Genome wide association mapping for resistance to multiple fungal pathogens in a panel issued from a broad composite cross-population of tetraploid wheat Triticum turgidum

Elsa Ballini, Muriel Tavaud, Aurélie Ducasse, Etienne Paux, Jonathan Kitt, Gilles Charmet, Delphine Audigeos, Pierre P. Roumet, Jacques David, Jean-Benoit Morel

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BALLINI[1] Elsa, TAVAUD[1] Muriel, DUCASSE Aurélie, SANCHEZ Dimitri, PAUX Etienne, KITT Jonathan, CHARMET Gilles, AUDIGEOS Delphine, ROUMET Pierre, DAVID[2] Jacques, MOREL[2] Jean-Benoit

1 Both first authors contributed equally to this publication., 2 both last authors contributed equally to the design

The affiliation(s), e-mail address, ORCID of the author(s):

BALLINI Elsa: elsa.ballini@supagro.fr; 0000-0002-2571-2069, BGPI, Univ Montpellier, CIRAD, INRAE, Institut Agro, Montpellier, France

TAVAUD Muriel: muriel.tavaud@supagro.fr; 0000-0001-9738-620X, AGAP, Univ Montpellier, CIRAD, INRAE, Institut Agro, Montpellier, France

DUCASSE Aurélie: aurelie.ducasse@inra.fr; BGPI, Univ Montpellier, CIRAD, INRAE, Institut Agro, Montpellier, France

SANCHEZ Dimitri : dimitri.sanchez@supagro.fr; AGAP, Univ Montpellier, CIRAD, INRAE, Institut Agro, Montpellier, France

PAUX Etienne ; etienne.paux@inra.fr, 0000-0002-3094-7129, GDEC, Université Clermont Auvergne, INRAE, Genetics Diversity & Ecophysiology of Cereals, 63000 Clermont-Ferrand, France

KITT Jonathan ; jonathan.kitt@inra.fr, 0000-0002-3817-4538, GDEC, Université Clermont Auvergne, INRAE, Genetics Diversity & Ecophysiology of Cereals, 63000 Clermont-Ferrand, France

CHARMET Gilles : gilles.charmet@inra.fr, 0000-0002-6618-1292, GDEC, Université Clermont Auvergne, INRAE, Genetics Diversity & Ecophysiology of Cereals, 63000 Clermont-Ferrand, France

AUDIGEOS Delphine : D.AUDIGEOS@arvalis.fr; Arvalis, Station expérimentale, Boigneville, France

ROUMET Pierre : pierre.roumet@inra.fr; 0000-0003-1054-2156, AGAP, Univ Montpellier, CIRAD, INRAE, Institut Agro, Montpellier, France

DAVID Jacques : jacques.david@supagro.fr; 0000-0002-2925-2258, AGAP, Univ Montpellier, CIRAD, INRAE, Institut Agro, Montpellier, France

MOREL Jean-Benoit: jean-benoit.morel@cirad.fr; 0000-0003-1988-956X, BGPI, Univ Montpellier, CIRAD, INRAE, Institut Agro, Montpellier, France

Abstract

Few resistance genes providing defence against the major fungal diseases Septoria tritici blotch (STB), Septoria nodorum blotch (SNB), Leaf rust (LR), and an emerging Wheat blast (WB) disease have been identified in durum wheat. We identified sixteen fungal disease-associated QTL through genome-wide association mapping of 180 inbred lines sampled from a durum wheat Composite Cross-population. Two STB resistance-associated QTL mapped to chromosome 3A, one of which colocalizes with *Stb6*, a known resistance gene previously identified in bread wheat. This partial resistance could be conferred by a new allele of *Stb6* or another paralogous gene. The second locus is associated with a reduction in pycnidia density, a recently identified and poorly understood form

of resistance. A resistance QTL strongly associated with LR, and colocalizing with *Lr61*, was observed in a 3.24 Mbp region on chromosome 6B. QTL mapping of LR resistance following treatment by chitin used in the context of inducer treatment was also investigated. Using a combination of resistance alleles at these loci could confer durable resistance to multiple fungal diseases and aid durum wheat breeders in their fight against these fungal pathogens.

Keywords

Triticum turgidum, wheat blast, leaf rust, septoria tritici blotch, septoria nodorum blotch, resistance inducers, GWAS, chitin

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Introduction

Durum wheat (*Triticum turgidum* subsp *durum*) is an important cereal in Mediterranean diets, mainly used for semolina and pasta. Because it is relatively well-adapted to arid climates, more than 50% of world production is in the Mediterranean region (Royo and Di Fonzo 2005). Fungal diseases of durum wheat cause significant crop loss, and the use of fungicides to control infection can have harmful effects on the environment and farmers (Figueroa et al. 2018). In France, the main fungal diseases infecting durum wheat are septoria (Fones and Gurr 2015) and leaf rust (Goyeau et al. 2012). European durum wheat is also facing a new fungal threat from the emergence of wheat blast (Ceresini et al. 2018). The use of varieties harboring particular resistance genes can be an effective means of managing fungal disease threats. However, it is often not sustainable (Priyamvada et al. 2011) as fungal pathogens evolve rapidly to overcome host resistance (McDonald and Linde 2002). The development of more effective and sustainable use of resistant varieties requires a wider range of resistance genes, and markers associated with allelic forms of these genes, for exploitation in breeding programs (Royo and Di Fonzo 2005). Genetic mapping of resistance genes to these diseases has mostly been done in the hexaploid bread wheat (*Triticum aestivum*) because of its greater economic interest. However, fungal diseases of wheat are often host specific, meaning that results obtained in studies of bread wheat are often not applicable to durum wheat (Kema et al. 1996; Goyeau et al. 2006). Durum wheat breeders, therefore, lack the specific markers of resistance alleles allowing for better-designed breeding programs to combat these diseases (Ghaffary et al. 2018).

Disease etiology of septoria can be confusing because two different pathogens cause similar necrotic symptoms and can co-infect the same plant: Septoria tritici blotch (STB) caused by *Zymoseptoria tritici* and Septoria nodorum blotch (SNB) caused by *Parastagonospora nodorum*. There are an estimated €1.7 bn in economic losses annually due to septoria diseases on durum and bread wheat in Europe (Fones and Gurr 2015). Although STB

appears to have gradually replaced SNB in France, SNB remains an important threat for durum wheat (Bearchell et al. 2005).

At least 21 STB resistance genes have been identified in bread wheat as well as 89 Meta-QTL (Brown et al. 2015). However, most likely due to host specificity, very few of these markers are useful in durum wheat breeding programs (Ghaffary et al. 2018). Recently, the bread wheat resistance gene *Stb6*, which codes for a wall-associated receptor kinase (WAK), and mapped to chromosome 3A (Saintenac et al. 2018), was shown to be locked in a gene-for-gene coevolutionary interaction with the associated *Z. tritici* avirulence gene *AvrStb6* (Zhong et al. 2017). Resistance to the necrosis phenotype caused by STB has been a focus of research. However, resistance may independently arise from reducing pathogen reproduction, as measured by a reduction in the number of fungal fruiting bodies called pycnidia (Karisto et al. 2017; Fones et al. 2015).

Both *Z. tritici* and *P. nodorum* produce toxins during infection, partly explaining necrotic symptoms and host susceptibility, with the absence or modification of proteins targeted by the toxins conferring resistance. Toxins can also be neutralized by detoxification mechanisms. However, few loci associated with toxin resistance have been identified in durum wheat (Virdi et al. 2016). In the case of SNB, nine toxin/host pairs have been identified (Francki 2013; Haugrud et al. 2019). The wheat gene *Snn1*, coding for a WAK on chromosome 1B, has recently been cloned (Shi et al. 2016) and the associated *S. nodorum* effector toxin *Tox1* isolated (Liu et al. 2012). The absence of *Snn1* reduces necrosis and strengthens resistance. Thus, toxin infiltration, instead of classical spore inoculation, is an efficient way of identifying resistance to SNB in wheat (Shi et al. 2016).

Another important disease of durum wheat is leaf rust (LR), caused by the biotroph *Puccinia triticina*. While durum wheat appears more resistant to leaf rust than bread wheat, aggressive strains of leaf rust have recently appeared in Europe that are detrimental to durum wheat production (Goyeau et al. 2006). Moreover, none of the 100 representative French elite lines are resistant to the leaf rust recently collected in a durum wheat production area in France (personal data). Leaf rusts infecting durum wheat are genetically different from those infecting bread wheat (Goyeau et al., 2006), and only twelve of the 68 genes associated with LR resistance in bread wheat appear important in durum wheat infections (Aktar-Uz-Zaman et al. 2017; Aoun et al. 2016). Thus, it is important to identify durum wheat resistance genes and QTL specifically associated with durum wheat LR isolates for wheat improvement (Ordonez and Kolmer 2007).

Wheat blast (WB) is an emerging hemibiotrophic disease caused by the *Magnaporthe oryzae Triticum* (MoT) pathotype (*Anamorph Pyricularia oryzae Triticum*) (Ceresini et al. 2019). The disease emerged in Brazil in 1985 and has recently spread to Bangladesh (Ceresini et al. 2019). Fungicides are relatively ineffective against WB, and the nine resistance genes that have been identified lack fine-mapping with markers easily exploitable by breeders (Anh et al. 2018; Krattinger et al. 2016; Tagle et al. 2015). Thus, these four fungal diseases are emerging as major threats to durum wheat production, they present different types of trophic development, and further genetic information is required to develop durable resistance management in the field (Figueroa et al. 2018).

Induction of plant defenses, for instance, through the use of chitin, is an alternative or complementary technique used to protect plants from some fungal diseases, including rust (Bohland et al. 1997; Popova et al. 2018; Vander et al. 1998). Indeed such induction increase basal immunity (Vander et al. 1998; Naz et al. 2014), and a positive effect of prophylactic induction in bread wheat has been reported (Cruz et al. 2011). However, to our knowledge, no study on wheat has examined genetic variation in resistance following prophylactic induction treatment.

In recent years with the full sequence availability of the wild emmer (*T. turgidum* subsp *dicoccoides* Thell.) and

durum wheat genomes (Appels et al. 2018; Avni et al. 2017; Maccaferri et al. 2019; Ling et al. 2018), there is a shift towards genomics-assisted breeding. Genome-wide association studies (GWAS) have already been used to map resistance to these four diseases in bread wheat (Crossa et al. 2007; Gurung et al. 2014; Jighly et al. 2016; Kertho et al. 2015; Tommasini et al. 2007; Neumann et al. 2011; Gao et al. 2016; Adhikari et al. 2011; Juliana et al. 2018; Muqaddasi et al. 2019). However, fewer studies of the genetic architecture of disease resistance in tetraploid durum wheat have been carried out (Letta et al. 2014; Liu et al. 2017; Maccaferri et al. 2010; Kidane et al. 2017; Aoun et al. 2016). While elite populations have often been used, the severe genetic bottleneck occurring during domestication (Haudry et al. 2007) may have left these populations with fewer resistance alleles than wild and primitive accessions. Composite cross-populations have been proposed as an effective alternative to remobilize diversity by crossing a large number of parents and allowing recombination (Enjalbert et al. 2011). Thus, the EPO durum wheat lines, derived from an Evolutionary Pre-breeding pOpulation, has the advantage of being highly diversified and weakly structured, making it an ideal panel for GWAS (David et al. 2014).

In this study, we used the EPO panel in a series of GWAS with the goal of identifying resistance loci, potentially rare or absent in modern domesticated populations, to three main fungal pathogens of durum wheat (STB, SNB, and LR) as well as to emerging WB disease. Moreover, associations with resistance to LR was evaluated both with and without prophylactic induction by chitin, an inducer of immunity, in order to evaluate the impact of this treatment on resistance.

Material and Methods

Plant Panels

The Evolutionary Pre-breeding pOpulation (EPO) is a genetically diverse, outcrossed tetraploid wheat population founded in 1997. It has since been cultivated with an outcrossing rate of 10%, artificially managed by maintaining sterile male plants in the population (David et al. 2014). In brief, genetic diversity in this population arises from the first generation artificial crossing of accessions from several subspecies of *Trititum turgidum* (subspecies *dicoccoides, dicoccum, turgidum, polonicum, carthlicum,* and *durum*). In 2009, a set of 180 lines was derived from the EPO and fixed by five generations of single seed descent. These lines are a subset of 425 original lines, and were chosen to eliminate genetic redundancy arising from sister lines derived from the same lineage.

A durum wheat collection of seventy-eight commercial lines provided by French private companies and genotyped on the 420 K Affymetrix Axiom genotyping array, TaBW280K (Rimbert et al. 2018), was screened for allelic diversity at two of the detected QTL (STB_Nec3A and STB_Pyc3A). Out of them, fourteen lines were selected for phenotyping based on their genotypes at these QTL.

All experiments were carried out in a glasshouse under 16 hours of light at 24°C/20°C, as described by Ballini et al. (2013). All wheat plants were grown in Neuhaus S soil with the addition of poudzolane (5L/70L soil) and slow-releasing fertilizer (Flocoat 90g/70L soil). Each pot was sown with six seeds of individual lines to ensure the growth of at least four seedlings.

Molecular Data

The EPO lines were genotyped using a 420 K Affymetrix Axiom genotyping array, TaBW280K, developed for the BreedWheat project (Rimbert et al. 2018). We used 168,725 high-resolution, publicly available reliable SNPs.

The genomic position for each SNP was identified by BLAST onto both the wild emmer (accession Zavitan) genome sequence (v2.0) and the Svevo sequence (v1.0)(Maccaferri et al. 2019; Zhu et al. 2019). Missing data, occurring at a low rate of 3.84%, were imputed by binomial sampling of alleles using allele frequencies as parameters.

The *Stb6* locus was genotyped using two previously published primer pairs and PCR conditions: 8311-16 (forward GCGACATGGTAGCTCAATCAAA; reverse TTCCTTCCATGGTCGGTAACTT) and 13609 (forward CTGAAAAAAAATACGAGGCCATGA: reverse GGCTTGTCTCCTCCGATCTTGT) (Saintenac et al. 2018). *AvrStb6* was sequence based on the available sequence MG018996 using following primers designed for this project (TCCACCTGTCGCCGAATC and CTAGGAAAGTGGGCGCAAAA).

Fungal Inoculations and Symptoms

The 180 lines from the EPO population were phenotyped for resistance to the different pathogens following the same adapted experimental design. For each genotype, six seeds were sown per pot, and each plant that grew was phenotyped. According to limited space in the glasshouse, we maximised the number of independent lines to increase our chance to detect QTLs (Korte and Farlow 2013). Nevertheless, to control for potential spatial variability in the greenhouse and to get an estimate of the error variance (evaluation of the broad sense heritability and computation of BLUPs), we chose to apply a p-rep design (Cullis et al. 2006) instead of repeating control checks. Thus, 20% of the genotypes were grown twice and these replicates distributed at random in the different sublocks (pots ware assemble in 16 pots trays). Pots were randomly divided and arranged in the greenhouse. All inoculations or infiltrations were conducted at seedling stage on 2-weeks old plants.

SNB resistance was evaluated using two complementary phenotyping methods: infiltration with Tox1 toxin and spore inoculation with an isolate not producing Tox1. The TOX1 toxin and the SNB fungal isolate were both obtained from Bioger (UMR INRA-AgroParisTech, Thiverval-Grignon, France). The SNB isolate SE15017 was collected by Arvalis from durum wheat in 2015 in Gomel (France). The WB isolates BR43 and BR32 were selected from the CIRAD strain collection (Cirad, Montpellier, France) and cultured and harvested as previously described (Ballini et al. 2013). STB and LR fungal strains were isolated in 2015 in Montpellier (France) by INRA-BGPI from durum wheat Pescadou (STB P1a and LR AD2015) and Miradoux (STB M1a). LR isolate AD2015 was genotyped (H. Goyeau, INRA Bioger) and identified as a haplotype currently circulating in France (data not shown).

The *Tox1* assay was conducted as previously described (Liu et al. 2004). Briefly, a 1-ml needleless syringe was used for infiltration on the last ligulated leaf. The boundaries of the infiltrated area were marked on the leaf with a nontoxic pen. Seven days after infiltration, disease reactions were scored on a scale from 1 to 4: 1 - a plant without any symptoms, 2 - a plant with some chlorosis, and 3 and 4 for different levels of necrosis. SNB inoculation with SE15017 involved dipping a paintbrush into a leaf spore suspension, made by adding one drop of Tween 20 per 10 mL of inoculum containing 10^6 conidial spores, and applying it to the last ligulated leaf with six strokes of the brush. Symptom severity was evaluated seven days after inoculation on a 0 to 5 scale as described previously, where 0 is highly resistant (Liu et al. 2004).

For STB inoculations with P1a or M1a, an inoculum of 10^6 spores per ml was prepared and applied using a paintbrush as described for SNB. We scored STB symptoms three weeks after inoculation. For each leaf, total necrotic leaf areas were automatically recorded from a scanned image using the image analysis program LeafTool,

available on GitHub (https://github.com/sravel/LeAFtool). Total necrotic areas with pycnidia were then manually recorded. From these measurements, we calculated two phenotypes used for QTL analysis: host damage (% of necrotic leaf area) and pathogen reproduction (density of pycnidia per unit area of lesion).

Wheat blast resistance was screened using two different fungal isolates from Brazil: BR43 and BR32. The isolates were cultured as in Berruyer *et al.* (2003). To establish infection, an inoculum solution of 50,000 spores/ml was prepared and sprayed onto the plants. A week after inoculation, disease symptoms were scored on a scale of 1 to 4: 1 - a plant without any symptoms, 2 - a plant with some chlorosis, and 3 and 4 for different levels of sporulating lesions on the leaf surface.

LR inoculation with strain AD2015 was conducted by spray with a solution containing: gelatin 0.5%, one drop of Tween 20 per 10 mL, and 8mg of spores per 150 plants. To test for the effect of prophylactic induction, four hours prior to inoculation plants were either mock-treated (gelatin 0.5%) or treated with chitin (100 µg/mL and gelatin 0.5%) following a previously established protocol (Cruz et al., 2011). The quantitative level of susceptibility was recorded ten days after inoculation using image analysis to evaluate the percentage of leaf surface with pustules using the image analysis program LeafTools.

Statistical Analysis

All inoculations were done on at least three independent plants and the mean values used as the susceptibility score for each line. To take into account potential variation arising from environmental differences within the glasshouse, we used two-dimensional P-spline mixed models implemented in the SpATS R package (Velazco et al. 2017).. Broad sense heritabilities based on the 1.2 repetitions per line were estimated using the SpATS model. We then used the best linear unbiased predictions (BLUPs) as genotypic values to perform the GWAS.

Genome-wide associations (GWA) were performed using GEMMA software (Zhou and Stephens 2012). QQ-plots were used to determine the most appropriate correcting method for each analysed phenotype. As the EPO panel is weakly structured, the association model including only a kinship matrix has been used for all the traits. We filtered SNPs for a minor allele frequency (MAF) of 5%. Kinship coefficients were calculated on R, using the Identity by State equation 2.3 (Astle and Balding 2009). Manhattan plots were made on R (qqman package), using SNP locations derived from the BLAST on the Zavitan wild emmer genome. The proportion of variance explained (PVE) by each QTL was calculated, following the GEMMA author's recommendations (Zhou 2017), as the ratio between variance on beta for the SNP considered, and the phenotypic variance for the trait analysed. We identified significant QTL using a threshold of 10e-5. The confidence interval was evaluated by keeping all SNP with a threshold above 10e-2 before and after significant SNP.

For gene annotation, the reference genome Zavitan v2.0 was used on GrainGenes database (https://wheat.pw.usda.gov/GG3/genome_browser) (Zhu et al. 2019). To search for colocalization with previously mapped genes, published markers were BLASTed onto the Zavitan v2.0 genome sequence.

Results and discussion

Mapping the SNB resistance gene Snn1 validates the usefulness of EPO lines for disease resistance studies

Resistance to Tox1 and to plant damage after inoculation by spores in the absence of Tox1 were evaluated

independently. Indeed, the presence of Tox1 can sometimes hide the effect of other toxins due to epistatic interactions (Phan et al. 2016). These two phenotypes were highly heritable (0.81 and respectively 0.53) and we identified four QTL (Fig1, Table 1, and online resource 1).

Two loci were associated with resistance to Tox1. The SNB_Tox1B locus maps to a 1,8 Mbp region at the beginning of chromosome 1B. The resistance allele is relatively frequent in the EPO lines (33%). The SNB_Tox1B peak (log(Pval) = 9,2) is located 66 kb from *Snn1*, a known resistance gene in bread wheat (Liu et al. 2012) (Fig2). It is thus very likely that SNB_Tox1B corresponds to Snn1 (Shi et al. 2016). This result validates using the EPO lines in a GWAS to identify resistance genes with strong phenotypic effects and alleles segregating at somewhat balanced frequencies. Its also further demonstrate that only replicating 20% of the lines can produce relevant mapping information. Another QTL for Tox1 resistance, SNB_Tox7A, was also identified in a 1,9 Mbp region on chromosome 7A. This region contains nine candidate genes and in particular, a cluster of five WAKs (online resource 3), suggesting that Tox1, which is known to target another WAK (Shi et al. 2016), may broadly target this gene family.

In the second assay using spore inoculation, two QTLs were identified: SNB_Nec4B and SNB_Nec7B (Fig1, Table1), that were associated with resistance to an SNB isolate that does not produce Tox1. SNB_Nec4B is located in a 1,8 Mbp region on chromosome 4B containing fifteen candidate genes, including two receptor kinases (online resource 3). A QTL was previously mapped in the same region when screening for resistance to the toxin Tox3 in the absence of Tox1 (Phan et al. 2016).

Identification of Wheat Blast resistance QTL

Resistance to each of the fungal isolates BR43 and BR42 was highly heritable (0.81 and 0.68 respectively) and we identified five QTL (Fig1, Table1, and online resource 1). Two QTL associated with resistance to BR43, WB_BR43_2A, and WB_BR43_2B, mapped closely to regions on chromosome 2 containing *Rmg7* and *Rmg8*, previously identified as resistance genes in bread wheat (Anh et al. 2018). The 450 kbp region of WB_BR43-2A co-localizes with the 2 Mbp locus of *Rmg7* (Fig3). If the two loci are allelic, this study suggests that finer mapping of this resistance gene is possible. Two chitinases, involved in biotic defense and present in this genomic region, could also be good candidates for WB43 resistance (online resource 3).

The QTL WB_BR32_7A maps to a 17 Mbp region on the same chromosome as *Rmg2*, which has not been finely mapped (Zhan et al. 2008). The SNP with the strongest association with BR32 resistance is located 75 kbp away from a Nod-like receptor (NLR) gene (TRIDC7AG077360). NLRs are known to confer resistance to rice blast (Saitoh et al. 2016), and TRIDC7AG077360 is thus a good candidate for WB_BR32_7A.

Identification of STB resistance QTL

EPO lines were screened for the resistance to the STB isolate P1a. Two resistance phenotypes were scored (online resource 4): 1) resistance to primary infection, evaluated as the percentage of leaf surface with necrosis (STB_Nec), and 2) resistance to septoria reproduction, evaluated as the percentage of necrotic surface with pycnidia (STB_Pyc). The heritability of these two traits was high (0.69 and 0.68, respectively). We found two QTL associated with resistance to primary infection. STB_Nec5A mapped to a 4,7 Mbp region on chromosome

5A containing 51 candidate genes, among which six code for receptor kinases and three for pathogenesis-related proteins (online resource 3). STB_Nec3A maps to a 327 kbp region on chromosome 3A containing a cluster of six WAK genes, one of which is homologous to the known resistance gene *Stb6* (online resource 3; see below). We found one QTL associated with pycnidia density. Notably, the QTL STB_Pycn3A mapped to a 326 kbp region on chromosome 3 containing nine candidate genes. Among these candidates is a wheat homolog of the rice negative regulator of defense NRR (Chern et al. 2005) and a tryptophan synthase orthologous to Chinese spring TraesCS3A01G041800 (Fig6 and online resource 3). A reduction in pycnidia density could be due to a higher basal defense level (Ors et al. 2018), and in this case, the NRR gene located in this region would be an excellent candidate. Tryptophan synthase is also a good candidate since this enzyme is involved in the biosynthesis of serotonin, a key player for defense in rice and wheat (Ishihara et al. 2015; Du Fall and Solomon 2013; Erland et al. 2016). Reductions in pycnidia may be linked to restricted access to nutrients (Zhan et al. 2005; Bancal et al. 2016). However, annotations of the nine genes in the region do not suggest a role in nutrient access.
The QTL STB_Nec3 and STB_Pycn3A are only 3,3 Mbp on chromosome 3 (Fig1, Table1, and online resource 2). We did not find a single EPO line in the panel carrying resistance alleles at all three of these loci (online resource 5A). Indeed, all the lines containing the STB_Pyc3A resistance allele developed extensive necrosis (Fig4), demonstrating that these two QTL are linked but distinct.

A recent GWAS in bread wheat reported a resistance QTL on chromosome 2 associated with STB reproduction (Yates et al. 2019), suggesting that this type of indirect resistance through controlling pathogen reproduction is common in wheat. In order to reduce the candidate gene number and to find recombinant lines at this locus, it could be worthwhile to come back to the still recombining EPO population. Due to the outcrossing rate in the population, the range of linkage disequilibrium in the 28th generation should be shorter, and we may find accessions with recombinant alleles at the target locus.
In order to validate the effect of STB_Pyc3A locus, we looked for the presence of the resistance allele in a French elite durum wheat population of 74 lines, and four lines were identified. It is noteworthy that because of the resistance allele's low frequency, a GWAS of this population would not have had sufficient power to identify this QTL. A total of 14 lines, four lines carrying the resistance allele at STB_Pyc3A and ten with the susceptibility allele, was screened using two different STB isolates, P1a and M1a (Fig5). All of the lines carrying the resistance allele completely inhibited pycnidia production whereas nine of the ten lines carrying the susceptible allele displayed a high level of pycnidia formation (Fig5A and B and online resource 5B). Thus, we established the reliability of the STB_Pyc3A marker and the presence of genetic resistance to STB in four elite lines: Coussur, Miradoux, Nefer and Plussur. These lines could be used in breeding programs designed to increase the durability of STB resistance. Indeed, a reduction of the production of pycnidia not only limits epidemic spread, by reducing the number of spores produced, but also reduces the inoculum size of a virulent strain that may appear during the season.

A possible relation between *Stb6* and STB_Nec3A

The same set of 14 elite lines was used for validation of the locus STB_Nec3A. From the two isolates used for phenotyping, only P1a triggered resistance to necrosis in EPO lines; thus, this locus seems to be isolate-specific (Fig5C and D) in a similar way than *Stb6* (Saintenac 2018). However, none of the three elite lines carrying the

resistant allele at the peak SNP for the STB_Nec3A locus were resistant to either isolated (Fig5A and B). Thus, the STB_Nec3A marker found in the EPO population is not a good predictor of resistance in the elite durum wheat population.

The resistance allele at *Stb6* identified in bread wheat is present in about 15% of European cultivars in this species (Brown et al. 2015). Three durum wheat lines carrying an orthologous *Stb6* resistance allele found in bread wheat have been previously described (Saintenac et al. 2018), but resistance to STB strains adapted to durum wheat has not been evaluated. In order to evaluate the possible relationship with STB_Nec3A, *Stb6* was genotyped in 14 durum elite lines and 23 EPO lines (online resource 5C and 6). Three different alleles for *Stb6* were detected and compared to previously described alleles (online resource 6) (Saintenac et al., 2018). One allele is similar to the published resistance-associated *Stb6*-1 allele. The other two alleles are similar to the alleles *Stb6*-7 and *Stb6*-12, for which no information is available on resistance in bread wheat. Only one of the 27 lines with either the *Stb6*-1 or *Stb6*-7 allele was resistant to the P1a isolate (online resource 5C). By contrast, the *Stb6*-12 allele is present in all EPO lines carrying the resistance allele at the STB_Nec3A locus (online resource 5C), suggesting that the presence of *Stb6*-12 allele at this candidate gene is highly predictive of resistance. However, the only elite line (TOMCLAIR) carrying both the *Stb6*-12 allele and the resistance allele at STB_Nec3A was susceptible to both STB isolates tested here, suggesting that *Stb6*-12 is not a good predictor in elite durum wheat.

The *Stb6*-12 allele (and STB_Nec3A) afforded only partial resistance in our study (Fig4), whereas previous research on this locus reported associations with complete resistance (Saintenac et al., 2018). In order to gain further insight, we sequenced *AvrStb6,* the cognate avirulence gene to *Stb6* (Brunner and McDonald 2018), in the P1a and M1a isolates. Neither isolate carried the *AvrStb6* allele known to be recognized by *Stb6* (online resource 7). The allele present in P1a isolates was similar to the virulent *virStb6* haplotypes 2 and 3 that are not recognized by the resistance allele Stb6-1 (Brunner and McDonald 2018). The M1a haplotype corresponded to another uncharacterized allele. Thus, strain-specific differences in cognate avirulence genes to *Stb6* in durum wheat could explain the observed interactions. Altogether, we cannot definitively conclude with our analysis if the observed partial resistance associated with STB_Nec3A is conferred by the *Stb6*-12 allele or by another paralogous WAK in this region that would recognize yet unknown alleles of *AvrStb6*. Because of the high recombination rate in EPO population (David et al, 2014) further genetic analysis of the STB_Nec3A is possible using these lines. Alternatively, durum wheat and STB strains could be genetically transformed with known alleles of *Stb6* and *AvrStb6* in order to directly test for gene-for-gene interactions affecting resistance.

Identification of a QTL for leaf rust resistance in the presence or absence of prophylactic induction

EPO lines were inoculated with a leaf rust isolate and a complete resistance phenotype was detected with a heritability of 0.49 (online resource 8A). In order to test the effect of chitin treatment on LR resistance, the experiment was repeated with prophylactic induction by chitin 4 hours prior to LR inoculation. Chitin treatment did not affect either complete resistance or partial resistance symptoms (online resource 8B). Two independent GWAS were conducted using LR symptoms on chitin treated and untreated plants. In total, 26 lines were completely resistant and this phenotype was confirmed in both experiments. Importantly, because none of the 78 French elite lines demonstrate resistance to this LR isolate (personal data), these 26 lines are potentially valuable

resources for breeders. Four QTL were identified in both experiments (Fig1, Table 1, and online resource 2). The QTL LR_6B11 on chromosome 6B conferred the strongest resistance and was identified in both experiments (Fig7). The locus is relatively large (3,24 Mbp), and contains 33 candidate genes (online resource 3), among which is a cluster of LRR-Kinases and a cluster of NLRs. There is a lack of SNPs between 10,15 and 11,15 Mbp near the NLR cluster; thus, we are not able to identify the best candidate based on the proximity with the most significant SNP. Three other QTL were identified on chromosome 2B, 6A, and 6B (Fig1, Table 1 and online resource 2). Several LR resistance genes have already mapped on 6BS chromosome: *Lr36* (Dvořák and Knott 1990), *Lrac104* (Hussein et al. 2005), *Lr53* (Dadkhodaie et al. 2011), *Lr61* (Kthiri et al. 2018; Herrera-Foessel et al. 2008; Maccaferri et al. 2010; Qureshi et al. 2017; Loladze et al. 2014) and two QTL (Maccaferri et al. 2010). *Lr36* and *Lr53* originate from wild grasses relatives (*T turgidum ssp. speltoides* and *ssp. dicoccoides* respectively). *Lr61* has been mapped in five different publications, originally in the Chilean durum wheat cultivar Guayacan INIA. *Lr61* can also be found in two ICARDA lines (Geromtel_3 and Tunsyr_2) and a Portuguese landrace (Aus26582). Based on marker position, it is difficult to conclude if LR_6B11 is new compared to *Lr36* and *Lr61*. The short arm of chromosome 6B also contains several resistance genes, but lack of fine mapping makes comparisons difficult. Indeed, two resistance genes have been identified in Geromtel_3: one is probably allelic to *Lr61*, but a second could be tightly linked to LR_6B11 (Kthiri et al. 2018). Our mapping of LR_6B11 has finer resolution in this genomic region and EPO lines could be useful to define this resistance locus further. Three other QTL have been identified but not further investigated: LR_6B135 on the short arm of the chromosome 6B, LR_2B on chromosome 2B, the latter colocalizing with *Lr50* (Brown-Guedira et al. 2003) and LR_6A on chromosome 6A which colocalizes with previously identified resistance QTL (Maccaferri et al. 2010; Aoun et al. 2016).

Conclusion

Our ability to easily map the SNB Snn1 resistance gene demonstrates the utility of the EPO lines for finding resistance QTL using a GWAS model and that this panel holds promise for discovering the genetic architecture of other phenotypes. In total, we identified sixteen QTL associated with resistance to the main fungal threats facing duram wheat growers. Among these were two QTL associated with resistance to Wheat Blast, possibly corresponding to finer positions of the *Rmg2* and *Rmg7* genes, that could provide valuable markers for durum wheat breeders for combatting this emerging disease. For STB, we identified a QTL associated with pycnidia density as well as a resistance QTL that may be allelic with the *Stb6* resistance gene that was previously found in bread wheat. Once combined, these two STB QTL hold promise for breeders in producing durable resistance against this major fungal pathogen. Finally, a resistance QTL associated with LR was detected on chromosome 6B, near *Lr61*. Finer mapping of these loci could easily be done using the source EPO population that is still evolving and recombining in the field.

Figure and table captions

Fig1 Physical map of fungal resistance QTL identified in durum wheat. The map distance is given on the left in megabase pairs (Mbp) based on the genomic position in the Zavitan map v2.0. QTL names are located on the right of the chromosome and are represented by a black segment on the chromosome. QTL are designated according to

Table1. QTL positions were defined by a support interval with a pvalue <0.00001. QTL were identified from association mapping in the EPO durum wheat panel of 180 lines for leaf rust (LR), septoria tritici blotch (STB), septoria nodorum blotch (SNB), wheat blast symptoms (WB). When previously mapped genes were identified at the same locus, they are represented with a skeleton segment on the right of the chromosome. Cloned *Snn1* and *Stb6* genes are shown as markers on chromosomes 1B and 3A respectively. The most relevant SNP markers are represented on the right of the chromosome for each QTL.

Fig2 Mapping of resistance to SNB toxin, Tox1 in EPO durum wheat population. **A** Manhattan plot of the QTL SNB_Tox1B. SNP positions are given in megabase pairs based on genomic position in the Zavitan map v2.0. The y-axis is plotted on a log scale. *Snn1* maps at 1,165 Mbp on Zavitan. **B** Boxplot for Tox1 phenotype based on the genotype at SNB_Tox1B locus. A scale from 1 to 4 was used for evaluation: 1 is complete resistance and 4 is leaf with 100 % necrosis symptoms. The number of lines carrying each allele is in parenthesis.

Fig3 Mapping of resistance to wheat blast in EPO durum wheat population. **A** Manhattan plot of the QTL WB_BR43-2A associated with resistance to WB isolate BR43. SNP positions are given in megabase pairs based on genomic position in the Zavitan map v2.0. The y-axis is plotted on a log scale. *Rmg7* is located between 771,35 and 773,37 in a 2,02 Mbp locus, based on published marker position blasted on Zavitan. **B** Boxplot for BR43 phenotype based on the genotype at WB_BR43-2A locus. A scale from 1 to 4 was used for evaluation, where 1 was complete resistance and 4 representing susceptibility. The number of lines carrying each allele is in parenthesis.

Fig4 Evaluation of quantitative resistance to STB in the EPO panel. **A** Boxplot for STB disease severity (necrotic area) based on the genotype at QTL STBNec_3A and STBNec_5A. S_S are lines without any alleles of resistance. S_STBNec_5A are lines without resistant allele on chromosome 3 but a resistant allele on chromosome 5. STBNec_3A_S are lines without resistant allele on chromosome 5 but a resistant allele on chromosome 3. **B** Boxplot for STB density of pycnidia based on the genotype at QTL STBPyc_3A. The number of EPO lines carrying the resistance allele are shown in parentheses.

Fig5 Evaluation of quantitative resistance to STB in a validation panel. STB resistance was evaluated in Elite lines and a subset of EPO lines using the isolate used for GWA (P1a) and a second isolate (M1a) **A** Boxplot of disease severity (necrotic area) in Elite lines and EPO lines inoculated with isolate M1a and P1a. Lines carrying a susceptible allele at STBNec_3A are in white, lines carrying the resistant allele are in black. **B** Boxplot of density of pycnidia in Elite lines and EPO lines inoculated with isolate M1a and P1a. Lines carrying a susceptible allele at STBPyc_3A are in white, lines carrying the resistant allele are in black. Significance was evaluated using a Wilcoxon test; *** pvalue <0.01.

Fig6 Manhattan plot on chromosome 3A for STB resistance: STB_Pyc3A. EPO lines were inoculated with STB isolate P1a and density of pycnidia was evaluated. The QTL position was defined by a support interval with a pvalue <0.00001. The SNP position is given in megabase pairs based on genomic position in the Zavitan map v2.0. The y-axis is plotted on a log scale.

Fig7 A Manhattan plot on chromosome 6B for LR resistance: LR_6B11. EPO lines were inoculated with LR isolate and the surface with pustule was evaluated. The QTL position was defined by a support interval with a pvalue <0.00001. The SNP positions are given in megabase pairs based on genomic position in the Zavitan map v2.0. The y-axis is plotted on a log scale. **B** Boxplot for LR phenotype based on the genotype at the LR_6B11 locus. The number of lines carrying each allele is in parenthesis.

Online resource

ESM_1 Manhattan plots from association mapping in the EPO durum wheat panel of 180 lines for SNB and WB traits. The map is based on Zavitan v2.0. A1 Septoria nodorum blotch SNB isolate inoculation chromosome A, B1 chromosome B. A2 Septoria nodorum blotch Tox1 infiltration chromosome A, B2 chromosome B. A3 Wheat blast BR43 isolate inoculation chromosome A, B3 chromosome B. A4 Wheat blast BR32 isolate inoculation chromosome A, B4 chromosome B.

ESM_2 Manhattan plots from association mapping in the EPO durum wheat panel of 180 lines for STB and LR traits. The map is based on Zavitan v2.0. A1 Septoria tritici blotch STB isolate inoculation, % of surface with necrose, chromosome A, B1 chromosome B. A2 Septoria tritici blotch STB isolate inoculation, % of surface with pycnidia chromosome A, B2 chromosome B. A3 Leaf rust isolate inoculation, mock treated 6 hours before inoculation, chromosome A, B3 chromosome B. A4 Leaf rust isolate inoculation, chitin treated 6 hours before inoculation, chromosome A, B4 chromosome B.

ESM_3 Annotation of candidate genes for SNB_Tox7A and SNB_Nec4B. Annotation and genome location are based on Zavitan v2.0.

ESM_4 Histogram of STB symptoms in EPO lines. The % of diseased leaf area was recorded for necrosis symptoms (dark grey) and % of surface with pycnidia for pycnidia production (light grey).

ESM_5 A Number of EPO lines carrying each allele at the STB_Pyc3A and STB_Nec3A loci. **B** Number of EPO lines and Elite lines resistant for pycnidia density, depending on their genotype at STB_Pyc3A. The number of resistant lines and the total number of lines carrying the corresponding allele are represented. The frequency of the resistant phenotype is in parenthesis. **C** Resistance of selected EPO lines and Elite lines based on percentage of necrotic leaf area and depending of their genotype at STB_Nec3A and at *Stb6*. The number of resistant lines and the total number of lines carrying the corresponding allele are represented. The frequency of the resistant phenotype is shown in parenthesis.

ESM_6 A Sequence of the kinase at *Stb6* locus. The three alleles identified in this study (*Stb6*-7, *Stb6*-1, and *Stb6*-12) were aligned with seven previously published alleles (Saintenac et al., 2018). **B A**mino acid changes due to the different nucleotide polymorphism are indicated.

ESM_7 Sequence alignment of *AvrStb6* allele in both isolates P1a and M1a. Two other isolates carrying *AvrStb6* allele (1E4, 323) and 1A5 (*VirStb6*) were sequenced and aligned on three of the published sequences (Hap2, Hap3 and Hap11) found in Brunner and McDonald 2018.

ESM_8 A Distribution of leaf rust severity evaluated as the percentage of leaf surface covered with pustules after prophylactic induction with chitin treatment (dark grey) or following mock treatment (light grey). **B** Boxplot of

leaf rust severity for completely resistant and partially resistant lines. Complete resistance was considered when no pustules were observed on the leaf surface.

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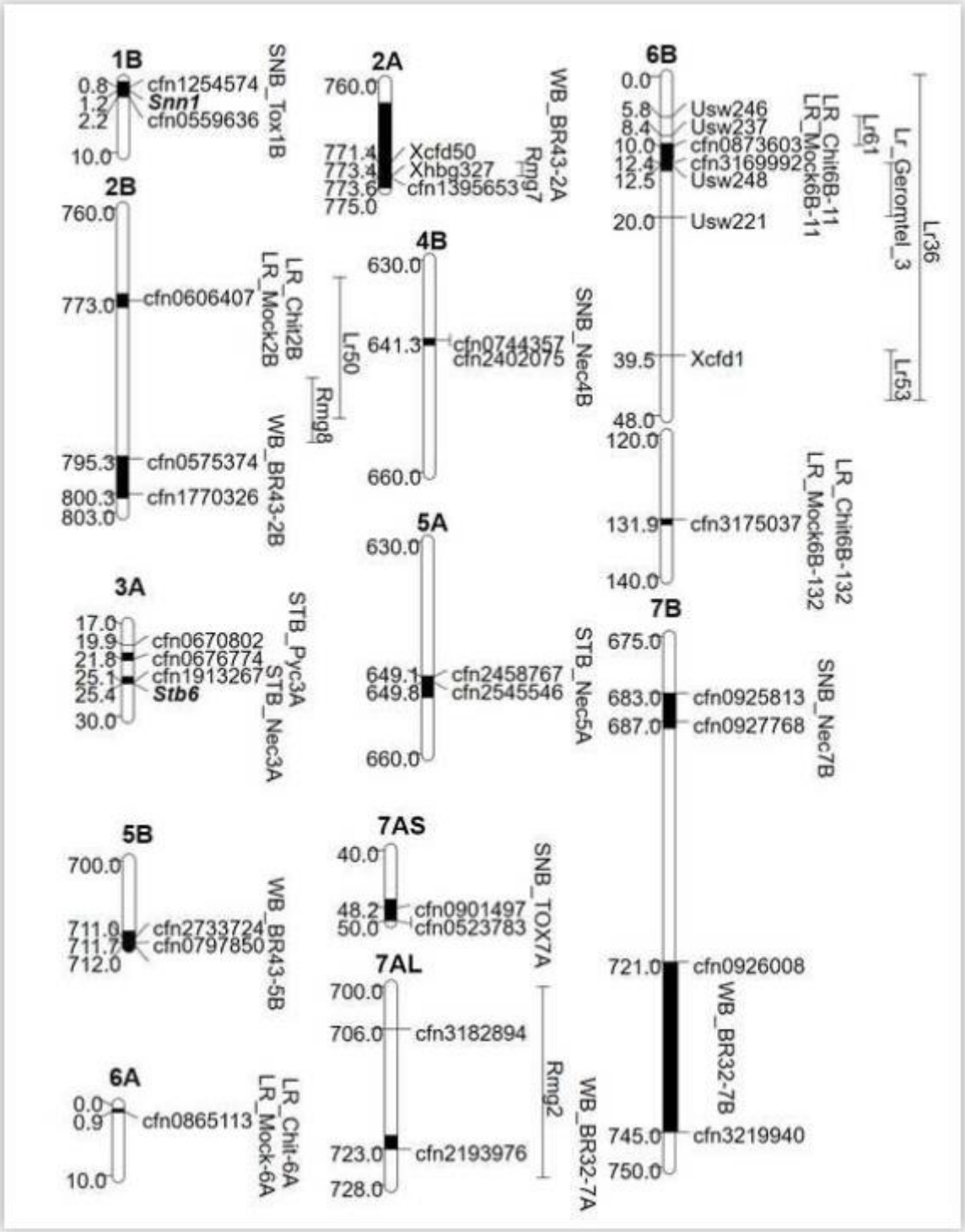
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795.3
800.3
803.0
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LR_Mock2B
WB_BR43-2B
3A
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25.4
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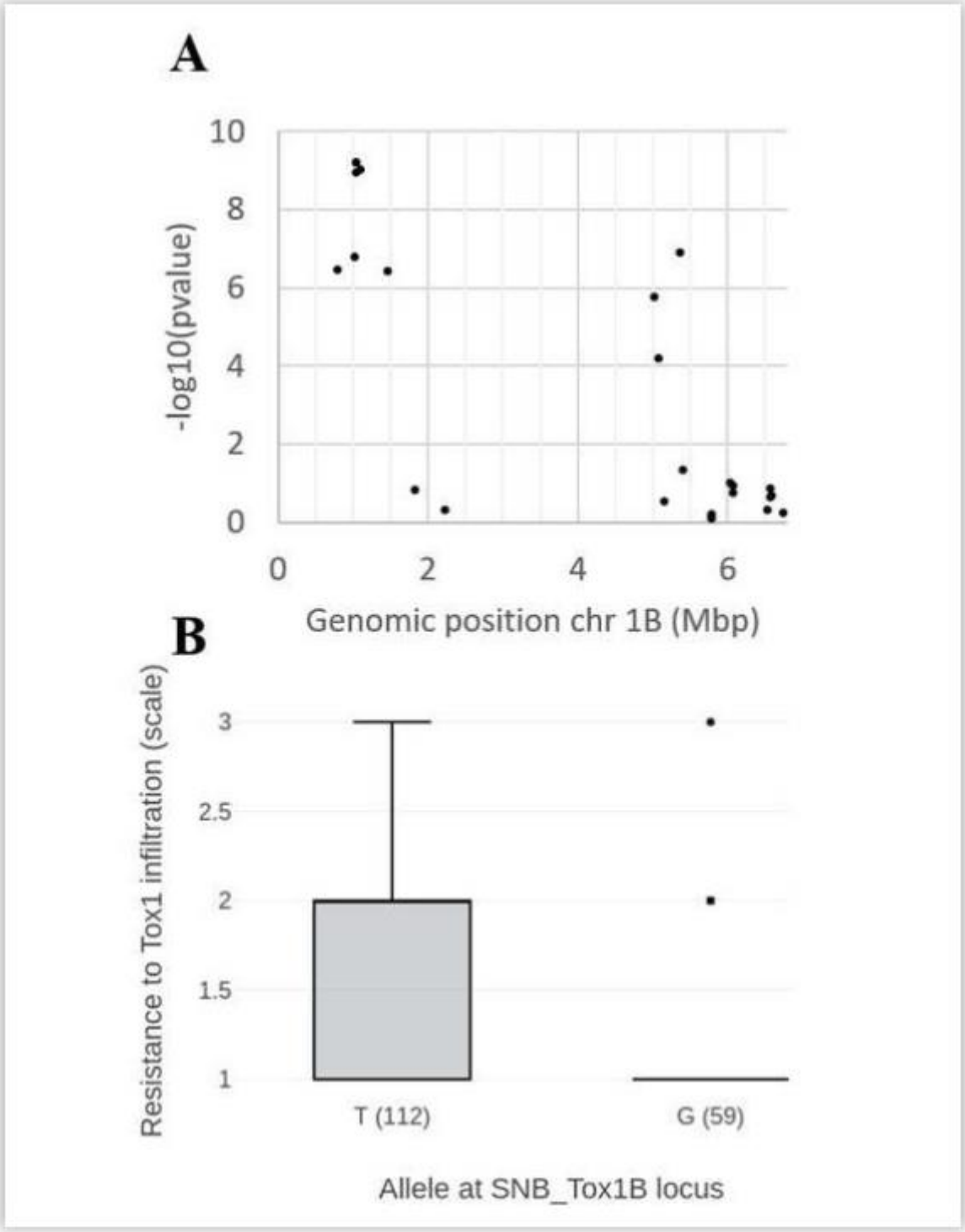
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6
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2
4
6
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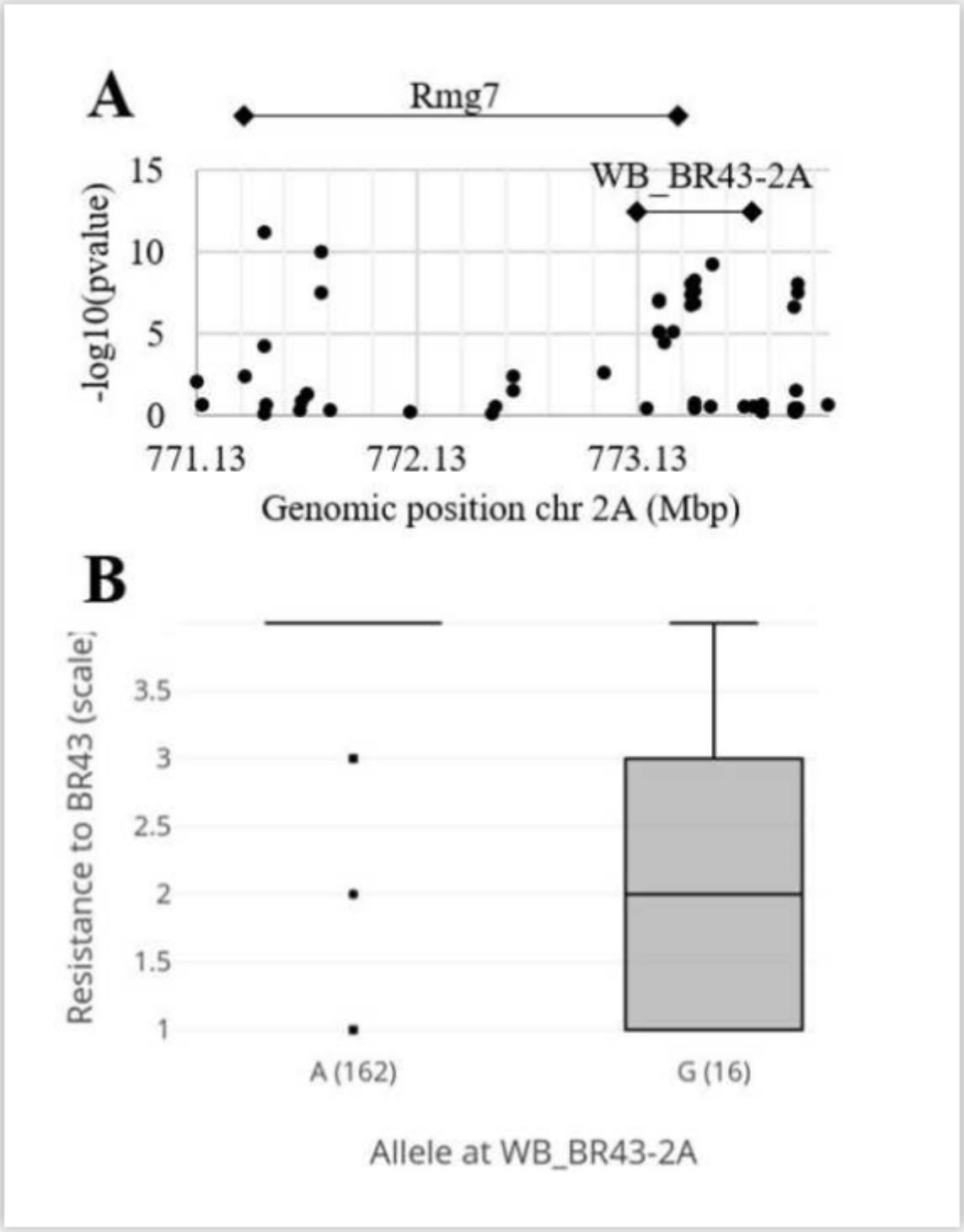
2
1.5
1
Resistance to Tox1 infiltration (scale)
T (112)
G (59)
Allele at SNB_Tox1B locus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3
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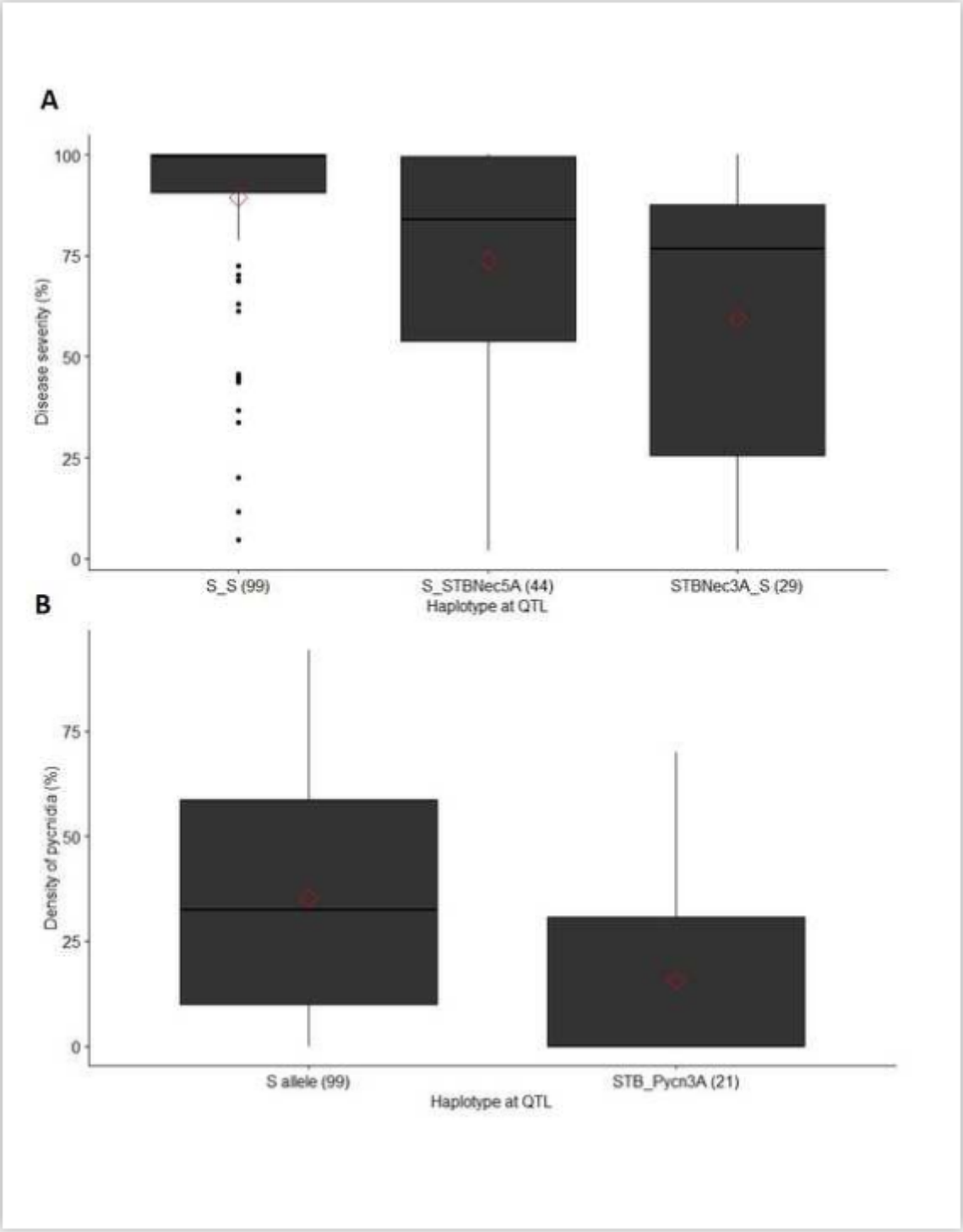
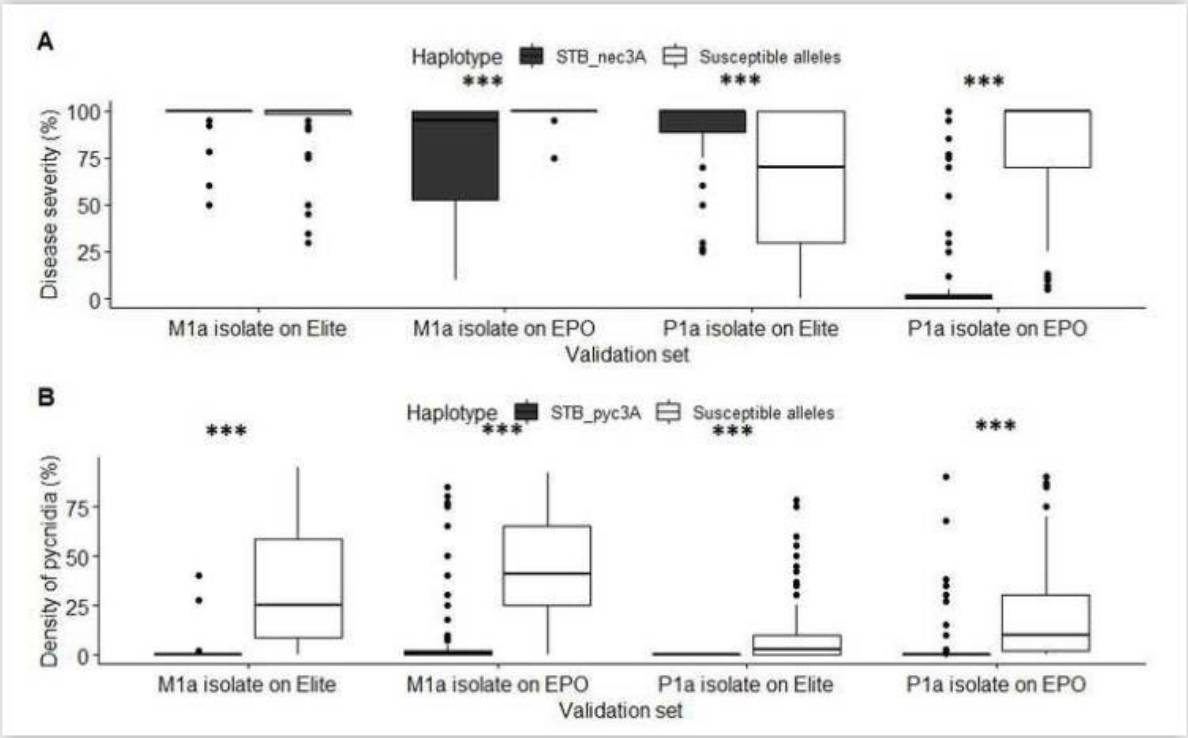
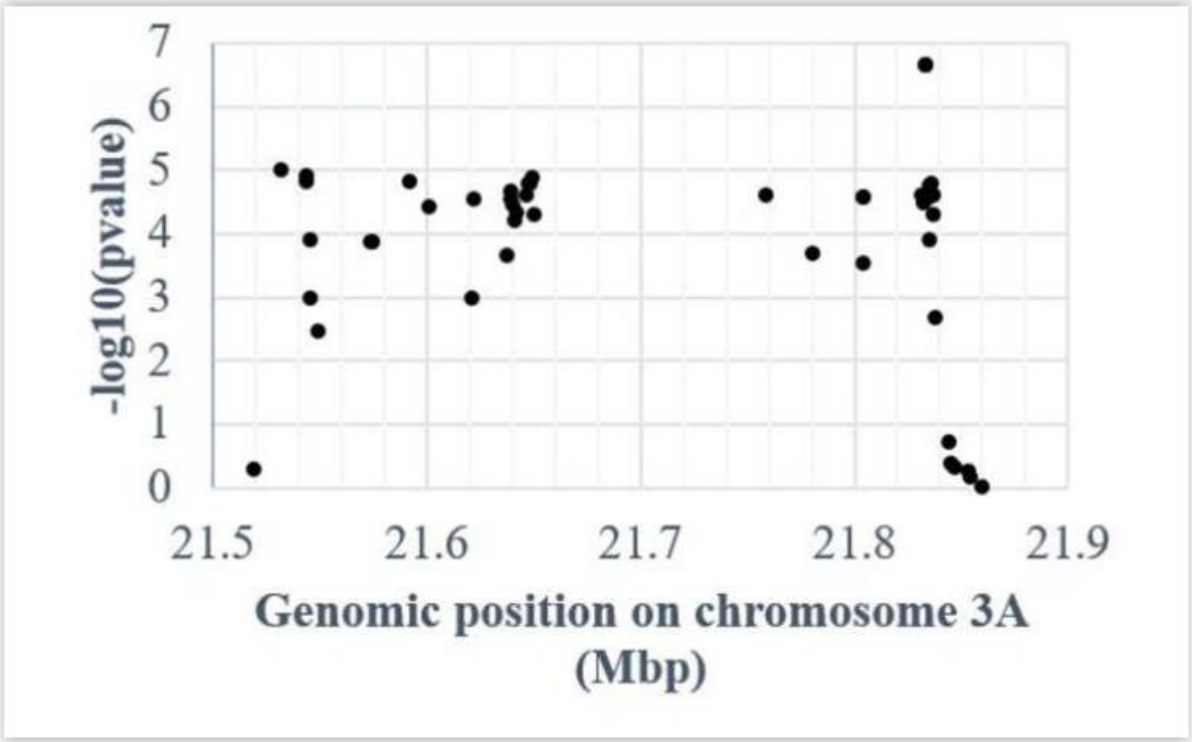
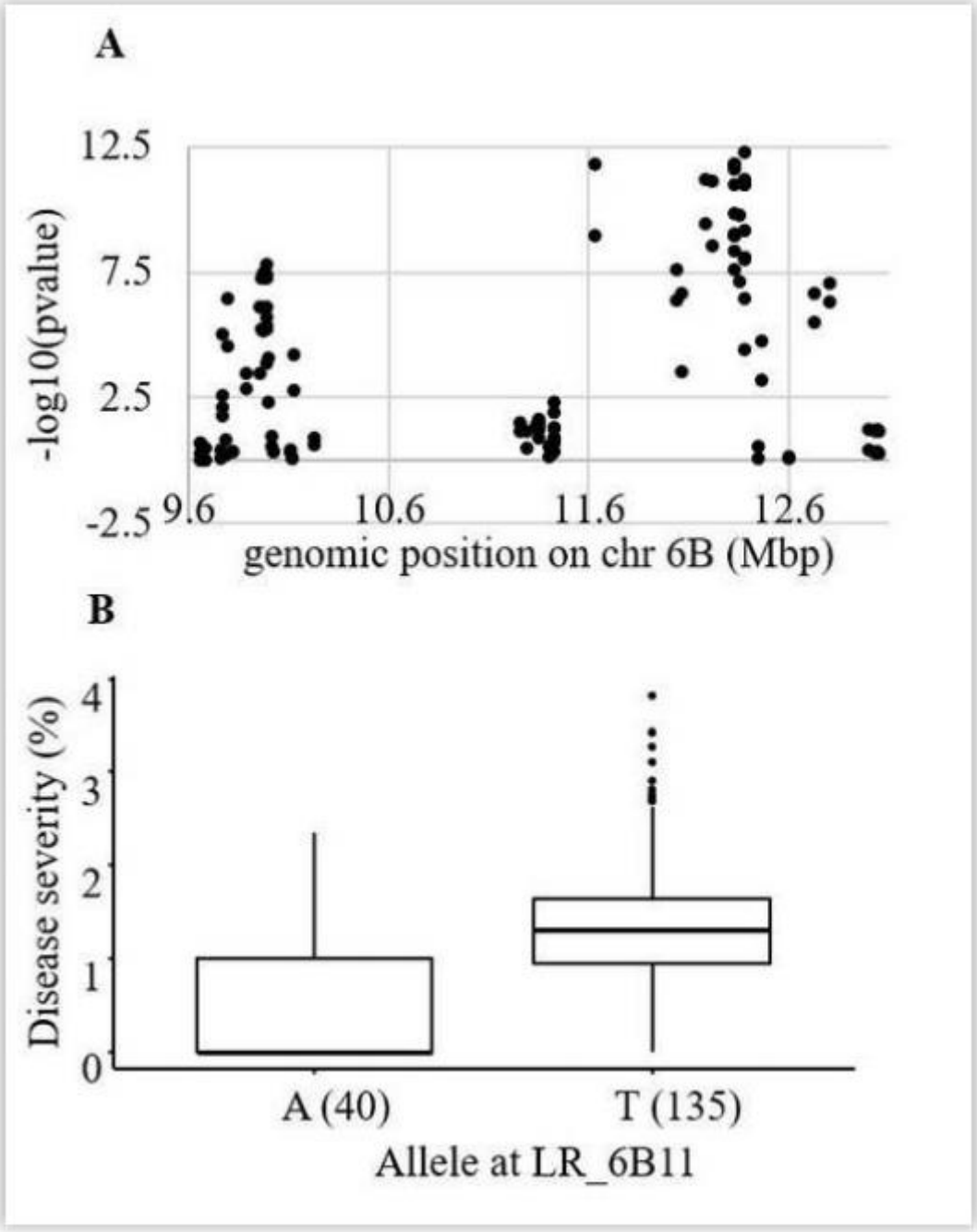
2
1.5
1
A (162)
G (16)
Allele at WB_BR43-2A


A
100
75
50
25
0
Disease severity (%)
S_S (99)
S_STBNec5A (44)
STBNec3A_S (29)
Haplotype at QTL
B
75
50
25
0
Density of pycnidia (%)
S allele (99)
STB_Pycn3A (21)
Haplotype at QTL


A
Haplotype
STB_nec3A
Susceptible alleles
***
***
***
Disease severity (%)
100
75
50
25
0
M1a isolate on Elite
M1a isolate on EPO
P1a isolate on Elite
P1a isolate on EPO
Validation set
B
Haplotype
STB_pyc3A
Susceptible alleles
***
***
***
***
Density of pycnidia (%)
75
50
25
0
M1a isolate on Elite
M1a isolate on EPO
P1a isolate on Elite
P1a isolate on EPO
Validation set


-log10(pvalue)
7
6
5
4
3
2
1
0
21.5
21.6
21.7
21.8
21.9
Genomic position on chromosome 3A (Mbp)


A
12.5
7.5
2.5
-2.5
-log10(pvalue)
9.6
10.6
11.6
12.6
genomic position on chr 6B (Mbp)
B
4
3
2
1
0
Disease severity (%)
A (40)
T (135)
Allele at LR_6B11

Trait (Phenotype)	QTL Name	Position (Mbp)	Chr	-log10 (pvalue)	Size (Mbp)	Allelic effect	R^2	Allele frequency
SNB isolate inoculation (Necrosis %)	SNB_Nec4B	641,3	4B	6.63	1,8	6.8	0.18	59%
	SNB_Nec7B	684,2	7B	4.73	2,2	9.9	0.09	69%
SNB Toxine inoculation (scale)	SNB_Tox1B	1,03	1B	9.2	1,8	-0.35	0.22	33%
	SNB_Tox7A	48,3	7A	8.0	1,9	-0.33	0.19	32%
WB BR43 inoculation (scale)	WB_BR43_2A	771	2A	11.2	0,5	1.43	0.23	8.9%
	WB_BR43_2B	795	2B	8.8	5	1.09	0.19	12.2%
	WB_BR43_5B	711	5B	6.7	0,1	1.39	0.16	5.6%
WB BR32 inoculation (scale)	WB_BR32_7A	722	7A	6.3	17	0.48	0.18	23.9%
	WB_BR32_7B	744	7B	5.1	24	-0.5	0.14	23.3%
STB isolate inoculation (Necrose %)	STB_Nec3A	25,1	3A	13.0	0,3	19	0.45	16%
	STB_Nec5A	649,3	5A	5.0	4,7	9	0.11	24%
STB isolate inoculation (Pycnidia %)	STB_Pyc3A	21,8	3A	6.7	0,3	18	0.11	12%
LR Mock treated (% of leaf area with pustules)	LR_Mock2B	773,2	2B	16.47	0,18	-0.6	0.22	21%
	LR_Mock6A	0,89	6A	9.11	0,01	0.57	0.21	19.1%
	LR_Mock6B11	12,3	6B	7.36	3,24	0.47	0.18	11%
	LR_Mock6B135	135,2	6B	8.78	2,4	0.33	0.14	28%
LR Chitin treated (% of leaf area with pustules)	LR_Chit2B	773,2	2B	17,33	0,18	-0.6	0.19	21%
	LR_Chit6A	0,89	6A	7.92	0,01	0.57	0.17	19.1%
	LR_Chit6B11	12,3	6B	5.39	3,24	0.39	0.13	11%
	LR_Chit6B135	135,2	6B	7.82	2,4	0.28	0.17	28%