Mapping of SnTox3-*Snn3* as a major determinant of field susceptibility to Septoria nodorum leaf blotch in the SHA3/CBRD x Naxos population

Anja Karine Ruud[1], Susanne Windju[1,2], Tatiana Belova[1], Timothy L. Friesen[3,4], Morten Lillemo[1]

[1] *Department of Plant Sciences, Norwegian University of Life Sciences, Post Box 5003, NO-1432 ÅS, Norway*

[2]*Graminor AS, Bjørke Gård, Hommelstadvegen 60, NO-2322 Ridabu, Norway*

[3] *Northern Crop Science Laboratory, USDA-ARS, 1307 North 18th Street, Fargo, ND 58102, USA*

[4] *Department of Plant Pathology, North Dakota State University, Walster Hall, Fargo, ND 58102, USA*

Corresponding author: Morten Lillemo, telephone: +47 67232775, e-mail: morten.lillemo@nmbu.no

Abstract

Parastagonospora nodorum is a necrotrophic pathogen of wheat, causing Septoria nodorum blotch (SNB) affecting both the leaf and glume. *P. nodorum* is the major leaf blotch pathogen on spring wheat in Norway. Resistance to the disease is quantitative, but several host-specific interactions between necrotrophic effectors (NEs) and host sensitivity (*Snn*) genes have been identified, playing a major role at the seedling stage. However, the effect of these interactions in the field under natural infection has not been investigated. In the present study, we saturated the genetic map of the recombinant inbred (RI) population SHA3/CBRD × Naxos using the Illumina 90K SNP chip. The population had previously been evaluated for segregation of SNB susceptibility in field trials. Here, we infiltrated the population with the purified NEs SnToxA, SnTox1 and SnTox3, and mapped the *Snn3* locus on 5BS based on sensitivity segregation and SNP marker data. We also conducted inoculation and culture filtrate (CF) infiltration experiments on the population with four selected *P. nodorum* isolates from Norway and North America. Re-mapping of quantitative trait loci (QTL) for field resistance showed that the SnTox3-*Snn3* interaction could explain 24 % of the phenotypic variation in the field, and more than 51 % of the variation in seedling inoculations. To our knowledge, this is the first time the effect of this interaction has been documented at the adult plant stage under natural infection in the field.

Keywords

Necrotrophic effectors, SnTox3-*Snn3*, *Parastagonospora nodorum*, plant resistance, wheat

Author contributions

AKR conducted seedling inoculation, culture filtrate infiltrations and validation of infiltration with purified effectors, analyzed the data from seedling experiments, refined linkage mapping of chromosome 5B in JoinMap, performed QTL mapping, reanalyzed the field data and wrote the manuscript.

SW analyzed and scored the SNP genotyping results in Genome Studio and performed linkage mapping in JoinMap.

TB performed linkage mapping in MSTmap and assigned linkage gruops to chromosomes based on BLASTn hits.

TF was responsible for seedling inoculations and infiltrations with isolate Sn4 and NOR4 and screening with purified SnToxA, SnTox1 and SnTox3.

ML obtained the funding, supervised the work and edited the manuscript.

Key message

The effect of the SnTox3-*Snn3* interaction was documented for the first time under natural infection at the adult plant stage in the field. Co-segregating SNP markers were identified.

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Conflict of interest

The authors declare that they have no conflict of interest.

Introduction

Parastagonospora (syn. *Septoria*, syn. ana *Stagonospora*) *nodorum* (Berk.) (Quaedvlieg et al. 2013) [teleomorph: *Phaeosphaeria* (syn. *Leptosphaeria*) *nodorum* (Müll), Hedjar.] is the causal agent of Septoria nodorum leaf and glume blotch (SNB), a disease that can cause yield losses of up to 31 % (Bhathal et al. 2003). The main hosts of *P. nodorum* are bread wheat (*T. aestivum*), durum wheat (*T. durum*) and triticale, but also other cereals and a range of wild grasses. The pathogen is common in major geographical regions where wheat is grown, including the USA, Australia and Europe (Solomon et al. 2006; Francki 2013), particularly in rainy climates, and is the major leaf blotch pathogen in Norwegian spring wheat.

QTL for flag leaf resistance have consistently been detected on chromosomes 1A, 1B, 2A, 2D, 3AS, 3B, 4A, 5A, 5B, 7A and 7B (Aguilar et al. 2005; Shankar et al. 2008; Friesen et al. 2009; Francki et al. 2011; Lu and Lillemo 2014). Most of the QTL explain less than 20 % of the phenotypic variation, as reviewed by Francki (2013).

Lately, it has been shown that host specific interactions play an important role in this pathosystem, at least at the seedling stage (Oliver and Solomon 2010). The necrotroph and the host interact in an inverse gene-for-gene manner based on necrotrophic effectors (NEs) and corresponding sensitivity loci (*Snn*) in the host (Friesen and Faris 2012). The effect of each SnTox-*Snn*-interaction is incomplete and usually additive in nature (Friesen and Faris 2010). However, epistatic interactions are also involved, affecting toxin expression, host gene action and cross talk between pathways (Friesen et al. 2008b). At least eight NE (SnToxA, SnTox1, SnTox2, SnTox3, SnTox4, SnTox5, SnTox6 and SnTox7) and nine corresponding *Snn* genes (*Tsn1*, *Snn1*, *Snn2*, *Snn3-5B*, *Snn3-5D*, *Snn4*, *Snn5*, *Snn6 and Snn7*) have been characterized (Friesen et al. 2006; Liu et al. 2006; Friesen et al. 2007; Abeysekara et al. 2009; Liu et al. 2009; Gao et al. 2015; Shi et al. 2015). *SnToxA*, *SnTox1* and *SnTox3* have been cloned into *Pichia pastoris* and the purified effectors are being used for seedling screenings (Friesen et al. 2006; Liu et al. 2009; Liu et al. 2012). In Australia, screenings with NEs has been implemented in wheat breeding programs (Tan et al. 2014). Two of the sensitivity genes have been cloned. *Tsn1* encodes a protein with N-terminal nucleotide binding site, leucine rich repeats (NBS-LRR) and a C-terminal serine/threonine protein kinase (S/TPK) (Faris et al. 2010) – representing a minor class of the classical NBS-LRR resistance genes typically conferring race specific resistance to biotrophs. The recent positional cloning of *Snn1* identified a wall-associated kinase class of receptor, which is also associated with biotrophic resistance (Shi et al. 2016b), supporting the hypothesis that the necrotrophic pathogens hi-jack biotrophic resistance pathways.

SnTox3-*Snn3* was the fourth NE-*Snn* interaction to be identified (Friesen et al. 2008a) and *SnTox3* the second necrotrophic effector from *P. nodorum* to be cloned (Liu et al. 2009). The gene encodes for a 693 bp small secreted

protein with no known homology to other proteins (Liu et al. 2009), and at least 11 haplotypes are known (McDonald et al. 2013). The SnTox3-*Snn3* interaction was first described by Friesen et al. (2008b), and the sensitivity locus mapped to the distal end of 5BS, with *cfd20* as the closest marker, but almost 30 cM from the next linked markers. In the BR34 × Grandin population the interaction explained up to 17 % of the phenotypic variation in disease after inoculation at the seedling stage. Recently, a saturated map covering the *Snn3-B1* region was also published, delineating the gene to a 1.5 cM interval (Shi et al. 2016a). At least two NB-LRR-like genes were linked to markers (*fcp652* and *fcp665, fcp666*) within this interval.

The SnTox3-*Snn3* interaction has been reported to be significant only in the presence of incompatible SnTox2-*Snn2* interaction, the SnToxA-*Tsn1* interaction is epistatic to SnTox3-*Snn3* (Friesen et al. 2008b; Cockram et al. 2015) and SnTox1 can suppress the expression of SnTox3 (Phan et al. 2016). A low, but significant negative correlation between sensitivity to SnTox3 and lower disease resistance ratings in Australian wheat cultivars has been reported (Waters et al. 2011; Francki 2013), indicating, but not confirming, that the interaction probably is significant in disease development also in the field.

Leaf infiltrations with single effectors have uncovered gene-for-gene-interactions, but the interactions are not always additive and the relative importance of each effector in a mixed natural pathogen population might change over time. Thus, it is necessary to investigate the relationships further. One study showed the significant effect of the SnToxA-*Tsn1* and SnTox2-*Snn2* interactions on adult plants in the field after inoculation with a single isolate (Friesen et al. 2009). An experimental design with naturally infected plants better explains the relationship between the natural pathogen population and the host. However, such a study is more complex and one can run the risk of not finding consistent effects across years due to fluctuations in the pathogen populations.

The damaging effect of SNB is largest in moist periods when the pathogen infects the flag and sub-ultimate leaf during grain filling (Francki 2013) and the milk stage in particular (Bhathal et al. 2003). Evaluation and genetic analysis of adult plants under field conditions are therefore of great importance, but also challenging. Considerable genotype × environment (G × E) interaction is expected, and many QTL have been detected in only one environment. To be relevant for breeders the QTL should be consistent in several environments (Francki 2013).

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, by overhead irrigation and/or inoculation with naturally infected straw gives a better estimate of host resistance under natural epidemics than inoculation of the nurseries with selected isolates.

The recombinant inbred line (RIL) population SHA3/CBRD × Naxos was previously analyzed for leaf blotch susceptibility (Lu and Lillemo 2014). Screenings with the cloned effectors showed that it most likely segregated for *Snn3*, but the sensitivity locus did not map to any linkage group, the population was monomorphic to linked markers *cfd20* and *gwm234*, and the effect of the interaction in the field could not be verified. To improve the map resolution, SHA3/CBRD × Naxos was genotyped with the Illumina iSelect 90K wheat SNP Chip (Wang et al. 2014) and QTL mapping was performed again on the field data. The population was also inoculated and infiltrated at the seedling stage with four *P. nodorum* isolates with different effector profiles (Table 1). This mapping revealed that the SnTox3-*Snn3* interaction indeed could explain a major proportion of the variation in resistance between genotypes. To our knowledge, this is the first time the effect of SnTox3 has been mapped under natural infection in the field.

The objectives of this study were to 1) perform new and more precise QTL mapping of the field data with high density SNP marker maps and 2) investigate to what degree these field QTL can be explained by seedling reactions to single isolates and infiltration with purified effectors.

Materials and methods

Plant material and foregoing field study

The development and field evaluation of Shanghai3/Catbird (SHA3/CBRD) × Naxos is described by Lu and Lillemo (2014). Briefly, it is an F_6 derived RIL population that segregates for SNB resistance in the field. The CIMMYT line SHA3/CBRD is highly resistant while the German spring wheat parent Naxos is susceptible. The main conclusion from Lu and Lillemo (2014) was that the field resistance was based on many minor effect genes. Although the population segregated for SnTox3 sensitivity, the position or any clear effect of the interaction in the field could not be mapped or verified in the study, which used a set of 564 SSR and DArT markers.

Linkage mapping

166 individuals from the SHA3/CBRD × Naxos RIL population were genotyped with the Illumina iSelect 90K wheat SNP Chip (Wang et al. 2014). Analyzing and scoring of the genotype results was performed manually for every SNP marker with the software Genome Studio Genotyping Module v1.0 from Illumina.

Markers scored as polymorphic were used for constructing linkage groups and genetic linkage maps. The markers were sorted in linkage groups with MSTmap (Wu et al. 2008). The linkage groups were assigned to chromosomes based on the best BLASTn hit from a comparison of SNP-flanking sequences with the Chinese Spring chromosome survey sequences (http://wheat-urgi.versailles.inra.fr/Seq-Repository). Previously developed SSR and DArT marker data in the population (Lu et al. 2012) were added to the SNP marker data.

Markers belonging to linkage groups assigned to the same chromosomes based on the BLASTn search were loaded into Join Map v. 4.0 (Van Ooijen 2006) and the linkage groups were refined using the maximum likelihood mapping algorithm. The genetic distances between markers were calculated by converting recombination fractions into map distances (cM) based on the Kosambi mapping function with minimum LOD score of 3.0 (Kosambi 1943).

QTL analysis

QTL analysis was performed using the software MapQTL6 (van Ooijen 2011). Multiple QTL mapping (MQM) was used, based on cofactors for major QTL initially detected with interval mapping (IM). The LOD significance threshold was set to 3.0. The software MapChart, v.2.2 was used to draw the genetic maps and LOD curves. For analysis of field resistance the confounding traits plant height, heading date and maturity were used as covariates to disease score in MapQTL6 as described by Lu and Lillemo (2014).

P. nodorum isolates: DNA extraction and screening for *SnTox* genes

Four isolates of *P. nodorum* were selected for the study (Table 1). Sn4 is a North American isolate known to produce SnToxA, SnTox1, SnTox2 and SnTox3, as described by Faris et al. (2011) and Crook et al. (2012). NOR4 was collected in Romerike, Akershus, Norway in 2011, from the spring wheat variety Zebra. Isolate 201593 was collected from the leaf blotch field trials at Vollebekk, Ås, Norway in 2014 from the Norwegian spring wheat cultivar Demonstrant (sensitive to SnTox3). Isolate 201618 was collected in Øsaker, Østfold in 2012 from the cultivar Quarna. The three Norwegian isolates were collected from leaves with visible leaf blotch symptoms, and grown on V8-PDA in 24h light (white + near ultraviolet (NUV)) to enhance sporulation before mycelial plugs were harvested with a cork borer and dried before storage at -80 ° C. For DNA extraction, the isolates NOR4, 201593 and 201618 were grown in the dark on PDA for 1-2 weeks and DNA extracted from the mycelium with the DNEasy plant kit (Qiagen). PCR screenings for *SnTox*-genes and actin were performed as described in Gao et al. (2015).

Inoculum preparation and seedling inoculation

Dried plugs of the *P. nodorum* isolates were plated on V8-PDA agar and grown for approximately one week in incubation chambers with constant light (white fluorescent + NUV) and temperature around 21 ° C until sporulation. The plates were flooded with distilled water and scraped with a sterilized inoculation loop to release pycnidiospores, and the final concentration of spores was adjusted to 1×10^6 spores/ml. One drop of Tween 20 (polyoxy-ethylene-20-sorbitan monolaureate) was added per 50 ml inoculum to reduce surface tension.

Seeds of the mapping population were planted in plastic conetainers (Stuewe and sons, Tangent, Orlando, USA), with potting mixture (peat soil with clay and sand, Gartnerjord, Tjerbo, Norway), and grown in the greenhouse

under 18° C day/15° C night temperature and 16 h light cycle until the second leaf was fully expanded – approximately 14 days after planting. Three seeds were planted per cone. The susceptible cultivar Brakar was used as a border to reduce edge effect.

The 14 days old plants were spray inoculated with a paint sprayer until runoff, placed in a mist chamber with 100 % RH for 24 h in constant light before they were returned to the greenhouse. Seven days after inoculation, the second leaf of each plant in the accessions was evaluated for disease reactions on a scale from 0 – 5 (Liu et al. 2004), where 0 is highly resistant and 5 is highly susceptible.

Infiltrations

Two seeds per RIL were planted in individual cones in racks fitting 98 cones and grown in the greenhouse under similar conditions as for the inoculation experiments. The experiments were repeated three times.

Liquid cultures of the isolates were produced in Fries 3 medium as described in Friesen and Faris (2012). After three weeks in stationary phase the cultures were filter sterilized and infiltrated into the fully expanded second leaf of 12-14 day old seedlings, using a 1 mL needleless syringe. The infiltrated areas were marked with a nontoxic felt marker. After five days the reactions were scored according to a 0-3 scale (Friesen and Faris 2012). These experiments were repeated three times with two infiltrated plants per genotype in each replicate.

Infiltration with purified SnToxA, SnTox1 and SnTox3

12-14 days old lines of the population were infiltrated with partly purified SnToxA, SnTox1 and SnTox3. Approximately 25 µL of the partly purified NE was infiltrated into the fully expanded secondary leaf using a needleless syringe. The infiltrations were done in Fargo, North Dakota in 2013 with effectors produced by *Pichia pastoris* using the pGAPzA expression vector (Liu et al. 2009), and repeated in Ås, Norway with effectors provided by Dr. Richard Oliver. SnToxA from Dr. Oliver was expressed in *Escherichia coli* BL21E using the pET21a expression vector (Tan et al. 2012), while SnTox1 and SnTox3 were produced as above. All protein preparations containing the expressed effectors were desalted (Waters et al. 2011) prior to infiltration (Liu et al. 2009).The plants were evaluated after 3 to 5 days and scored on a 0 – 3 scale (Friesen and Faris 2012).

Gene annotations

The contextual sequences of the SNP markers with the closest linkage to *Snn3* were downloaded from https://triticeaetoolbox.org/ and BLASTED at http://plants.ensembl.org/Multi/Tools/Blast and https://urgi.versailles.inra.fr/Tools/BLAST. Annotated genes were identified, and the sequences were aligned

against rice orthologues available through the rice genome annotation project http://rice.plantbiology.msu.edu/ in order to compare the results with previously reported genes in Shi et al. (2016a).

Results

Seedling inoculations and infiltrations

The frequency distribution histograms (Figure 1) show that inoculation with isolate 201593 produced more severe necrosis (reaction type 5) than inoculation with the other isolates. Correlations between the SnTox3-positive isolates were highly significant after inoculation (Pearson's correlations 0.623-0.785, P< 0.0001, table 2), while correlations between the SnTox3-negative isolate 201618 and the others were lower, but still significant. Also, the correlation between seedling inoculations and sensitivity data based on purified SnTox3 infiltration was high except for the SnTox3-negative isolate, as expected (Table 2).

Correlation between infiltration experiments with different isolates indicated that SnTox3 was the single effector produced in liquid culture by SnTox3-positive isolates causing sensitivity in the SHA3/CBRD × Naxos population (Table 3). Based on reactions on differential lines we assume that Sn4 and NOR4 also produced SnTox1 and SnTox2 and 201593 and 201618 produced SnTox2 and SnTox6 (data not shown) as well as unpublished effectors, but the population did not segregate for sensitivity to these.

Correlation between adult plant and seedling stage results

The correlation was highly significant (P<0.0001) between disease reaction scores based on single isolate inoculations with SnTox3 positive isolates NOR4, Sn4 and 201593 and field disease severities in 2010 and 2011 and for the mean over years (Table 4). The correlation was lower between these isolates and field scores for 2012 and 2013. The correlation between field scores and the North American isolate Sn4 was as significant as the Norwegian isolates except for 2012. Correlation between 20168 and field scores was only significant in 2010.

Frequency distribution and mapping of *Snn3*

The RILs segregated for SnTox3 sensitivity as either completely sensitive (reaction type 3) or insensitive (reaction type 0), with 75 insensitive to 82 sensitive, which is not significantly different from 1:1 ($\chi^2 = 0.312$, P=0.576). 11 lines (of 168) were coded as missing, due to inconsistent reactions, to avoid misclassification of the alleles. The susceptibility was inherited from parent Naxos.

The phenotypic scores for SnTox3 sensitivity were used to infer allele variants (a and b for parent SHA3/CBRD and Naxos, respectively) and the position of the sensitivity locus mapped with linkage analysis (Figure 2). The locus could not previously be mapped with SSR markers polymorphic in the population (Lu and Lillemo 2014).

Only with the improved resolution and coverage provided by the SNP markers, the locus could be mapped as Figure 2 shows. The population was insensitive to SnToxA and SnTox1.

QTL – seedling resistance

The major QTL at the *Snn3* locus on 5BS explained up to 51.8 % of the phenotypic variation when the population was inoculated with SnTox3-positive, SnTox1-negative isolate 201593, and was also highly significant after inoculation with SnTox1-positive Sn4 and NOR4 (table 2, figure 3) where suppressed expression of SnTox3 was expected according to the literature (Phan et al. 2016). The QTL on 5BS was the only significant genomic region after inoculation with isolates NOR4 and 201593 (Table 5, Figure 3). After inoculation with Sn4 a QTL on 7B was also detected, but not after infiltration. After inoculation with 201618, QTL were detected on 1A, 1B and 2D. However, all three had only moderate or minor effects and did not correspond to the adult plant QTL on 1A and 1B (Table 7, Figure S1). Interestingly, the QTL showing significance on 7B after Sn4-inoculation corresponded to the only significant QTL after infiltration with 201618 (Tables 5, 6).

QTL – adult plant resistance

Seven significant and one putative QTL for adult plant resistance to SNB were previously reported in the population, based on the field evaluations from 2010-2013 (Lu and Lillemo 2014). The major QTL was found on 3BL flanked by *wpt-4933*. However, improved map resolution and re-analysis of QTL captured a total of 11 significant QTL, with four being new (Table 7, Figure S1).

The QTL explaining most of the variation in any environment was located on the telomeric end of 5BS (table 7, figure 4), not mapped with the initial set of SSR and DaRT markers in the study by Lu and Lillemo (2014). This QTL is located at the *Snn3* locus (Figure 2) and explained as much as 24.0 and 9.0 % of the phenotypic variation in 2010 and 2011, respectively. It was also significant across years (mean), and had an effect in 2013. However, in 2012 the Snn3 region was not significant in QTL analysis. These results are also reflected by the correlations between infiltration with purified SnTox3 and field trials (Table 6), where the correlation is highly significant ($p < 0.0001$) between SnTox3-sensitivity for 2010 and across years, and significant at $p < 0.05$ in 2011, but not significant for 2012.

A novel QTL was detected on 1A in 2012 (Table 7). Higher map resolution and MQM mapping also revealed that 3A harbors at least two QTL (3AS.1 and 3AS.2), the most significant QTL in 2013. The 3AS.2 QTL was also significant in 2011 and across years (mean). The region covering 3AS.2 was not well covered in the SSR/DArT map.

The originally putative QTL on 3BS, important in 2013 (3BS.1) and 2013 (3BS.2), respectively, appear to be two distinct QTL although located approximately 8 cM apart. The QTL on 3BL was highly significant in 2011 and marker *wPt-4933* showed an effect in all years except 2012. In addition to the major QTL explained by *Snn3*, The QTL on 5B flanked by *wPt-5346* detected before, was also significant in 2013.

Gene annotations

Most of the SNPs cosegregating with *Snn3* could be matched to genes on scaffold TGACv1_scaffold_423631_5BS (Table 8). Although *Traes_6DL_388658304.1* was reported to be located on 6DL and *Traes_5AS_905D6F817.1:1* on 5AS, our mapping results as well as Wang et al. (2014) indicate that they are located on 5BS. Some of the genes share hallmarks of R-genes, i.e. coiled-coil (CC) (*Traes_5BS_C460CEDFB*), leucine rich repeats (LRR) (*Traes_5BS_E0680D15E.2.path1*) and nucleotide binding sites (NBS) (*Traes_5BS_C460CEDFB*, *Traes_5AS_905D6F817.1:1*) domains (Table 8).

Discussion

General

In this study, we mapped the *Snn3* locus (Figure 2) in the SHA3/CBRD × Naxos population and identified it as a major determinant of susceptibility to SNB both under natural field infection at the adult stage and single spore isolate inoculations of seedlings (Tables 5-7, Figures 3-4). In the previous study by Lu and Lillemo (2014) the effect of this interaction was not identified, due to lack of segregating SSR and DART markers in the chromosome area. Although the locus has been mapped in other populations, this is, to our knowledge, the first time the effect of the SnTox3-*Snn3* interaction has been detected under natural infection in the field (Table 7, Figure 4). We also identified SNP markers tightly linked to *Snn3*, some of which are located within putative NBS-LRR genes (Table 8).

Seedling QTL

The most significant interaction after seedling inoculation was SnTox3-*Snn3*, explaining as much as 51.8 % of the phenotypic variation (Table 5) and producing strong necrosis on the leaves of susceptible lines after inoculation with SnTox3-positive isolates. Prior to screening the entire population, a selection of differential lines from SHA3/CBRD × Naxos, segregating for single field resistance QTL, were screened with several locally collected isolates to test for differential segregation (data not shown). However, very few isolates produced higher reaction scores than 2.5 on the lines unless they were also SnTox3-positive. One exception was isolate 201618 which was selected to possibly capture different QTL than the one explained by *Snn3*. QTL on 1A, 1B and 2D

were detected after inoculation with 201618 (Table 5, Figure 3). The QTL on 1A overlaps partly with the QTL on 1A detected in 2012 (Table 7), but the resistance source was opposite. The QTL on 1B also seems to be specific to this particular isolate. After infiltration, a QTL on 7B corresponding to the QTL detected after inoculation with Sn4 was discovered, indicating a putative new NE/*Snn* interaction that will be investigated in further studies.

Of the three major interactions SnToxA/*Tsn1*, SnTox1/*Snn1* and SnTox3-*Snn3*, SHA3/CBRD × Naxos only segregated for *Snn3*. The limited number of genes segregating in a two-parent cross is a limitation to the range of the results and several important interactions may not be detected due to monomorphism in the population. On the other hand, it also allows better investigation of interactions that may be statistically undetectable in the presence of other genes and epistatic interactions.

It has been suggested that presence of SnTox1 suppresses SnTox3 production (Phan et al. 2016). We found that the SnTox3-*Snn3* interaction was highly significant in all relevant inoculation experiments, and that infiltration with CF with SnTox3 positive isolates produced the same necrotic symptoms regardless of SnTox1-prescence. However, the frequency of RIL with reaction type 5 was much higher after inoculation with the SnTox1-negative isolate 201593 (Figure 1).

Effect of *Snn3* in the field

Saturation of the genetic map with the 90 K SNP chip showed that *Snn3* can explain up to 24 % of the phenotypic variation in the field (Table 7, Figure 4: 2010). The results favor the hypothesis that host-specific interactions also play a role in adult plant susceptibility to *P. nodorum* leaf blotch. It also serves as a confirmation that the multiple regression approach where confounding traits (plant height, heading date and maturity) are included as covariates, works well. However, the SnTox3-*Snn3* interaction was only significant in two out of four years of field trials – illustrating the complexity of the disease. One definition of a robust QTL is that it is significant in two or more environments (Francki 2013). Under this definition, selection against lines carrying *Snn3* would be recommended based on our findings.

Since the field experiments depended on natural infection, the results capture a more realistic picture of the situation in farmers' fields rather than after artificial inoculation with single isolates. Nevertheless, very few QTL studies rely on natural inoculum, where one takes a higher risk of large variability between environments.

Mapping of other QTL for field resistance

The fine-mapping improved the coverage of the chromosomes, and led to the discovery of a significant novel QTL for field resistance on 3A (3A.2, Table 7, Figure S1). Lu and Lillemo (2014) reported that MQM or CIM mapping did not improve the results for the field resistance QTL. However, with the new maps, we found that the significance and precision increased with MQM mapping for several field QTL (1B, 3A, 3BL, 5B (Table 7, Figure 4, Figure S1), although different cofactors were used for different years. In 2012 the use of cofactors did not improve the results. Improved coverage of the chromosomes also revealed that some QTL are probably linked and that different underlying genes may be involved in different years, for instance the two on 3BS (Table 7, Figure S1). The novel QTL detected on 1A (Table 7) was below significance threshold when mapped on the original SSR and DArT map.

Although the effect of SnTox3-*Snn3* was highly significant in 2010 and in 2011, the variation between years shown both in correlation coefficients and relative importance of individual QTL, also emphasizes the need to screen the plants in multiple environments and/or locations as discussed by Francki (2013), before selecting genotypes or markers for marker assisted selection (MAS). The variation illustrates the complexity of the trait and diversity of the natural pathogen population. For some QTL the % explained variation was lower with the new maps.

Correlation field – seedling trials

A main objective of this study was to investigate the correlation between seedling and adult plant resistance to SNB. Based on the Pearson correlation coefficients between field years and single isolates (Table 6) the correlation seems to be highest between SnTox3-producing isolates and years where *Snn3* was significant (2010, 2011 and mean). However, correlation was also significant between the SnTox3-negative isolate 201618 and the field scores in 2010, indicating that other infection mechanisms or effectors may also play a role. Interestingly, the correlation between this isolate and field resistance was negligible for all other years. Although the correlation between 201593 and 2013 was significant ($p < 0.0001$), no significant QTL were shared between the field and seedling resistance. In other words, correlation alone is a fairly rough mean to compare experiments compared to genetic analysis. Interestingly, the correlation between the North American isolate and the field trials conducted in Norway was as high as for Norwegian isolates, illustrating the global relevance of the disease and host resistance mechanisms.

Genetic mapping of *Snn3*

The markers linked to *Snn3* mapped to the telomeric end of 5BS, about 30 cM from the nearest markers in SHA3/CBRD × Naxos (Figure 2, Figure 4). In the consensus map (Wang et al. 2014) several markers that clustered in this distal group were not assigned to any chromosome, or mapped to different chromosomes (like *Kukri_c6784_718*, assigned to 6DL) in the different populations used to build the consensus map. The high recombination frequency in this region challenges the mapping algorithms and we want to underline the importance of including unassigned and unmapped markers in the analysis (i.e. association mapping or linkage maps) before filtering.

We did not observe recombination between *Snn3* and the markers *BS00091518_51*, *BS00091519_51*, *BobWhite_c4838_58*, *Excalibur_c47452_183* or *GENE-3324_338* in the RIL lines. However, A small number of missing data points contributed to the minor distances between the markers in the map (Figures 2- 4).

Gene annotations

The SNP markers *BS00091518_51* and *BS00091519_51* are located 20 bp apart from each other in an exon of a P-loop containing nucleoside triphosphate hydrolases superfamily protein (Table 8, *Traes_5BS_C460CEDFB*, https://triticeaetoolbox.org/jbrowse). The P-loop is a common motif in NTP-binding proteins including NBS-LRRs (Marone et al. 2013). *Excalibur_ c47452_183* is located within a gene (*Traes_5BS_E0680D15E.2.path1*) expressing a protein with leucine-rich repeats (LRR, Table 8), also a feature of the classical R-genes. The genes in which *Excalibur_ c47452_183* and *BobWhite_c4838_58* are located, corresponded to rice orthologue *Os12g44000* (http://rice.plantbiology.msu.edu/) (Table 8). This rice gene was also reported by Shi et al. (2016a). Indeed, the sequence for marker *XTC266536* (Table 1) in Shi et al. (2016a) corresponded to the same gene, *TRIAE_CS42_5BS_TGACv14236631_AA1380950.1*, as *Excalibur_c47452_183* and *BobWhite_c4838_58*. Interestingly, this gene has been annotated both as an NBS-LRR (PTHR23155) and ubiquitin-conjugating enzyme.

In the case of *BobWhite_c4838_58* the rice orthologue is identified as *Os06g30380.1* by the International Rice Sequencing Project (IRGSP) (http://rgp.dna.affrc.go.jp/IRGSP/) , which corresponds to the gene in which SNPs *BS00091518_51*, *BS00091519_51* and possibly *GENE-3324_338* are located (Table 8). We speculate whether the orthologues in reality correspond to different motifs in the same gene, allelic or splice variants or if more than one gene belonging to the same gene family are clustered within the scaffold.

The markers *Excalibur_c47452_183*, *Kukri_c6784_718*, *BobWhite_c4838_58* and *GENE-3324_338* also co-segregates with the loose smut resistance gene *UtBW278*, conferring resistance to *Ustilago tritici* race T9 (Kassa et al. 2015). Since the *Snn*-genes confer dominant susceptibility and the NE-*Snn*-interactions are described as hi-jacking traditional R-genes to biotrophs, it has been speculated that they may counteract with these. However, SnTox3-resistant cultivars like BR34 are also resistant to T9 (Kassa et al. 2015), while T9-susceptible lines like Sumai3 and Grandin also carry *Snn3*. Clustering of NBS-LRR genes after duplications and the following evolution through local rearrangements and gene conversions is common, as is the irregular distribution of the gene family across chromosomes (Marone et al. 2013). Screening of SnTox3-sensitivity in a wide association mapping panel of spring wheat (MASbasis) revealed that the markers are not diagnostic or that there may be more than one sensitivity locus present (data not shown). Hence, it is likely that several NBS-LRR-like genes, including *UtBW278*, *Traes_5BS_C460CEDFB* and *Traes_5BS_E0680D15E.2.path1* are clustered within scaffold TGACv1_scaffold_423631_5BS, and further work is needed to identify *Snn3*, potential splice variants, allelic variants and other genes within its proximity.

References

Abeysekara N, Friesen T, Keller B, Faris J (2009) Identification and characterization of a novel host–toxin interaction in the wheat–Stagonospora nodorum pathosystem. Theoret Appl Genetics 120:117-126

Aguilar V, Stamp P, Winzeler M, Winzeler H, Schachermayr G, Keller B, Zanetti S, Messmer MM (2005) Inheritance of field resistance to Stagonospora nodorum leaf and glume blotch and correlations with other morphological traits in hexaploid wheat (Triticum aestivum L.). Theoret Appl Genetics 111:325-336

Bhathal J, Loughman R, Speijers J (2003) Yield reduction in wheat in relation to leaf disease from yellow (Tan) spot and septoria nodorum blotch. Eur J Plant Pathol 109:435-443

Cockram J, Scuderi A, Barber T, Furuki E, Gardner KA, Gosman N, Kowalczyk R, Phan HP, Rose GA, Tan K-C, Oliver RP, Mackay IJ (2015) Fine-Mapping the Wheat Snn1 Locus Conferring Sensitivity to the Parastagonospora nodorum Necrotrophic Effector SnTox1 Using an Eight Founder Multiparent Advanced Generation Inter-Cross Population. G3: Genes|Genomes|Genetics 5:2257-2266

Cowger C, Murphy JP (2007) Artificial Inoculation of Wheat for Selecting Resistance to Stagonospora Nodorum Blotch. Plant Disease 91:539-545

Crook AD, Friesen TL, Liu ZH, Ojiambo PS, Cowger C (2012) Novel necrotrophic effectors from Stagonospora nodorum and corresponding host sensitivities in winter wheat germplasm in the southeastern United States. Phytopathology 102:498-505

Faris JD, Zhang Z, Lu H, Lu S, Reddy L, Cloutier S, Fellers JP, Meinhardt SW, Rasmussen JB, Xu SS, Oliver RP, Simons KJ, Friesen TL (2010) A unique wheat disease resistance-like gene governs effector-

triggered susceptibility to necrotrophic pathogens. Proceedings of the National Academy of Sciences 107:13544-13549

Faris JD, Zhang Z, Rasmussen JB, Friesen TL (2011) Variable expression of the Stagonospora nodorum effector SnToxA among isolates is correlated with levels of disease in wheat. Molecular plant-microbe interactions : MPMI 24:1419-1426

Francki MG (2013) Improving Stagonospora nodorum Resistance in Wheat: A Review. Crop Sci 53:355-365

Francki MG, Shankar M, Walker E, Loughman R, Golzar H, Ohm H (2011) New Quantitative Trait Loci in Wheat for Flag Leaf Resistance to Stagonospora nodorum Blotch. PHYTOPATHOLOGY 101:1278-1284

Fraser DE, Murphy JP, Leath S, Van Sanford DA (2003) Effect of Inoculation with Selected Isolates of Stagonospora nodorum on Field Evaluations of Host Resistance in Winter Wheat. Plant Disease 87:1213-1220

Friesen T, Chu CG, Liu ZH, Xu SS, Halley S, Faris JD (2009) Host-selective toxins produced by Stagonospora nodorum confer disease susceptibility in adult wheat plants under field conditions. Theoret Appl Genetics 118:1489-1497

Friesen T, Faris J (2012) Characterization of Plant-Fungal Interactions Involving Necrotrophic Effector-Producing Plant Pathogens. In: Bolton MD, Thomma BPHJ (eds) Plant Fungal Pathogens. Humana Press, pp 191-207

Friesen TL, Faris JD (2010) Characterization of the wheat-Stagonospora nodorum disease system: what is the molecular basis of this quantitative necrotrophic disease interaction? Can J Plant Pathol-Rev Can Phytopathol 32:20-28

Friesen TL, Faris JD, Solomon PS, Oliver RP (2008a) Host-specific toxins: effectors of necrotrophic pathogenicity. Cellular microbiology 10:1421-1428

Friesen TL, Meinhardt SW, Faris JD (2007) The Stagonospora nodorum-wheat pathosystem involves multiple proteinaceous host-selective toxins and corresponding host sensitivity genes that interact in an inverse gene-for-gene manner. The Plant journal : for cell and molecular biology 51:681-692

Friesen TL, Stukenbrock EH, Liu Z, Meinhardt S, Ling H, Faris JD, Rasmussen JB, Solomon PS, McDonald BA, Oliver RP (2006) Emergence of a new disease as a result of interspecific virulence gene transfer. Nat Genet 38:953-956

Friesen TL, Zhang Z, Solomon PS, Oliver RP, Faris JD (2008b) Characterization of the Interaction of a Novel Stagonospora nodorum Host-Selective Toxin with a Wheat Susceptibility Gene. Plant Physiology 146:682-693

Gao Y, Faris JD, Liu Z, Kim YM, Syme RA, Oliver RP, Xu SS, Friesen TL (2015) Identification and Characterization of the SnTox6-Snn6 Interaction in the Parastagonospora nodorum-Wheat Pathosystem. Molecular plant-microbe interactions : MPMI 28:615-625

Kassa MT, Menzies JG, McCartney CA (2015) Mapping of a resistance gene to loose smut (Ustilago tritici) from the Canadian wheat breeding line BW278. Molecular Breeding 35:1-8

Kersey PJ, Allen JE, Armean I, Boddu S, Bolt BJ, Carvalho-Silva D, Christensen M, Davis P, Falin LJ, Grabmueller C, Humphrey J, Kerhornou A, Khobova J, Aranganathan NK, Langridge N, Lowy E, McDowall MD, Maheswari U, Nuhn M, Ong CK, Overduin B, Paulini M, Pedro H, Perry E, Spudich G, Tapanari E, Walts B, Williams G, Tello–Ruiz M, Stein J, Wei S, Ware D, Bolser DM, Howe KL, Kulesha E, Lawson D, Maslen G, Staines DM (2016) Ensembl Genomes 2016: more genomes, more complexity. Nucleic Acids Research 44:D574-D580

Kosambi D (1943) The estimation of map distances from recombination values. Annals of Eugenics 12:172-175

Liu Z, Faris JD, Oliver RP, Tan KC, Solomon PS, McDonald MC, McDonald BA, Nunez A, Lu S, Rasmussen JB, Friesen TL (2009) SnTox3 acts in effector triggered susceptibility to induce disease on wheat carrying the Snn3 gene. PLoS pathogens 5:e1000581

Liu Z, Friesen TL, Ling H, Meinhardt SW, Oliver RP, Rasmussen JB, Faris JD (2006) The Tsn1-ToxA interaction in the wheat-Stagonospora nodorum pathosystem parallels that of the wheat-tan spot system. Genome 49:1265-1273

Liu Z, Zhang Z, Faris JD, Oliver RP, Syme R, McDonald MC, McDonald BA, Solomon PS, Lu S, Shelver WL, Xu S, Friesen TL (2012) The Cysteine Rich Necrotrophic Effector SnTox1 Produced by Stagonospora nodorum Triggers Susceptibility of Wheat Lines Harboring Snn1. PLoS pathogens 8:e1002467

Liu ZH, Friesen TL, Rasmussen JB, Ali S, Meinhardt SW, Faris JD (2004) Quantitative Trait Loci Analysis and Mapping of Seedling Resistance to Stagonospora nodorum Leaf Blotch in Wheat. Phytopathology 94:1061-1067

Lu Q, Bjornstad A, Ren Y, Asad MA, Xia X, Chen X, Ji F, Shi J, Lillemo M (2012) Partial resistance to powdery mildew in German spring wheat 'Naxos' is based on multiple genes with stable effects in diverse environments. Theor Appl Genet 125:297-309

Lu Q, Lillemo M (2014) Molecular mapping of adult plant resistance to Parastagonospora nodorum leaf blotch in bread wheat lines 'Shanghai-3/Catbird' and 'Naxos'. Theoret Appl Genetics 127:2635-2644

Marone D, Russo MA, Laidò G, De Leonardis AM, Mastrangelo AM (2013) Plant Nucleotide Binding Site–Leucine-Rich Repeat (NBS-LRR) Genes: Active Guardians in Host Defense Responses. International Journal of Molecular Sciences 14:7302-7326

Mayer KFX, Rogers J, Doležel J, Pozniak C, Eversole K, Feuillet C, Gill B, Friebe B, Lukaszewski AJ, Sourdille P, Endo TR, Kubaláková M, Číhalíková J, Dubská Z, Vrána J, Šperková R, Šimková H, Febrer M, Clissold L, McLay K, Singh K, Chhuneja P, Singh NK, Khurana J, Akhunov E, Choulet F, Alberti A, Barbe V, Wincker P, Kanamori H, Kobayashi F, Itoh T, Matsumoto T, Sakai H, Tanaka T, Wu J, Ogihara Y, Handa H, Maclachlan PR, Sharpe A, Klassen D, Edwards D, Batley J, Olsen O-A, Sandve SR, Lien S, Steuernagel B, Wulff B, Caccamo M, Ayling S, Ramirez-Gonzalez RH, Clavijo BJ, Wright J, Pfeifer M, Spannagl M, Martis MM, Mascher M, Chapman J, Poland JA, Scholz U, Barry K, Waugh R, Rokhsar DS, Muehlbauer GJ, Stein N, Gundlach H, Zytnicki M, Jamilloux V, Quesneville H, Wicker T, Faccioli P, Colaiacovo M, Stanca AM, Budak H, Cattivelli L, Glover N, Pingault L, Paux E, Sharma S, Appels R, Bellgard M, Chapman B, Nussbaumer T, Bader KC, Rimbert H, Wang S, Knox R, Kilian A, Alaux M, Alfama F, Couderc L, Guilhot N, Viseux C, Loaec M, Keller B, Praud S (2014) A chromosome-based

draft sequence of the hexaploid bread wheat (Triticum aestivum) genome. Science (New York, NY) 345:1251788

McDonald MC, Oliver RP, Friesen TL, Brunner PC, McDonald BA (2013) Global diversity and distribution of three necrotrophic effectors in Phaeosphaeria nodorum and related species. The New phytologist 199:241-251

Mi H, Poudel S, Muruganujan A, Casagrande JT, Thomas PD (2016) PANTHER version 10: expanded protein families and functions, and analysis tools. Nucleic Acids Res 44:D336-342

Oliver RP, Solomon PS (2010) New developments in pathogenicity and virulence of necrotrophs. Current Opinion in Plant Biology 13:415-419

Phan HT, Rybak K, Furuki E, Breen S, Solomon PS, Oliver RP, Tan KC (2016) Differential effector gene expression underpins epistasis in a plant fungal disease. Plant J 87:343-354

Quaedvlieg W, Verkley GJ, Shin HD, Barreto RW, Alfenas AC, Swart WJ, Groenewald JZ, Crous PW (2013) Sizing up Septoria. Studies in mycology 75:307-390

Shankar M, Walker E, Golzar H, Loughman R, Wilson RE, Francki MG (2008) Quantitative Trait Loci for Seedling and Adult Plant Resistance to Stagonospora nodorum in Wheat. Phytopathology 98:886-893

Shi G, Friesen TL, Saini J, Xu SS, Rasmussen JB, Faris JD (2015) The Wheat Gene Confers Susceptibility on Recognition of the Necrotrophic Effector SnTox7. The Plant Genome 8:1-10

Shi G, Zhang Z, Friesen TL, Bansal U, Cloutier S, Wicker T, Rasmussen JB, Faris JD (2016a) Marker development, saturation mapping, and high-resolution mapping of the Septoria nodorum blotch susceptibility gene Snn3-B1 in wheat. Molecular genetics and genomics : MGG 291:107-119

Shi G, Zhang Z, Friesen TL, Raats D, Fahima T, Brueggeman RS, Lu S, Trick HN, Liu Z, Chao W, Frenkel Z, Xu SS, Rasmussen JB, Faris JD (2016b) The hijacking of a receptor kinase–driven pathway by a wheat fungal pathogen leads to disease. Science Advances 2:e1600822

Solomon PS, Lowe RGT, Tan K-C, Waters ODC, Oliver RP (2006) Stagonospora nodorum: cause of stagonospora nodorum blotch of wheat. Molecular Plant Pathology 7:147-156

Tan K-C, Waters ODC, Rybak K, Antoni E, Furuki E, Oliver RP (2014) Sensitivity to three Parastagonospora nodorum necrotrophic effectors in current Australian wheat cultivars and the presence of further fungal effectors. Crop and Pasture Science 65:150-158

Tan KC, Ferguson-Hunt M, Rybak K, Waters OD, Stanley WA, Bond CS, Stukenbrock EH, Friesen TL, Faris JD, McDonald BA, Oliver RP (2012) Quantitative variation in effector activity of ToxA isoforms from Stagonospora nodorum and Pyrenophora tritici-repentis. Molecular plant-microbe interactions : MPMI 25:515-522

Van Ooijen J (2006) JoinMap 4. Software for the calculation of genetic linkage maps in experimental populations Kyazma BV, Wageningen, Netherlands

van Ooijen J (2011) MapQTL 6: software for the mapping of quantitative trait loci in experimental populations of diploid species. Wageningen, The Netherlands.

Voorrips RE (2002) MapChart: Software for the Graphical Presentation of Linkage Maps and QTLs. Journal of Heredity 93:77-78

Wang S, Wong D, Forrest K, Allen A, Chao S, Huang BE, Maccaferri M, Salvi S, Milner SG, Cattivelli L, Mastrangelo AM, Whan A, Stephen S, Barker G, Wieseke R, Plieske J, International Wheat Genome Sequencing C, Lillemo M, Mather D, Appels R, Dolferus R, Brown-Guedira G, Korol A, Akhunova AR, Feuillet C, Salse J, Morgante M, Pozniak C, Luo M-C, Dvorak J, Morell M, Dubcovsky J, Ganal M, Tuberosa R, Lawley C, Mikoulitch I, Cavanagh C, Edwards KJ, Hayden M, Akhunov E (2014) Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. Plant Biotechnology Journal 12:787-796

Waters OD, Lichtenzveig J, Rybak K, Friesen TL, Oliver RP (2011) Prevalence and importance of sensitivity to the Stagonospora nodorum necrotrophic effector SnTox3 in current Western Australian wheat cultivars. Crop and Pasture Science 62:556-562

Wu Y, Bhat PR, Close TJ, Lonardi S (2008) Efficient and accurate construction of genetic linkage maps from the minimum spanning tree of a graph. PLoS genetics 4:e1000212

Figure captions

Fig.1 Frequency distributions of disease reaction types for the SHA3/CBRD × Naxos RIL, after seedling inoculations. Parental phenotypes are indicated by arrows.

Fig.2 Left: Mapping of the *Snn3* locus on chromosome 5BS in SHA3/CBRD × Naxos based on segregation of SnTox3-sensitivity. Right: Region of 5BS in the Wang et al. (2014) consensus map covered by polymorphic SNPs in SHA3/CBRD × Naxos. Common markers are indicated in green. The maps are drawn in Mapchart v. 2.2 (Voorrips 2002).

Fig.3 From top: QTL detected on 1A, 1B and 2D after inoculation with 201618. QTL on 5B after inoculation with NOR4, Sn4 and 201593. QTL on 7B after detected after infiltration with 201618 and inoculation with Sn4. Genetic distances are shown in centimorgans to the left of the chromosomes. A threshold of 3.0 is indicated by a dashed vertical line in the LOD graphs. The maps are drawn in Mapchart v.2.2 (Voorrips 2002).

Fig.4 Linkage group 5B with LOD curves for the major QTL for field susceptibility to SNB at the *Snn3* locus detected in the field trials at Vollebekk, Ås, Norway in 2010, 2011 and across years (mean). Genetic distances are shown in centimorgans to the left of the chromosomes. A threshold of 3.0 is indicated by a dashed vertical line in the LOD graphs. The maps are drawn in Mapchart v.2.2 (Voorrips 2002).

Fig.S1 Chromosomes with significant QTL for field resistance to SNB, with corresponding LOD curves. LOD threshold of 3.0 is indicated by the dashed vertical lines on the graphs. Marker names in green indicate SSR or DArT markers from the earlier version of the map.

Tables

Table 1 List of isolates included in the study, with SnTox-profile (presence/absence based on PCR) and disease range and mean in the RIL population.

Isolate	Presence (+) or absence (-) of SnToxA, SnTox1 and SnTox3, respectively	Disease range in the RILs	Population mean reaction
Sn4	+ + +	0.17-3.83	2.23
NOR4	+ + +	0.00 – 4.00	2.13
201593	- - +	0.00 – 5.00	3.37
201618	- - -	0.00 – 4.80	2.7

Table 2 Pearson correlation coefficients between single isolate inoculations at the seedling stage and correlation with reaction to purified SnTox3

	NOR4	201593	Sn4	SnTox3
201618	0.260**	0.300***	0.325***	0.062
Sn4	0.785***	0.623***		0.559***
201593	0.670***			0.741***
NOR4				0.626***

*** < 0.0001, ** < 0.001, * < 0.01

Table 3 Pearson correlation coefficients between sensitivity scores after single isolate culture filtrate (CF) infiltration and correlation between CF reactions and reactions to purified SnTox3 infiltration

	NOR4	201593	Sn4	SnTox3
201618	0.012	-0.097	-0.002	-0.07
Sn4	0.924***	0.863***		0.912***
201593	0.890 ***			0.952***
NOR4				0.935***

*** < 0.0001, ** < 0.001, * < 0.01

Table 4 Pearson correlation coefficients between corrected leaf blotch severities in the field trials (years, 2010-2013 and mean) and disease reactions after seedling inoculations with single isolates, and infiltration with purified SnTox3

Year	Inoculation with single spore isolates				
	NOR4	Sn4	201593	201618	SnTox3
2010	0.486***	0.519***	0.615***	0.335***	0.486***
2011	0.344***	0.360***	0.291***	0.092	0.222**
2012	0.262**	0.182	0.243*	0.036	0.080
2013	0.235*	0.264**	0.334***	0.161	0.205**
mean	0.387***	0.366***	0.432***	0.154	0.262**

*** < 0.0001, ** < 0.001, * < 0.01

Table 5 Significant QTL (LOD > 3.0) for seedling resistance to SNB in inoculation experiments with single isolates, after MQM mapping. % phenotypic variance (PEV) explained for significant QTL is listed .

		Isolate				
Chromosome	markers (cofactors)	Sn4	NOR4	201593	201618	R-source
1A	*RAC875_c10083_800*				**11.7**	Naxos
1B	*psp3000*				**10.4**	SHA3/CBRD
2D	*wsnp_RFL_Contig3960_4401914*				**11.1**	Naxos
5B (*Snn3*)	*BS00091518_51*	**27.5**	**35.4**	**51.8**		SHA3/CBRD
7B	*wsnp_BE498662B_Ta_2_5*	**15.5**				Naxos

Table 6 Marker correlations after infiltration with culture filtrate from single isolates. The % phenotypic variance (R^2 values) is listed for the significant interactions.

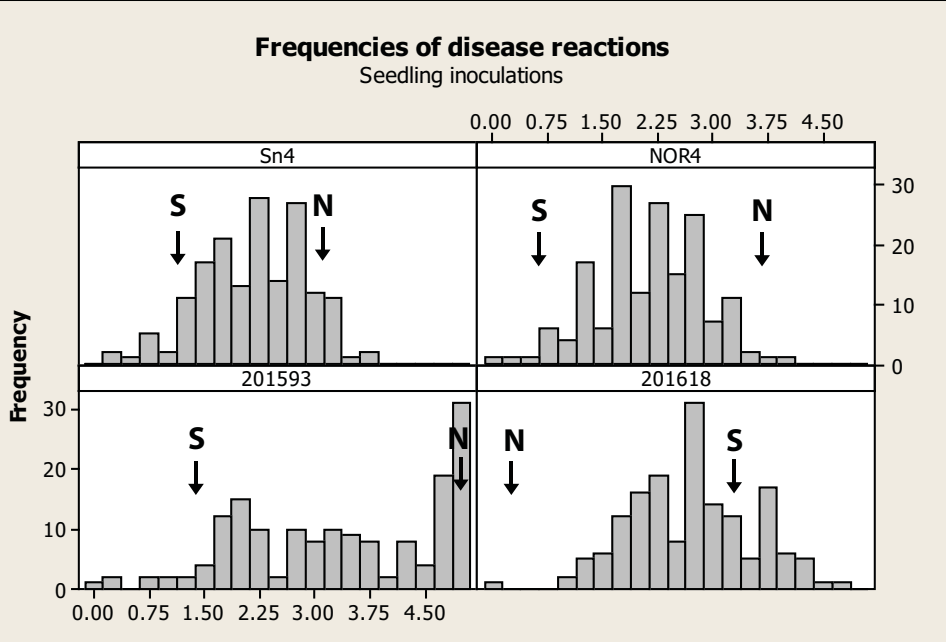
		Isolate				
Chromosome	markers	Sn4	NOR4	201593	201618	R-source
5BS	*BS00091518_51*	**82.7**	**87.2**	**73.4**		SHA3/CBRD
7B	*wsnp_BE498662B_Ta_2_5*				**32.6**	Naxos

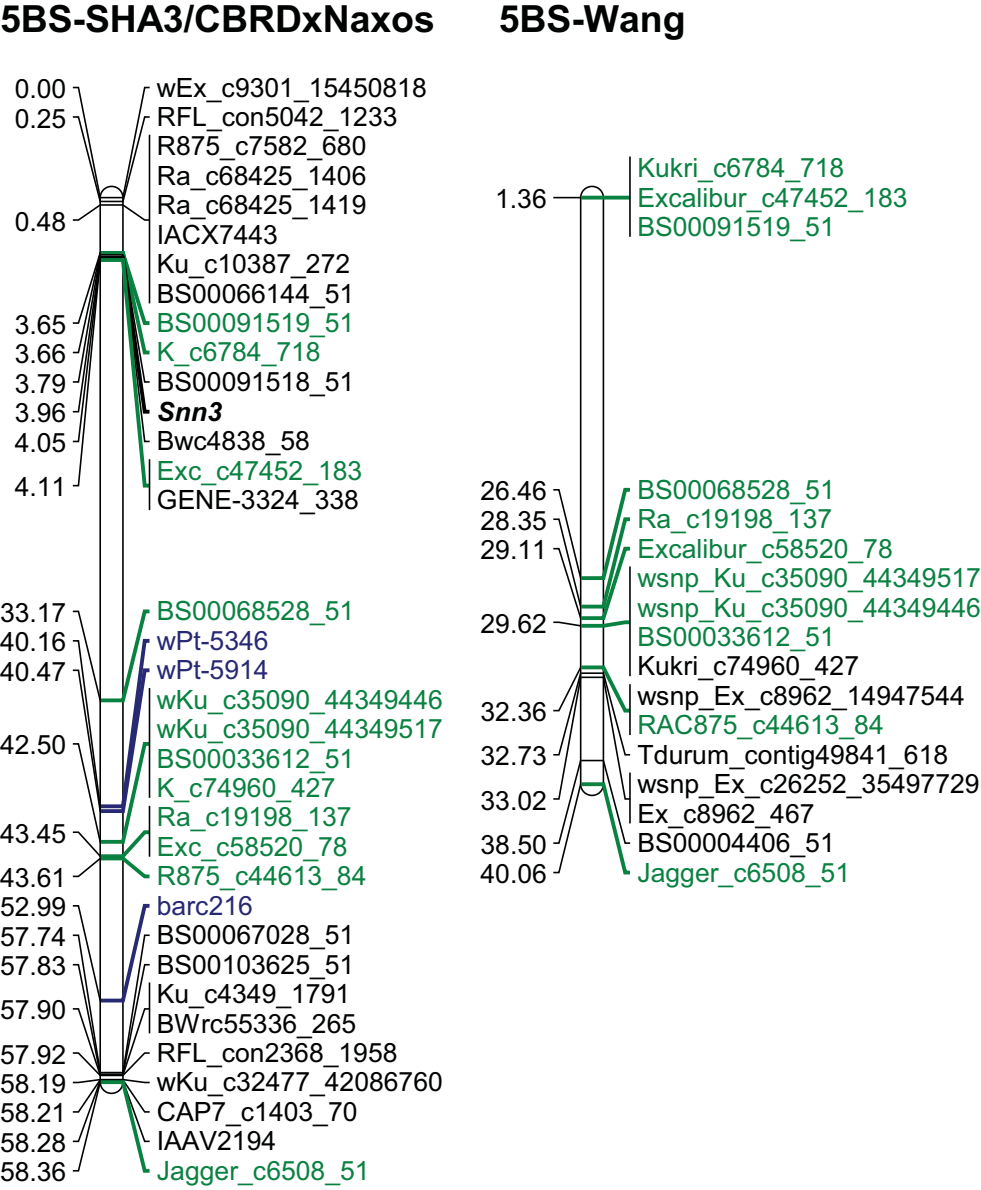
Table 7 List of significant QTL with close markers based on four years and the mean of field scorings at Vollebekk, Norway. The % explained phenotypic variation (R^2) is listed if above the LOD threshold of 3 in at least one environment. QTL detected above the LOD threshold in the corresponding environment are indicated in bold. The phenotypic data is identical to the dataset used for the analysis published by Lu and Lillemo (2014)

Chr.	Markers	2010	2011	2012	2013	mean	R-source
1A	*wsnp_Ex_c25734_34995416*		2.4	**10.3**		3.0	SHA3/CBRD
1B.1RS	*SCM9*		5.2		**8.1**	**7.7**	Naxos
3AS.1	*gwm2*				**11.5**		Naxos
	IAAV6676	6.5				3.7	
3AS.2	*Ku_c41007_116,*		**6.6**			2.2	SHA3/CBRD
	Excalibur_c52446_519				**9.4**		
3BS.1	*BS00030534_51*				**5.7**		SHA3/CBRD
3BS.2	*wBE445348B_Ta_2_1*	**6.9**					
3BL	*wPt-4933*	4.6	**11.2**		3.5	3.9	Naxos
5BS	*BS00091518_51*	**24.0**	**9.0**		4.7	**9.9**	SHA3/CBRD
5B.2	*wPt-5914*	4.8	3.4		**5.6**	2.4	SHA3/CBRD
7A	*RAC875_c14195_1155*	2.9	4.1	3.4	**6.5**	**6.2**	Naxos
7B	*BobWhite_rep_c50229_413*			**8.4**		2.7	Naxos

Table 8 List of SNPs tightly linked to *Snn3* in the SHA3/CBRD × Naxos population, and gene annotations based on the draft genome sequence (Mayer et al. 2014) unless otherwise noted (In hexaploid wheat within scaffold TGACv1_scaffold_423631_5BS).

SNP marker	NCBI *Triticum aestivum* gene	Rice orthologue	Function	Reference
BS00091519_51	*Traes_5BS_C460CEDFB*	*Os06g30380.1*	P-loop containing nucleoside triphosphate hydrolases superfamily protein GTP-binding domain GTPase	http://plants.ensembl.org/ (Kersey et al. 2016) http://www.uniprot.org/uniprot/Q656A4
Excalibur_c47452_183	*Traes_5BS_E0680D15E.2.path1* *TRIAE_CS42_5BS_TGACv14236631_AA1380950.1*	*Os12g44000*	Ubiquitin-conjugating enzyme 15-like Panther: Leucine-rich repeat-containing protein (PTHR23155) (Traes_5BS_E0680D15E.2.path1)	http://plants.ensembl.org/ (Kersey et al. 2016) https://urgi.versailles.inra.fr http://www.uniprot.org/ http://www.pantherdb.org/ (Mi et al. 2016)
Kukri_c6784_718	*Traes_6DL_388658304.1*	*Os05g05354*	Trypsin-like cysteine/serine peptidase domain superfamily	http://plants.ensembl.org/ (Kersey et al. 2016)
BS00091518_51	*Traes_5BS_C460CEDFB*	*Os06g30380.1*	P-loop containing nucleoside triphosphate hydrolases superfamily protein	http://plants.ensembl.org/ (Kersey et al. 2016)
BobWhite_c4838_58	100% BLAST match to *Traes_5BS_C460CEDFB*	*Os12g44000 (MSU)* *Os06g30380.1 (IRGSP)*	Coiled-coil superfamily (based on *Arabidopsis thaliana* match)	http://plants.ensembl.org/ (Kersey et al. 2016) http://rice.plantbiology.msu.edu/ http://rgp.dna.affrc.go.jp/IRGSP/
GENE-3324_338	*Traes_5AS_905D6F817.1:1*	*Os06g30380.1*	Non-translating coding sequence (CDS) GTP binding domain P-loop NTPase	https://urgi.versailles.inra.fr/ http://www.uniprot.org/


Frequencies of disease reactions
Seedling inoculations
Sn4
NOR4
201593
201618
S
N
Frequency
0.00 0.75 1.50 2.25 3.00 3.75 4.50
0 10 20 30


5BS-SHA3/CBRDxNaxos
0.00
0.25
0.48
3.65
3.66
3.79
3.96
4.05
4.11
33.17
40.16
40.47
42.50
43.45
43.61
52.99
57.74
57.83
57.90
57.92
58.19
58.21
58.28
58.36
wEx_c9301_15450818
RFL_con5042_1233
R875_c7582_680
Ra_c68425_1406
Ra_c68425_1419
IACX7443
Ku_c10387_272
BS00066144_51
BS00091519_51
K_c6784_718
BS00091518_51
Snn3
Bwc4838_58
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GENE-3324_338
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Ra_c19198_137
Exc_c58520_78
R875_c44613_84
barc216
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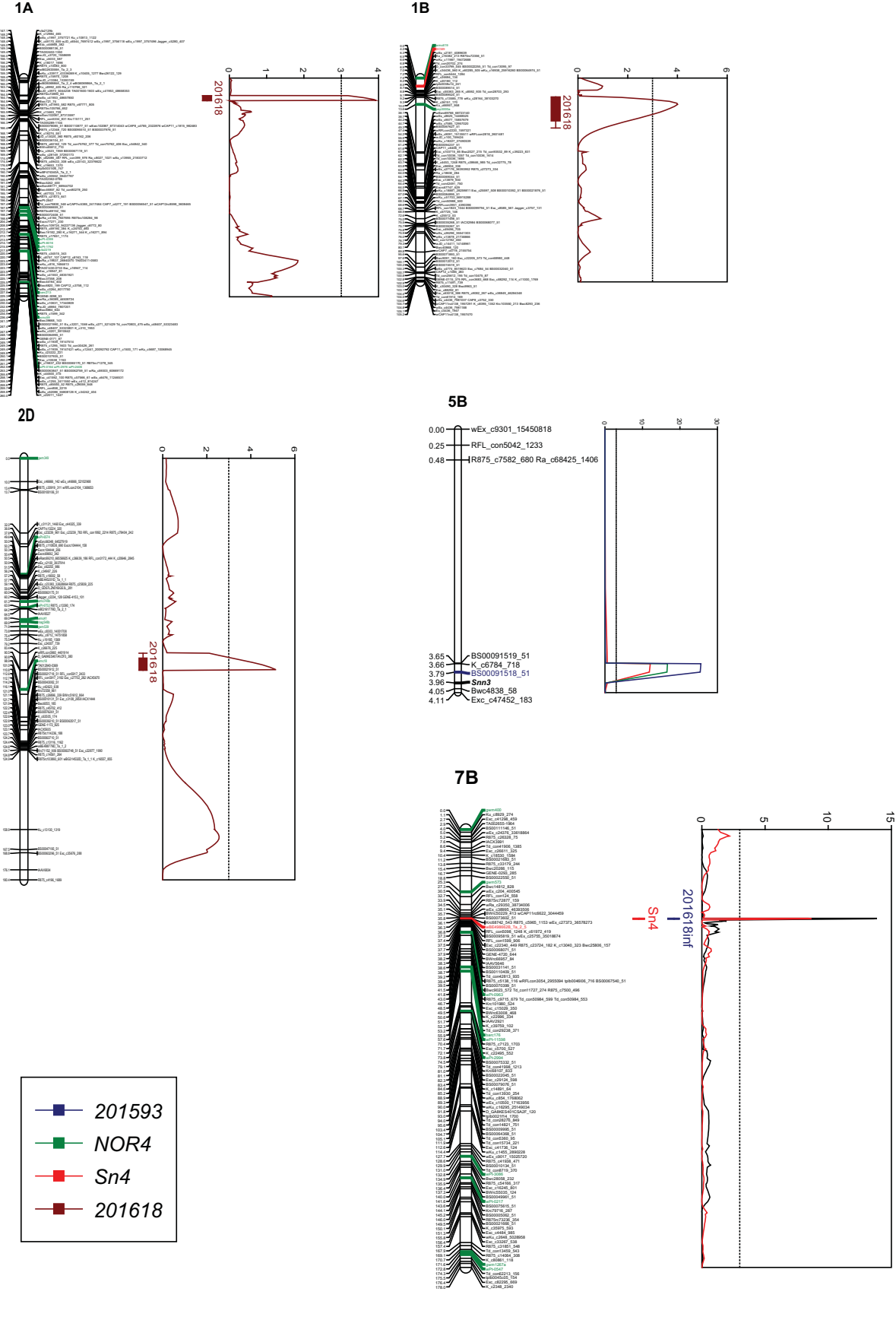
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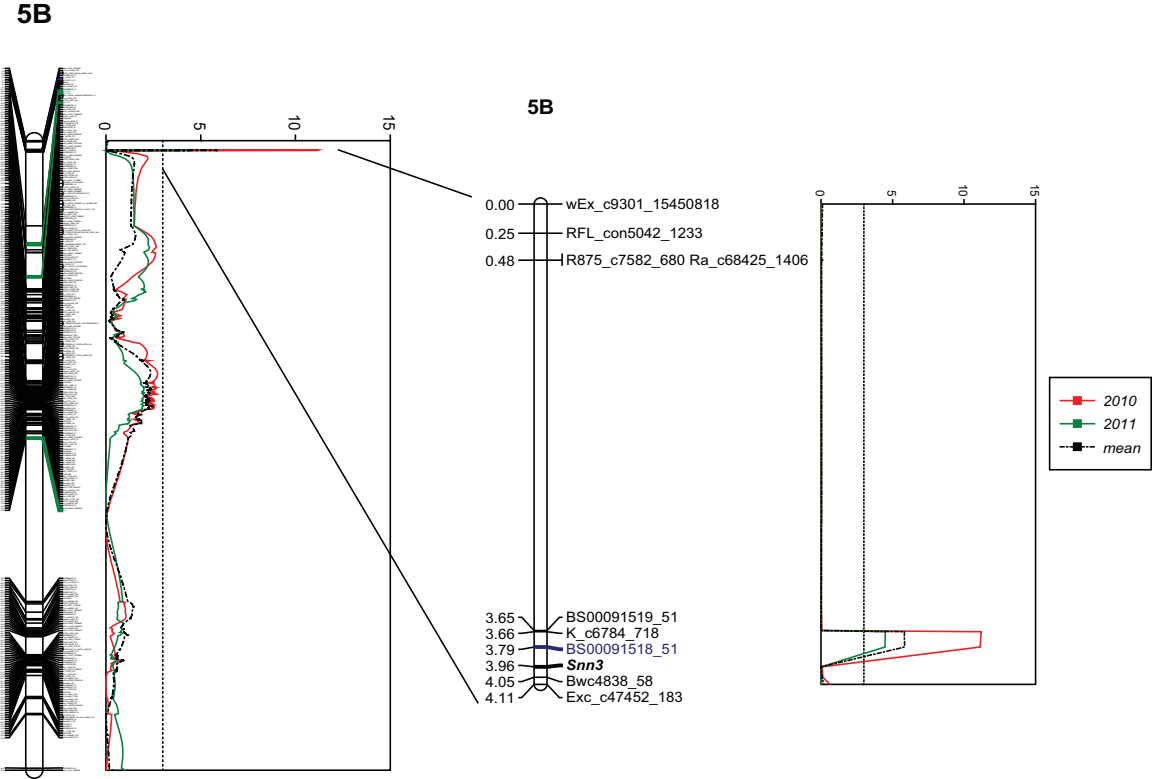
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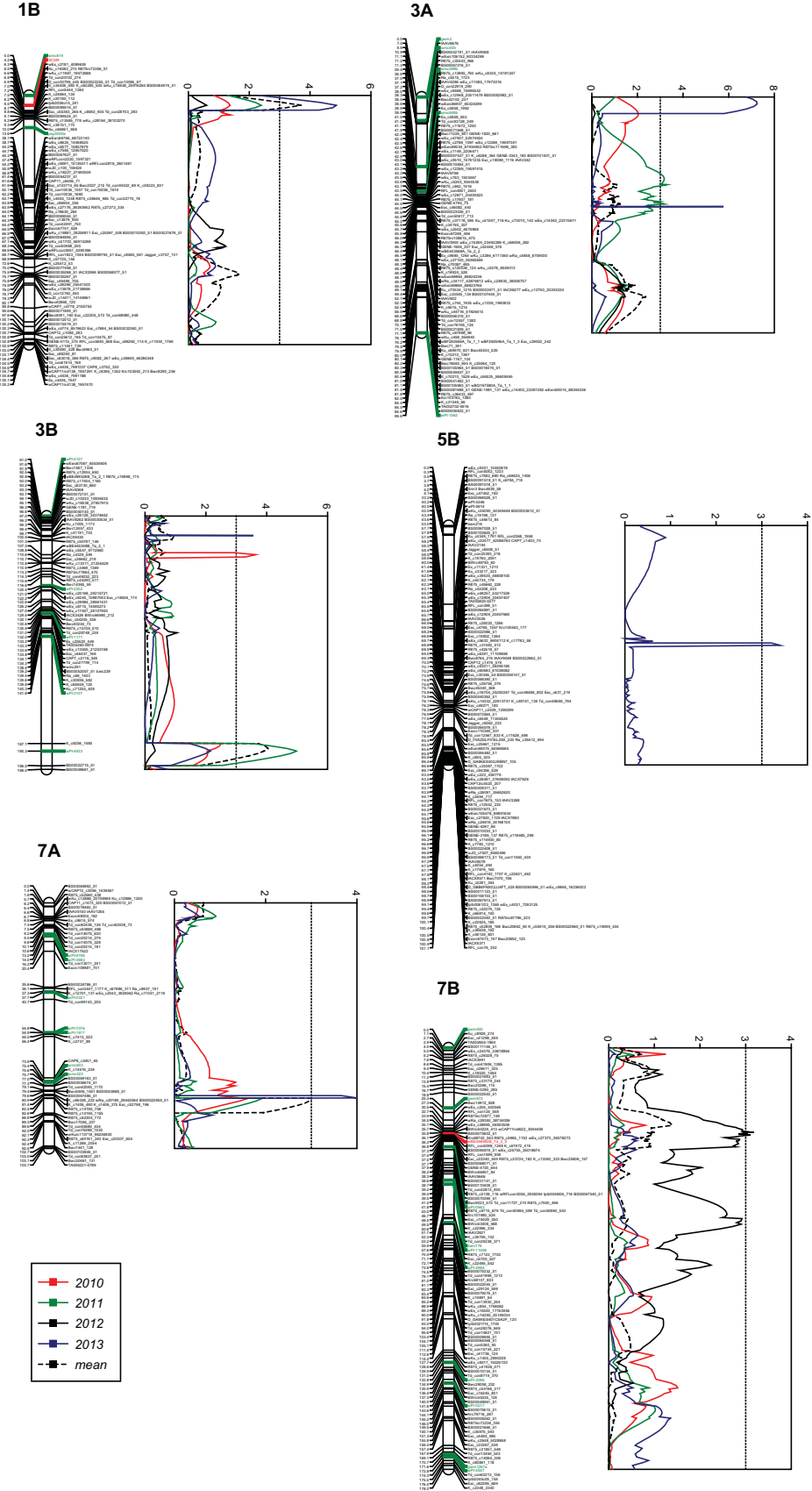
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