungicides at the heading stage. The main fungicide groups are strobilurins and triazoles.

Strobilurins inhibit fungal respiration by binding to cytochrome *b* complex III at the Q_0 site in the mitochondrial electron transport chain (Bartlett et al., 2002). Loss of strobilurin sensitivity is associated with a mutation in the pathogen's mitochondrial genome leading to an amino acid (aa) change in cytochrome *b* (Gisi et al., 2002). In 2002, this mutation was found in *Z. tritici* isolates in several European countries (Leroux et al., 2007) and the same aa substitution was carried by the majority of Swedish *P. nodorum* isolates collected between 2003 and 2005 (Blixt et al., 2009).

Triazoles inhibit the 14-α-demethylation of lanosterol in the ergosterol biosynthesis pathway (Siegel, 1981; Vanden Bossche et al., 1995) and target CYP51, a cytochrome 450 enzyme responsible for the 14-α-demethylation. Reduced sensitivity to azoles can be caused by three mechanisms: Point mutation in the target gene *CYP51*, overexpression of CYP51 and up-regulation of efflux proteins leading to reduced accumulation of the fungicide inside the cells (Leroux et al., 2007).

Two non-synonymous amino acid substitutions in CYP51 associated with reduced azole sensitivity were recently reported in European and Chinese *P. nodorum* isolates (Pereira et al., 2017), including 25 % of Swedish isolates. A higher number of mutations has been found in *Z. tritici* CYP51 indicating that reduced sensitivity developed earlier in *Z. tritici* than *P. nodorum*,

and the effectiveness of all classes of this fungicide group is threatened (Cools et al., 2011), while still mostly effective against *P. nodorum* (Pereira et al., 2017).

Perhaps the shift in abundance from *Z. tritici* to *P. nodorum* in many European countries in recent years may be due to the greater speed that the latter pathogen adapted to triazole fungicides (McDonald, B, personal communication)? In Norway, *P. nodorum* remains the major leaf blotch pathogen in spring wheat. But also here, *P. nodorum* isolates have been reported with resistance to strobilurins and with reduced sensitivity to triazoles (Abrahamsen, 2013; Ficke et al., 2011a). The potential loss of effective fungicides underlines the need to control the disease by other and more sustainable methods.

3.3. Use of resistant cultivars

Growing cultivars with sufficient genetic resistance towards diseases is the most sustainable and economic strategy to prevent losses. Experiments conducted to measure the losses caused by leaf blotch disease in Norwegian wheat, showed that the gain from fungicide treatment was lower in the most resistant cultivars like Zebra and, in particular, Mirakel (data for 2012). For these cultivars the yield was high also in the untreated plots, illustrating the potential of cultivars with genetic resistance to reduce the need for fungicide spraying (Abrahamsen, 2013). A quantitative difference in genetic resistance to the disease, allows a quantitative reduction in need for fungicide application.

4. The genetics of resistance

Breeding for resistance to SNB is challenged by the lack of major and dominant resistance genes. The inheritance of resistance is complex (Mullaney et al., 1982; Scharen and Krupinsky, 1978) and strong genotype × environment (G×E) interactions can mask the relatively small contributions of the individual genes. Plant height and maturity are also associated with the development of the disease (Rosielle and Brown, 1980; Scott et al., 1982). However, significant residual resistance is also observed that is not associated with the confounding traits (Scott et al., 1982). This residual, "true" genetic resistance, and the discovery that host specific gene-for-gene interactions are important determinants of susceptibility in the *P.nodorum*-wheat pathosystem (Liu et al., 2004a) provide the potential for genetic gain in SNB resistance breeding.

4.1. The gene-for-gene models

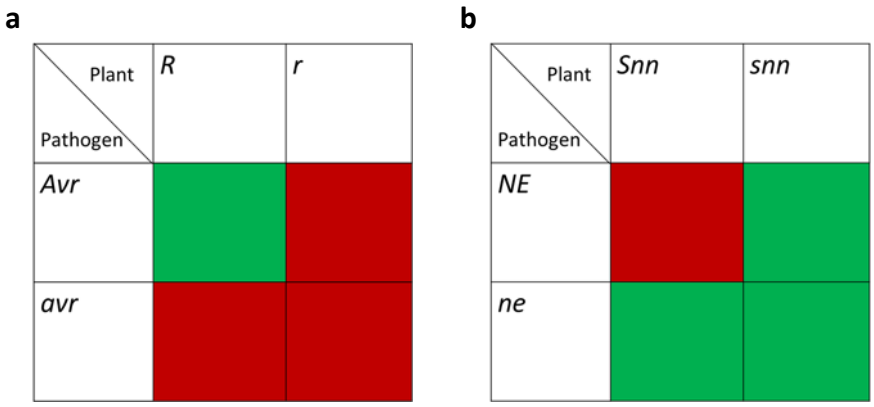


Figure 4 a. The classical gene-for-gene model adapted from Flor (1971). In a biotrophic system, resistance is conferred when the product of a resistance (*R*) gene in the host recognizes an avirulence (*Avr*) gene product secreted by the pathogen. **b.** The "inverse" model adapted from Friesen et al. (2007). In a necrotrophic system, HR is induced upon recognition of a necrotrophic effector (NE) by the product of a sensitivity (*Snn*) gene, and leads to increased susceptibility.

Flor's studies of the biotrophic pathosystem flax rust (*Melampsora lini*) and flax (*Linum marginale*) led to the classical gene-for-gene model of resistance (Flor, 1956; Flor, 1971) (Figure 4a). The resistance is conferred when the product of an avirulence gene (*Avr*) from the pathogen is recognized by a resistance (*R*) gene in the host and hypersensitive response (HR) and programmed cell death is initiated (Figure 4a).

4.2. Host specific toxins and necrotrophic effectors

The first host specific toxins (HSTs) were also discovered in the 1930-40s, for instance AK toxin produced by *Alternaria alternata* (Tanaka, 1933) and victorin produced by *Cochliobolus victoriae* (Meehan and Murphy, 1947). While resistance genes in the classical model are dominant (Figure 4a), susceptibility is usually caused by a dominant *susceptibility* gene and is referred to as an inverse or mirror model (Figure 4 b) (Friesen et al., 2007; Wolpert et al., 2002)

HSTs produced by fungi are, like *Avr*-gene products in biotrophic systems, also diverse in structure and biosynthetic mechanisms (Wolpert et al., 2002). Some peptide HSTs act as effectors by inducing HR (Faris et al., 2010; Oliver and Solomon, 2010) and are called necrotrophic effectors (NEs).

4.3. NE and sensitivity gene interactions in the *P. nodorum* – wheat pathosystem

It has been known for almost 40 years that *P. nodorum* produces phytotoxic compounds inducing plant cell death prior to hyphal growth (Bird and Ride, 1981). However, the role of these phytotoxins in relation to resistance was not understood by the end of the last century (Cunfer, 1999).

Liu et al. (2004a) characterized the first host specific protein secreted by *P. nodorum*, and named it SnTox1. Earlier, Tomas and Bockus (1987) had described that the causal agent of tan spot, *P. tritici-repentis,* secretes a host-specific toxin, Ptr toxin, later renamed ToxA (Tomas et al., 1990). The corresponding sensitivity locus *Tsn1/tsn1* was mapped to chromosome 5BL and reported as a "dominant susceptibility" locus (Faris et al., 1996). Friesen et al. (2006) discovered a *P. nodorum* gene that shared 99.7 % sequence similarity to the *ToxA* in *P. tritici-repentis* described above, and the sensitivity also mapped to *Tsn1* (Liu et al., 2006). It was estimated that the *ToxA*-gene was introduced from *P. nodorum* into *P. tritici-repentis* through horizontal gene transfer before 1941 when *P. tritici-repentis* emerged as a pathogen on wheat (Friesen et al., 2006).

The *ToxA* gene is also present in *P. avenaria* f.sp. *tritici*, closely related to *P. nodorum* (McDonald et al., 2013; McDonald et al., 2012). Recently, the *ToxA* gene was also discovered in *Bipolaris sorokiniana*, the causal agent of spot blotch in wheat (McDonald et al., 2017). The *ToxA* region in *B. sorokiniana* showed more similarities with *P. tritici-repentis* than *P. nodorum*.

The characterized *P. nodorum* NEs are small, secreted proteins, and virulence factors rather than true pathogenicity factors (Friesen et al., 2007), i.e. they affect the degree of disease in the host. So far, at least eight NEs and nine corresponding *Snn* loci have been characterized (Table 2).

Table 2. Overview of the mapped and characterized necrotrophic effectors (NEs) from *P. nodorum* and corresponding host sensitivity loci in wheat.

Necrotrophic effector (NE)	Protein size (kD)	Host sensitivity gene	Wheat chromosome arm	Type of gene/ mapping status	Reference
SnToxA	13.2	*Tsn1*	5BL	NBS-LRR-PK	(Faris et al., 2010; Liu et al., 2006)
SnTox1	9	*Snn1*	1BS	WAK	(Liu et al., 2004a; Liu et al., 2004b; Liu et al., 2012; Shi et al., 2016b)
SnTox2	7-10	*Snn2*	2DS	Mapped	(Friesen et al., 2007)
SnTox3	19	*Snn3-B1*	5BS	Fine-mapped	(Friesen et al., 2008; Liu et al., 2009; Ruud et al., 2017; Shi et al., 2016a)
		Snn3-D1	5DS	Fine-mapped	(Zhang et al., 2011)
SnTox4	10-30	*Snn4*	1AS	Mapped	(Abeysekara et al., 2009)
SnTox5	10-30	*Snn5*	4BL	Mapped	(Friesen et al., 2012)
SnTox6	12	*Snn6*	6AL	Mapped	(Gao et al., 2015)
SnTox7	10-30	*Snn7*	2DL	Mapped	(Shi et al., 2015)

P. nodorum is a good model organism for genomic studies due to its willingness to grow on artificial media, its importance as a crop pathogen and its relatively small sized genome (≈ 37 Mb). The first *P. nodorum* reference genome was published by Hane et al. (2007), and it has later been re-sequenced (Syme et al., 2013) using isolates with different effector profiles and annotated (Syme et al., 2016). Naturally, an important objective of the genomic studies has been to investigate the genetic and evolutionary basis of the fungus' pathogenicity. Bioinformatic tools have been used to search for novel effector candidate genes and were successful in identifying the *SnTox1* gene (Liu et al., 2012). The criteria used to predict candidate effector genes are: 1) Small secreted protein (< 30 kDa), 2) cysteine rich, 3) located near repetitive DNA regions or scaffold ends, 4) no blast matches. In addition, criteria like presence/absence of genes in virulent versus non-virulent isolates and evidence of positive selection can be applied when isolates with known differences in pathogenicity are compared (Syme et al., 2013). By applying these criteria on genomic data from three isolates (SN15, Sn4 and Sn79 (non-virulent)), a candidate list of 159 potential effector genes was the result (Syme

et al., 2013). Although two of the known effectors ranked top of the list, the number of genes that would need further investigation and validation is somewhat discouraging. Also, since few similarities have been identified between different effectors, as discussed above, the prediction criteria may not capture all the real candidate genes. In addition, although acknowledging that effector genes are often located in repetitive regions (criterion 3), such regions have presented technical challenges and been filtered out in many next-generation-sequencing studies (Alkan et al., 2011), which means that many true candidate genes may be missed (Treangen and Salzberg, 2011). More recently, technologies that also capture these regions, i.e. sequence longer continuous pieces of DNA, like PacBio (Pacific Biosciences), have been developed (Goodwin et al., 2016).

The cloning of NEs and their corresponding host sensitivity genes has revealed quite distinct and diverse molecular functions. SnToxA was shown to be internalized into the chloroplasts of sensitive (*Tsn1*) host cells where it induces alterations in photosynthetic electron transport leading to a burst in reactive oxygen species (ROS) (Manning et al., 2009). However, the Tsn1 protein does not interact directly with SnToxA (Faris et al., 2010). On the other hand, SnTox1 was found to be localized in the fungal cell walls where it likely serves to protect the pathogen against wheat chitinases, and it does not enter the plant cell (Liu et al., 2016). The effector is recognized directly by the Snn1 protein, which spans the plasma membrane and contains extra-cellular binding domains (Shi et al., 2016b). Although the outcome is the same – induction of programmed cell death – it appears that *Snn1* activates PAMP-triggered immunity (PTI) upon direct recognition by SnTox1 at the host cell surface, while Tsn1 activates effector-triggered immunity (ETI) upon indirect recognition of SnToxA inside the plant cell.

The relative contributions of NE-*Snn* interactions to disease under field conditions are still discussed (Francki, 2013), although more and more evidence supports that at least some of the interactions are important: SnToxA-*Tsn1* and SnTox2-*Snn2* were identified after spray inoculation of the flag leaf with a single *P. nodorum* isolate in the field (Friesen et al., 2009). The effect of SnToxA-*Tsn1* was also likely to underlie a significant QTL in the 05Y001 doubled haploid mapping population one year, but not in the subsequent trial (Francki et al., 2011). Waters et al. (2011) found a lower difference in resistance rating between ToxA-insensitive and sensitive cultivars than Oliver et al. (2009) and suggested that reduction in SnToxA-sensitive cultivars could have triggered a shift in the NE frequencies in the pathogen population. Waters et al. (2011) also found a low, but significant correlation between sensitivity to SnTox3 and disease resistance ratings in Australian wheat cultivars. The identification of

Snn3 as a major susceptibility factor in the SHA3/CBRD × Naxos population in the field was the first to validate the importance of this locus in adult plant resistance (Ruud et al., 2017).

In Australia SnToxA has been delivered to the breeders since 2009 (Vleeshouwers and Oliver, 2014). By 2012, 30 000 doses of SnToxA and 6 000 doses each of SnTox1 and SnTox3 were provided annually (Vleeshouwers and Oliver, 2014). The area of SnToxA sensitive wheat in Australia fell from 30.4 % in 2009-2010 to 16.9 % within three years. The estimated economic gain was approximately 50 million AUD, assuming a yield loss of 0.3 tons per hectare in susceptible cultivars (Vleeshouwers and Oliver, 2014).

4.4. The nature of resistance and sensitivity genes

Most of the R-genes encode proteins with a nucleotide binding site (NBS) and leucine-rich repeats (LRRs). Upon direct or indirect recognition of a pathogen effector (i.e. the product of a *Avr*-gene), the NBS-LRR initiates signaling pathways, in most cases leading to HR and cell death (Jones and Jones, 1997; van't Slot et al., 2003).

Less is known about the genes conferring susceptibility to NEs. However, the molecular cloning of a number of sensitivity genes including *Tsn1* involved in ToxA sensitivity, have showed that they often have NBS and LRR domains associated with effector triggered immunity (ETI) (Faris et al., 2010; Lorang et al., 2007; Nagy and Bennetzen, 2008). Recently, also *Snn1* conferring sensitivity to SnTox1 was cloned and shown to encode a wall-associated kinase (WAK) (Shi et al., 2016b). Receptor kinases are usually pattern recognition receptors (PRR) involved in pathogen-associated molecular patterns (PAMP) triggered immunity. Responses to ETI and PAMP overlap, including the HR response (Dodds and Rathjen, 2010).

Figure 5. Left: Infiltration with culture filtrate with a needleless syringe. Right, top: SnToxA-insensitive leaf 5 d.p.i (days past inoculation). Right, bottom: Necrotic tissue developed in the infiltrated area in a SnToxA-sensitive leaf 5 d.p.i..
Photos: Anja K. Ruud (left), Min Lin (right).

These gene-for-gene interactions, inducing HR upon recognition, forms the framework for identifying resistance and sensitivity to SNB at the seedling stage. Single *P. nodorum* isolates can be grown in liquid medium, and are expected to secrete NEs into the medium. When seedling leaves are infiltrated with filter sterilized culture filtrate (CF) (Figure 5), sensitive lines develop chlorosis or necrosis, while resistant lines remain healthy. The reaction types are usually scored on a 0-3 scale (Friesen and Faris, 2012) and the sensitivity locus can be genetically mapped if a segregating mapping population is used. Typically, symptoms develop after 3 to 5 days in the greenhouse.

Since the NE is a virulence factor, it should also have an effect on disease development after inoculation with a conidiospore suspension. Historically, different methods have been used to evaluate the role of different components of resistance at the seedling stage. The methods include latency period, lesion expansion and development and number of pycnidia in the lesions (Czembor et al., 2003; Eyal and Scharen, 1977; Eyal et al., 1987). Quantitative measurements have also been used, for instance in Eyal and Scharen (1977), Karjalainen (1985) and Jönsson (1985). However, a reaction type scale from 0 to 5 (Liu et al., 2004b) emphasizing the extent of chlorosis and necrosis associated with the lesions is now commonly applied. This method is believed to be accurate in capturing the effect of potential NE-*Snn* interactions (Friesen and Faris, 2012).

Adult plant resistance to SNB is mainly quantitative and additive (Bostwick et al., 1993; Fried and Meister, 1987; Wicki et al., 1999). Dominant SNB resistance is also observed, and the segregation patterns of intermediate reactions can indicate the presence of modifier genes (Kleijer et al., 1977; Ma, 1993; Ma and Hughes, 1993, 1995). General mechanisms like pathogen production of cell wall degrading enzymes (Lehtinen, 1993; Magro, 1984), host lignification and papilla formation to reduce hyphae penetration (Bird and Ride, 1981) also explain variation in resistance.

Epistatic interactions among NEs seem to be common. For instance, it was shown that the SnToxA-*Tsn1* interaction is epistatic to SnTox3-*Snn3* and that SnTox3-*Snn3* is significant only in the presence of an incompatible SnTox2-*Snn2* interaction (Friesen et al., 2008). This was further corroborated by generation of knockout-strains of *P. nodorum* isolate SN15 whereby

the *SnToxA*, *SnTox1* and *SnTox3* genes were sequentially deleted through genetic transformations (Tan et al., 2015). In the Calingiri x Wyalkatchem mapping population, the SnTox1-*Snn1* interaction was paramount when inoculated with SN15, and no effect was observed for the SnTox3-*Snn3* interaction. However, this interaction became significant when plants were inoculated with *tox1-6,* a knockout strain for *SnTox1* (Phan et al., 2016). Apparently, this was due to upregulated *SnTox3* expression in *tox1-6* (Phan et al., 2016). The triple knockout strain (*toxa13*) lacking all three effector genes was still able to cause disease (Tan et al., 2015). When used in QTL mapping it unveiled a significant QTL on 2DS where *Snn2* is located, which was not detected with SN15 or *tox1-6* (Phan et al., 2016), which indicated that the 2DS QTL was epistatic to SnToxA-*Tsn1* and/or SnTox3-*Snn3*. Interestingly, the SnTox5-*Snn5* and SnTox6-*Snn6* interactions are also epistatic to SnTox3-*Snn3* (Friesen et al., 2012; Gao et al., 2015).

4.5. Escape mechanisms and environmental impacts on resistance

Morphological traits that reduce the contact between the pathogen and the plant can be identified as disease escapes (Parlevliet, 1977), and often lead to misinterpretation of true association with resistance. These include variation in plant height and timing of heading (earliness) (Scott et al., 1982). Taller plants may escape from rain driven spread of disease in the canopy. Early maturing plants may escape the highest disease pressure (Francki, 2013), but on the other hand, later plants may appear more resistant at the time of disease scoring since the disease develops faster in more mature plants.

The development of disease is affected by weather conditions like temperature, rainfall and humidity. Variation in these factors within and between growth seasons can have a strong effect on the relative resistance rankings (Kim and Bockus, 2003).

5. Genetic mapping of resistance and use of markers in breeding

5.1. Molecular markers

Breeding for improvement of complex traits is not straightforward. The contribution of each individual locus is moderate and can be masked by other, dominant loci or epistatic effects. However, the development of molecular markers could provide a help in overcoming some of these difficulties. Co-dominant markers can distinguish between all genotypes (Tanksley, 1983). Markers can be either hybridization or polymerase chain reaction (PCR) based. In the

first case, a probe is hybridized to the DNA. In PCR based systems small fragments of DNA are amplified with polymerase enzymes.

Simple sequence repeat (SSR) markers have many advantages. They can be non-anonymous, abundant, reproducible and show a high degree of inter- and intra-specific polymorphism (Mammadov et al., 2012; Semagn et al., 2014).

Single nucleotide polymorphisms (SNPs) are the most abundant of molecular markers, estimated to occur for every 100-300 bp in any genome (Gupta et al., 2001), and for wheat every 96-483 bp, depending on the stringency criteria and germplasm used (Manickavelu et al., 2012). Chip based SNP platforms are oligonucleotide based DNA microarrays that cover thousands of SNP markers that can be genotyped in one go. Recent genome sequencing efforts have enabled the development of low-cost high density arrays in wheat, such as the Illumina 90K wheat chip (Wang et al., 2014) and the Affymetrix 35K breeders array (Allen et al., 2017).

Genotyping-by-sequencing (GBS, also called next generation sequencing, NGS) can be an alternative to chip based arrays. GBS is particularly suitable for projects where the genomes of several specimens are sequenced to discover large numbers of SNPs. No prior knowledge of the genome is necessary and the cost is lower than for chip-based arrays (Elshire et al., 2011).

SNPs are quite easily transferred from one platform to another, and for genotyping of only a few markers on many samples, KASP (Kompetitive Allele Specific PCR) is a highly flexible and cost-effective alternative commonly used in wheat breeding (Semagn et al., 2014).

5.2. Linkage QTL mapping

Linkage mapping is the most widely used method to dissect complex traits and identify markers linked to them. The genomic regions associated with such a trait are called quantitative trait loci (QTL). The development of molecular marker technologies in the 1980s facilitated the construction of genetic linkage maps (Collard et al., 2005) and complex traits could be separated into discrete QTL (Paterson et al., 1988).

Maps can be constructed for specific, segregating populations. Preferably, recombinant inbred lines (RILs) or double haploids (DHs) are used since they are homozygous and can be maintained and reproduced forever (Collard and Mackill, 2008). To identify QTL, the population is phenotyped for the traits of interest, and the phenotypic and genotypic data is analyzed to uncover linkage between a certain phenotype and genetic regions.

After the initial detection, additional steps to confirm a QTL and validate associated markers are usually required (Langridge et al., 2001). The effect and position of a QTL can be inaccurate due to sampling bias (Melchinger et al., 1998) and flanking markers may not be polymorphic in other genotypes.

Table 3 shows an overview of SNB resistance QTL detected in QTL mapping studies. For field-based studies, only QTL significant (LOD ≥ 3 or threshold calculated by permutation test) in at least two environments are included.

Table 3 Overview of quantitative trait loci (QTL) for SNB resistance, including necrotrophic effector-sensitivity (NE-*Snn*) gene interactions. Adapted from Francki (2013) and updated with more recent studies. *T. aestivum* unless otherwise noted below. Adult plant resistance QTL from field trials are only listed if they have been reported significant in at least two environments (years, locations).

Plant tissue	**Population**	**NE-*Snn***	**QTL, chromosome**	**Markers**	**Reference**
Seedling leaf	Liwilla × Begra, DH		*QSnl.ihar-2B*	*gwm501 –gwm410*	(Czembor et al., 2003)
			QSnl.ihar-5B	*barc32 – gwm499*	
			QSnl.ihar-5D	*gwm205 - gwm212*	
	W7984 × Opata85	SnTox1-*Snn1*	1B	*mwg938 – snn1 fcp618, psp3000*	(Liu et al., 2004b)
			4B	*cdo1312*	
	Alba × Begra		*QSnl.ihar-6A*	*gwm570 –mwg934*	(Arseniuk et al., 2004)
	BR34 × Grandin	SnToxA-*Tsn1*	5BL	fcp1, fcp2, fcp394, fcp620	(Friesen et al., 2006)
	BR34 × Grandin	SnTox2-*Snn2*	2DS	*TC253803, cfd51*	(Friesen et al., 2007)
		SnTox3-*Snn3-B1*	5BS	*gwm234, cfd20*	
	LDN × LDN (DIC-1B) (*T. turgidum*)		5BL	*bcd9 – fbb237*	(Gonzalez-Hernandez et al., 2009)
	Arina × Forno	SnTox4-*Snn4*	1AS	*BG262267, BG26975, cfd58*	(Abeysekara et al., 2009)
	Aegilops tauschii	SnTox3-*Snn3-D1*	*5D*	*cfd18 - hbg337*	(Zhang et al., 2011)
	Lebsock × PI 94749 (*T. turgidum* subsp. *durum* × *T. turgidum* subsp. *carthlicum)*	SnTox5-*Snn5*	4BL	*wmc349 - cfd22, barc163*	(Friesen et al., 2012)
	W7984 × Opata85	SnTox6-*Snn6*	6AL	*BE424987 - BE403326*	(Gao et al., 2015)
	Chinese Spring × Timstein	SnTox7-*Snn7*	2D	*cfd44 – gwm311*	(Shi et al., 2015)
	Calingiri x Wyalkatchem	SnTox1-*Snn1*	*Q.snb.fcu-1BS*	*gpw7059a - wPt-2654*	(Phan et al., 2016)
			Qsnb.cur-2AS1	*gwm339 - gwm312*	
			Qsnb.cur-2DS	*cfd36 - wPt-669517*	
			Qsnb.cur-3AL	*tPt-1143 - wPt-4859*	
			Qsnb.fcu-4BL	*barc163 - wPt-4243*	
		SnTox3-*Snn3-B1*	*Qsnb.fcu-5BS*	*TC282809a - wPt-666323*	
	Calingiri x Wyalkatchem		*Qsnb.cur-2AS2*	*wmc382a - barc124a*	(Rybak et al., 2017)
			Qsnb.cur-2DS	*cfd36 - wPt-669517*	

			Qsnb.cur-3AL	*tPt-1143 - wPt-4859*	
			Qsnb.cur-6AS	*gpw4329 - wPt-4270*	
	SHA3/CBRD x Naxos		1A	*IWB52902*	(Ruud et al., 2017)
			1B	*psp3000*	
			2D	*IWA8544*	
		SnTox3-*Snn3-B1*	5B	*IWB11709*	
			7B	*Wsnp_BE498662B_Ta_2_5*	
	Altar 84 x Langdon (*T. turgidum* ssp. *durum*)	SnToxA-*Tsn1*	*Qsnb-fcu.5B*	*Tsn1*	(Virdi et al., 2016)
	GWAS 567 spring wheat landraces		2D	*wPt-665317*	(Adhikari et al., 2011)
			3B	*wPt-6047*	
			5B	*wPt-1149*	
			6A	*wPt-7330*	
			7A	*wPt-4515*	
	GWAS 528 spring wheat landraces		2D	*IWA7348*	(Gurung et al., 2014)
			3A	*IWA4075*	
			5B	*IWA7024*	
	GWAS 120 winter wheat cultivars and breeding lines		5A	*IWB67424*	(Liu et al., 2015)
			5B	*IWB36366*	
			5B	*IWB38178*	
			5D	*IWB45668*	
	GWAS 320 synthetic hexaploid wheat lines		7B	*1208964*	(Jighly et al., 2016)
	GWAS 121 spring wheat lines		1A	*IWB56511*	(Ruud et al., 2018a)
			1B	*IWB6550*	
			3A	*IWB9350*	
			4B	*IWB6422*	
		SnTox3-*Snn3-B1*	5B	*IWB26869*	
		SnToxA-*Tsn1*	5B	*IWB67424, IWB67425, IWB3660*	
			6B	*IWB73405*	
			7A	*IWB21459*	
			7B	*IWB64015*	
			7D	*IWA732*	
Seedling and adult pant leaf	BR34 × Grandin	SnTox2-*Snn2*	*QSnb.fcu-2DS*	*gwm614 – cfd53*	(Friesen et al., 2009)
			QSnb.fcu-5AL	*barc151 – fcp13*	
		SnToxA-*Tsn1*	*QSnb.fcu-5BL*	*barc1116 – barc43*	
Adult plant leaf	Forno x Oberkulmer		*QSnl.eth-2D*	*psr932 – psr331a*	(Aguilar et al., 2005)
			QSnl.eth-4B	*glk348 – psr921*	
			QSnl.eth-7B	*mwg710a – glk576*	
	WAWHT2074 x 6HRWSN125		*QSnl.daw-2D*	*cfd11 – gwm30*	(Shankar et al., 2008)
	BR34 x Grandin		*QSnb.fcu-1BS*	*fcp267 – barc240*	(Friesen et al., 2009)
	P92201D5 x P91193D1		*QSnl.daw-2A*	*gwm614a – wPt-7056*	(Francki et al., 2011)
	EGA Blanco x Millewa		*QSnl.daw-1B*	*wPt-8949 – wPt-2575*	
			QSnl.daw-5B	*wPt-3457 – wPt-0935*	
	SHA3/CBRD x Naxos		*1B*	*wmc619*	(Lu and Lillemo, 2014)
			3AS	*gwm2*	
			3B	*wPt-4127*	
			3BL	*wPt-4933*	
			5BS	*wPt-5346*	
			5BL	*fcp1*	
			7A	*wmc603*	
			7B	*wPt-0963*	
	SHA3/CBRD x Naxos		*1A*	*IWA2995*	(Ruud et al., 2017)
			1B	*SCM9*	
			3AS.1	*gwm2, IWB35234*	

			3AS.2	*IWB39383, IWB27319*	
			3BL	*wPt-4933*	
			5BS	*IWB11709*	
			5B.2	*wPt-5914*	
			7A	*IWB53887*	
	Calingiri x Wyalkatchem	SnTox1-*Snn1*	*Qsnb.fcu-1BS*	*gpw7059a - wPt-2654*	(Phan et al., 2016)
			Qsnb.cur-6BS	*wPt-3168 - barc146a*	
	GWAS 121 spring wheat lines		2B	*IWB2427*	(Ruud et al., 2018a)
			2D	*gwm301, IWB35134, IWB30879, IWB30880, IWB34359*	
			4B	*IWB6422*	
			7B	*IWB57207, IWB73685, IWB70085*	
Glume blotch	Arina x Forno		*QSng.sfr-3B*	*gwm389 – cfd79c*	(Schnurbusch et al., 2003)
			QSng.sfr-4B	*gwm165 – glk335*	
	Arina x Forno and GWAS 44 winter wheat cultivars		*Qsng.sfr-3B*	*Sun2-3B*	(Tommasini et al., 2007)
	Arina/3*Forno NILs		3BS1	*wmm756*	(Shatalina et al., 2014)
			3BS2	*swm01210*	
	P92201D5 x P91193D1		*QSng.pur-2DL.1*	*gwm526a – cfd50b*	(Uphaus et al., 2007)
			QSng.pur-2DL.2	*cfd50c – wPt9848*	
	WAWHT2074 x 6HRWSN125		*QSng.daw-4B*	*Rht1 – gwm495*	(Shankar et al., 2008)
	Forno x Oberkulmer		*QSng.eth-5AL*	*psr1194 - psr918*	(Aguilar et al., 2005)
	GWAS 320 synthetic hexaploid wheat lines		2AL	*wPt-1657*	(Jighly et al., 2016)
			2BL	*1107710*	
			2DL	*wPt-7825*	
			3BS	*wPt2757, wPt-8079, wPt-3921*	
			4BL	*1094836*	
			5AL	*wPt-8262*	
			6B	*1019982*	
			7DS	*1263913*	
			7DL	*1216888, 1233921, 1227840*	

5.3. Association mapping

Association mapping (AM, also called genome wide association mapping, GWAS) emerged in the early 2000s as an alternative to biparental linkage mapping (Gupta et al., 2014). Only a handful of GWAS studies have investigated SNB resistance (Table 3). AM was used to fine map a region on 3BS associated with Septoria nodorum glume blotch in 44 European winter wheat varieties (Tommasini et al., 2007). Adhikari et al. (2011) detected unique SNB seedling resistance QTL on 6A and 7A in a GWAS panel consisting of 576 land races from the USDA Small Grains Collection. A novel QTL on 3A was identified in a set of 528 spring wheat landraces from the same USDA Small Grains Collection in a study by Gurung et al. (2014), while two other QTL on 2D and 5B were described previously by Adhikari et al. (2011). Also,

Liu et al. (2015) identified seedling QTL on 5A, 5B and 5D in a panel of 120 hard red winter wheats. To our knowledge, the GWAS on Nordic wheat germplasm (Ruud et al., 2018a) is the only study so far focusing on adult plant leaf resistance. It showed that field resistance is based on many small-effect QTL and only a few loci showed effect across field seasons (Table 3).

5.4. Marker assisted selection and resistance breeding

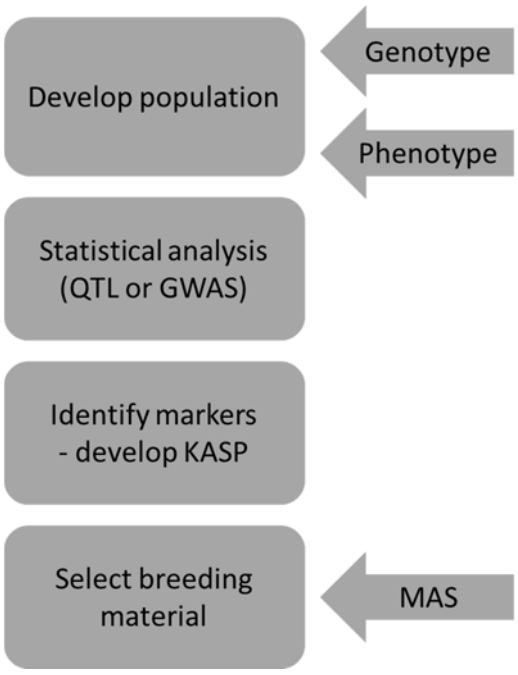


Figure 6 Typical workflow of a genetic mapping study with marker assisted selection (MAS) as the goal.

Figure 6 shows the typical workflow for a genetic mapping study. A suitable population is genotyped with genetic markers (usually SNPs or SSRs), and phenotyped for the traits of interest. QTL mapping or GWAS is performed to identify significant marker trait associations (MTA). The most promising markers can then be validated in other populations and converted to economical KASP markers and used to screen and select breeding material (marker-assisted selection, MAS).

Economic and practical constraints decide whether MAS is feasible in a resistance breeding program. Compared to phenotypic selection at the adult plant stage, MAS can provide higher accuracy and save time in the breeding cycle, if markers are closely linked or diagnostic for QTL that explain a substantial amount of the phenotypic variation (typically at least 10-20%). For quantitative disease resistance, the best strategy is suggested to be MAS followed by

phenotypic selection. The subsequent step of phenotypic selection allows for the inclusion of minor QTL (Miedaner and Korzun, 2012).

5.5 Genomic prediction and selection

Genomic prediction and selection (GS) (Meuwissen et al., 2001) is an approach that predicts the best individuals based on genetic values. Compared to traditional models where only markers significantly linked to the trait are considered, genomic selection considers ALL markers across the genome to predict breeding values (BV). Known QTL with large effects can be included in the genomic prediction models as fixed effects and further increase the prediction accuracy (Bernardo, 2014). The genomic selection is performed on a different population than the reference (training) set on which the genetic marker effects were calculated. Although initially used to predict BVs of animals, genomic selection also has the potential to improve genetic gain in crops like wheat (Crossa et al., 2010; Ornella et al., 2012; Storlie and Charmet, 2013).

For a complex trait like SNB seedling resistance, genomic prediction models performed better than a MAS approach using only the markers significantly associated with the trait (Juliana et al., 2017). The accuracy for prediction of adult plant resistance has not yet been reported for SNB. However, prediction values for adult plant resistance to comparable diseases like tan spot and Septoria tritici blotch showed that although the prediction accuracy was generally lower than for seedling resistance, genome-wide prediction models performed significantly better than the MAS approach which only considered the significant markers (Juliana et al., 2017).

6. A case study on SNB resistance in Norwegian wheat

SNB is one of the most important diseases in spring wheat in Norway (Ficke et al., 2011b; Lillemo and Dieseth, 2011). The disease is mainly controlled by fungicides since the use of other measures like crop rotation and autumn ploughing is limited. The potential loss of sensitivity towards fungicides (Abrahamsen, 2013; Ficke et al., 2011a) and concern about health and environmental risks underlines the need for more sustainable control.

As outlined in sections 4 and 5, the recent discoveries of the NEs and their role in the wheat-SNB pathosystem opened up new possibilities for resistance breeding by elimination of sensitivity loci in the germplasm (Vleeshouwers and Oliver, 2014; Zhang et al., 2009). On this

background, research efforts were initiated in Norway in 2009 to characterize the local pathogen population, investigate the role of NEs in causing susceptibility under field conditions and map major resistance/susceptibly loci in relevant germplasm for wheat breeding. In the following, we give a brief outline of the research approach and main results so far, with focus on implications for disease management and resistance breeding.

6.1. Survey of the pathogen population

During the 2010 and 2011 field seasons, a small survey was conducted by collecting leaf samples from unsprayed wheat fields with leaf blotch symptoms across the wheat growing regions in Norway. These were analyzed by PCR with species-specific primers and indicated that *P. nodorum* was present in almost all the collected samples from both spring wheat and winter wheat fields. *P. tritici-repentis* was detected in about half of the samples of both spring and winter wheat, while *Z. tritici* was less common and mostly found in winter wheat. These unpublished results proved a very similar situation as in Finland, where also *P. nodorum* was the dominating pathogen on spring wheat and present in almost every sample that was analyzed in 2009 (Jalli et al., 2011). The frequent occurrence of all three leaf blotch pathogens on the same samples was well in line with similar results obtained from winter wheat fields in Sweden (Blixt et al., 2010).

P. nodorum isolates were also collected from unsprayed fields in the major wheat growing areas in 2012 to 2014. The majority (69 %) of the Norwegian *P. nodorum* isolates investigated (n = 62) harbored the *SnToxA* gene, which may be an adaptation to the sensitive host populations (Ruud et al., 2018b). The frequency was much higher than in Swiss isolates (McDonald et al., 2013). The high frequency of the *SnToxA* gene in the pathogen population can also explain why the sensitivity was detected as significant under natural infection in our field trials. Also, the frequencies of isolates producing SnTox3 (76 %) and SnTox1 (53 %) were high in the Norwegian *P. nodorum* population (Ruud et al., 2018b). This demonstrated that the known effectors are common in the pathogen population, and that elimination of corresponding sensitivity alleles in the wheat germplasm might be a promising avenue to follow in resistance breeding.

6.2. Establishment of reliable field testing methodology

To identify genetic SNB resistance, the first steps are to collect and screen diverse germplasm in the field and in the greenhouse. In the field, one can either rely on natural infection by the ambient pathogen population, or inoculate with single isolates or a mixture of isolates. The reproducibility across locations or seasons may be higher when the same isolates are used. On the other hand, one or a few isolates may not be representative of the situation in the farmers' fields. Breeders usually rely on natural infection in the field for evaluation of leaf blotch resistance (Cowger and Murphy, 2007). Fraser et al. (2003) suggested that promotion of infection by natural inoculum gives a better estimate of host resistance under natural epidemics than inoculation of the nurseries with selected isolates. In the field trials that will be referred to, we relied on natural infection promoted by mist irrigation and naturally infected straw.

Correction for confounding traits

Development of SNB at the adult plant stage in the field is influenced by variation in plant height and earliness (timing of heading). It is important to account for the effects of these traits. This can be done in several ways:

1) Score the plants and/or spray inoculate the flag leaves at the same developmental stage, in the greenhouse or in a tunnel to avoid effects of plant height by rain-splash spread spores. These are good measures, but very labor intensive and time-consuming when large populations are screened
2) Choose or develop mapping populations with little variation in earliness and height.
3) Score all traits of interest separately and only consider QTL for SNB resistance that does not co-locate with QTL for the confounding traits. However, true resistance QTL under the threshold can go undetected by this method
4) Include the confounding traits as covariates in a regression model with SNB severity as the dependent trait. This is the method we used. The QTL detected when the corrected values are analyzed are assumed to capture the true residual, genetic resistance to SNB. The corrected resistance was annotated as "corrected SNB severity".

The effect of plant height on SNB development varied from year to year. As described in 2.1., the pathogen has certain rainfall and relative humidity requirements in order to sporulate, spread by rain-splash and successfully infect new leaves (Eyal et al., 1987). In years with

moderate rainfalls during mid-June to July, the effect of plant height on disease severity was usually significant in our field trials. In years with low correlation between plant height and SNB development, extreme rainfalls (i.e. 76 mm in 24 h in 2015) seemed to reduce the differences between tall and short plants, by spreading the spores to the flag leaf also in tall cultivars.

Temperature affects plant development, like the timing of heading. Although spring wheat normally does not require vernalization, i.e. a cooler period after germination to induce heading and flowering, some of the lines we tested harbor the *Vrn-A1* gene (Yan et al., 2003). This gives them a weak vernalization requirement. In particular, this applies to several lines and cultivars originating from CIMMYT. When the spring is warm, the requirement may not be met, and the induction of heading and flowering is significantly delayed. Thus, the effects of the individual vernalization genes vary between years, depending on temperature.

How can we use the correlation between field and seedling experiments to select resistant genotypes?

To be relevant for breeding purposes, the genetic resistance needs to act at the adult plant stage in the field. However, disease evaluation in the field is resource demanding and is often complicated by genotype by environment interactions and sometimes by confounding traits. Thus, if sufficiently representative results are possible to score at the seedling stage, it would save time and money. A typical example of the relationships that can be obtained in mapping populations and germplasm collections is shown in Fig. 7. Significant correlations can be obtained by the use of representative isolates that reflect the natural pathogen population, but R^2 values rarely exceed 20-25 % when field data is based on natural infection. When less relevant isolates are used, such as 201618, there might not be any relationship at all. *Therefore, good care should be taken in screening isolates beforehand if seedling inoculations are to be used as a selection tool in breeding, and results should always be confirmed by field testing.*

As will be shown later in this section, we have also detected some QTL that were significant both at the seedling and adult plant stage. In particular, the *Snn3-B1* locus in SHA3/CBRD × Naxos (Ruud et al., 2017) and the QTL on 4B and 7A in our association mapping panel (Ruud et al., 2018a). We also have evidence that the *Tsn1* locus has an effect across developmental stages in our germplasm (Ruud et al., 2018b).

On the other hand, many of the QTL we have detected are *either* seedling stage *or* adult plant stage QTL, giving a strong indication that resistance at the two growth stages are only partially controlled by the same genes.

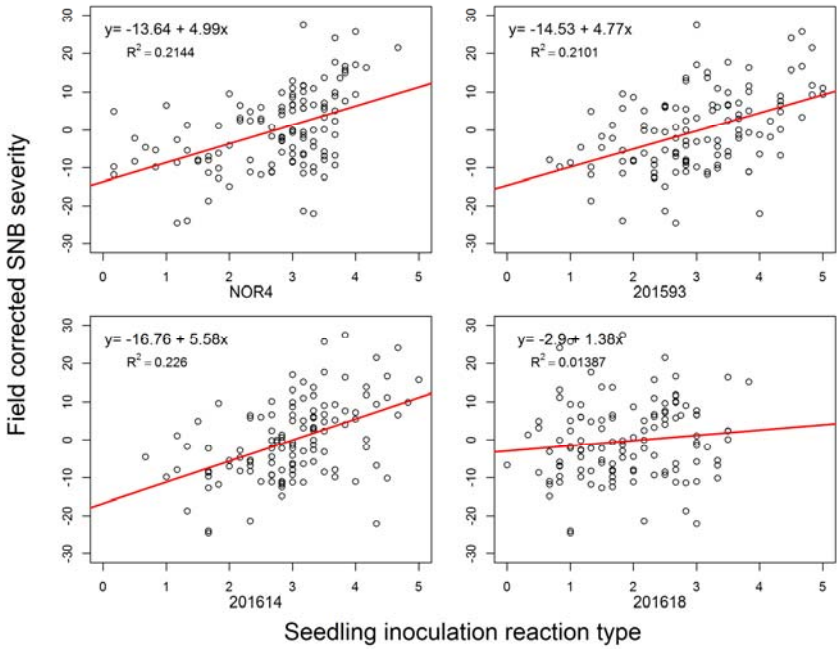


Figure 7 Correlation between seedling inoculation disease reaction type and the mean corrected SNB severity from seven years of field trials in a Nordic spring wheat collection (Ruud et al., 2018a; Ruud et al., 2018b).

6.3. The role of known effector sensitivities in field resistance

A crucial issue for resistance breeding was to investigate the relationship between sensitivity to the cloned effectors SnToxA, SnTox1 and SnTox3 and their potential effect on disease severity under the field conditions. A Nordic collection of spring wheat cultivars, landraces and breeding lines was used for this purpose. The plants were screened in mist irrigated field trials from 2010 to 2016, and phenotyped for plant height, earliness (days from sowing to heading) and leaf blotch severity. Sensitivity to SnToxA and SnTox3 was common in the material (45 and 55 %, respectively) while sensitivity to SnTox1 was only present in 12 % of the lines. For SnTox3 sensitivity, we identified two distinct reaction types in our germplasm. While full necrosis and tissue collapse (reaction type 3) was common in CIMMYT germplasm and some of the Norwegian lines, most lines of Nordic origin showed weaker, chlorotic reaction to SnTox3 (reaction type 2) (Ruud et al., 2018b). Sensitivity to SnToxA was significantly correlated to higher SNB severity in the field, while sensitivity to SnTox1 and

SnTox3 were not significant in the field in this material (Ruud et al., 2018b). Since the SnToxA-*Tsn1* interaction masks the SnTox3-*Snn3* interaction, it may have contributed to the lack of significance of SnTox3-sensitivity in this material. In a QTL mapping population that did not segregate for *Tsn1* and *Snn1* (both parents were SnToxA and SnTox1 insensitive), we detected a large and significant effect of the SnTox3-*Snn3* interaction in naturally infected field trials (Ruud et al., 2017, see below).

6.4. Understanding the genetics of resistance in relevant germplasm

In our research efforts, several mapping populations have been screened over several years under natural infection of *P. nodorum* in mist irrigated field nurseries. The isolates used for seedling inoculations were selected based on SnToxA, SnTox1 and SnTox3-profile from PCR, virulence on differential lines and subsets of the mapping populations. In the two cases that will be reported here, we also inoculated and infiltrated with the North Dakotan isolate Sn4, which has been sequenced (Syme et al., 2013) and used in previous NE-*Snn*-characterization studies (Liu et al., 2015; Zhang et al., 2011). In both studies, the plants were also infiltrated with culture filtrates from the isolates used for inoculation in order to unveil potential new NE-*Snn*-interactions and correlation between sensitivity to the culture filtrate, seedling inoculation and field susceptibility.

Case 1: QTL mapping

Initial field testing identified the line SHA3/CBRD ('Shanghai3/Catbird') from CIMMYT to be a promising source of SNB resistance. From previous projects, we already had a mapping population available of recombinant inbred lines (RILs) from a cross between SHA3/CBRD and the German spring wheat cultivar Naxos that we previously had successfully used for mapping powdery mildew (Lu et al., 2012) and Fusarium head blight resistance (Lu et al., 2013).

The population was tested for four years with our established field testing methodology, and we also screened the parents for sensitivity to the known NEs SnToxA, SnTox1 and SnTox3. The population was found to segregate for SnTox3 sensitivity, and the existing genetic map of 567 SSR and DArT markers was used to perform a QTL analysis (Lu and Lillemo, 2014). Several QTL for field resistance were identified, but only a few of them were significant across years, and we failed to identify the *Snn3-B1* locus, which is known to be the causal locus for SnTox3 sensitivity in bread wheat.

In parallel efforts to fine map and validate powdery mildew resistance loci (Windju et al., 2017), the same mapping population was later genotyped with the Illumina iSelect 90K wheat SNP chip (Wang et al., 2014). With the improved linkage map of 4177 markers in total, we could map the *Snn3-B1* locus with tight linkage to a small group of SNP markers at the telomeric end of 5BS (Ruud et al., 2017). The corrected SNB severity data from the field trials was re-analyzed with the new map, and the SnTox3-*Snn3* interaction was identified as a major determinant of susceptibility in the field, explaining up to 24 % of the phenotypic variation. This is the first report of the significance of the SnTox3-*Snn3* interaction in the field. We also inoculated the population with four *P. nodorum* isolates at the seedling stage and infiltrated with filter sterilized culture filtrates (CFs) from the same isolates. The locus explained up to 51% of the phenotypic variation after seedling inoculations, and was also the major determinant of sensitivity to CFs (Ruud et al., 2017).

Case 2: Genome-wide association mapping

A genome wide association mapping study (GWAS) was carried out on 121 lines from the same spring wheat collection as used for the investigating the role of NEs on field resistance (Ruud et al., 2018b). The aims were to investigate whether SnTox-*Snn*-interactions could be detected with significant marker-trait associations in GWAS analysis, to identify stable adult plant SNB resistance in the Norwegian spring wheat material and determine to which degree seedling and adult plant resistance overlapped.

The population was genotyped with the 90 K SNP chip and the data combined with previously genotyped SSR and other gene-specific markers. A total of 22 031 polymorphic markers were included in the study. The population was also inoculated with four *P. nodorum* isolates and infiltrated with filter sterilized culture filtrate (CF) from the same isolates, at the seedling stage in the greenhouse. GWAS was performed on the corrected SNB severities from the field trials, infiltrations with the purified SnToxA, SnTox1 and SnTox3, infiltrations with CFs from and inoculations with single isolates.

Markers associated with *Tsn1* conferring sensitivity to SnToxA were highly significant at the seedling stage, but only detected below the significance threshold at the adult plant stage. Significant QTL for seedling resistance were located on 1A, 1B, 3A, 4B, 5B, 6B, 7A and 7B. At the adult plant stage the most robust QTL were located on 2B, 2D, 4A, 4B, 5A, 6B, 7A and 7B. The most stable QTL in the field was located on 2DL and was significant in all years except 2012. QTL on 4B and 7A were significant both after seedling inoculations in the greenhouse

and at the adult plant stage in the field (Ruud et al., 2018a). The QTL on 7A corresponded to the same QTL detected in the SHA3/CBRD × Naxos population (Ruud et al., 2017). This study highlighted the quantitative nature of field resistance to SNB in wheat, and that many QTL need to be combined in order to breed cultivars with high levels of field resistance.

6.5. Conclusions

As outlined in this case study, we have with relatively limited use of resources at a national level been able to identify crucial information that will be useful in disease management and resistance breeding. The main results can be summarized as follows:

1. We have shown that *P. nodorum* is the most important pathogen causing leaf blotch on wheat in Norway – hence information on SNB resistance in current cultivars is important information for the farmers, and leaf blotch resistance breeding should focus on this pathogen.
2. Isolates were identified with reduced sensitivity to important fungicides – hence there is a need to diversify disease management strategies with a stronger focus on disease resistance and sustainable use fungicides based on alternative active ingredients
3. A reliable field testing methodology was established by mist irrigation in combination with straw inoculum in hillplots – this has provided important data on field resistance in relevant cultivars and breeding lines.
4. Seedling inoculation results showed only a weak association with field resistance – hence resistance breeding should rely mostly on disease evaluations in the field.
5. Our field results together with leaf infiltrations showed that SnToxA and SnTox3 can contribute significantly to increased disease severities under field conditions – hence breeding efforts should focus on eliminating sensitivities to these NEs in the germplasm.
6. The frequencies of SnToxA and SnTox3 producing *P. nodorum* isolates are high in the local pathogen population – hence cultivars with sensitivity to these NEs should be avoided by farmers.
7. Important QTL for field resistance have been identified in relevant germplasm – these could be utilized in resistance breeding in combination with the elimination of NE sensitivities to build up higher levels of quantitative resistance.

7. Future trends

The potential loss of fungicide sensitivity, and concern about negative impacts of fungicides on the environment and human health, provide strong incentives for breeding and growing SNB resistant wheat cultivars. The discovery of the importance of NE sensitivities on host resistance led to a paradigm shift in the way of thinking about resistance breeding. Rather than searching for sources of resistance, there is a focus on eliminating susceptibility. Either by use of markers (Zhang et al., 2009) or infiltration assays (Vleeshouwers and Oliver, 2014). This approach is based on available polymorphisms in the breeding germplasm. With the recent developments in genome editing technologies (Gil-Humanes et al., 2017), we anticipate that breeders in the future will be able to eliminate NE sensitivities by editing or knocking out host receptors. The genomic resources now available, with a newly released, improved version of the wheat genome (Clavijo et al., 2017), allow for putative identification of gene functions and simplify the steps to candidate gene validation. The genetic background for potential *Snn* genes and the two reaction types for SnTox3 sensitivity observed in the Nordic spring wheat collection, could be further investigated in this respect. While clearly some of the NE-*Snn* interactions show effect and are important at the adult plant stage, there are likely also many other mechanisms at play which are still poorly understood. It is expected that the available reference genome sequences of both the host and the pathogen will be useful in unraveling these mechanisms as well. Deeper insights into the biological mechanisms behind plant-pathogen interactions will likely come from pan genome studies where the whole genome sequences of hundreds of wheat lines with various levels of disease resistance can be compared using bioinformatics pipelines. And likewise, similar comparisons of the genome sequences of hundreds of isolates with various levels of virulence and/or aggressiveness on host cultivars (Gao et al., 2016).

As we have shown in this chapter, the genetics of host resistance under field conditions at the adult plant stage is quite complex and involves many genes. Even if all the most important genetic factors can be identified, it will still be a challenge to handle more than a handful QTL by MAS or through effector screening. Further advancements in breeding methodology will therefore be needed. A quantitative trait like SNB resistance will be a good case for genomic selection. As genomic selection methodology is still in its infancy when it comes to wheat breeding, the coming years will likely see big advancements in the methodology. A challenge for resistance breeding will be how to develop reliable prediction models and integrate those with other agronomically important traits into a cost-effective breeding program. In all cases,

it will serve as a necessary fundament to have a reliable field testing methodology in place in order to make progress in resistance breeding to this challenging wheat disease.

8. Where to look for further information

Here are a few recommended papers for further reading on different topics related to SNB in wheat. The relatively short review paper by Solomon et al. (2006) gives an overview of the disease from the pathogen side. For more detailed information about the *P. nodorum* reference genome at the molecular level, the recent publication by Syme et al. (2016) can be recommended. A comprehensive review of SNB resistance in wheat from a breeding perspective is given by Francki (2013) while Oliver et al. (2012) summarized the recent molecular understanding of host-pathogen interactions for the disease. Friesen and Faris (2012) provide detailed protocols for dissecting plant-pathogen interactions and identification of necrotrophic effectors in this pathosystem, and examples of their use in breeding is given by Vleeshouwers and Oliver (2014).

9. References

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