Abdulqader Jighly[1, 2, 3]; Manickavelu Alagu[1, 4]; Farid Makdis[1], Murari Singh[1]; Sukhwinder Singh[5]; Livinus C. Emebiri[6], Francis C. Ogbonnaya[1, 7*]

Genomic regions conferring resistance to multiple fungal pathogens in synthetic hexaploid wheat

[1] *International Centre for Agricultural Research in the Dry Areas (ICARDA), P. O. Box 5466, Aleppo, Syria*

[2] *AgriBio, Centre for Agribioscience, Department of Economic Development, Jobs, Transport and Resources, 5 Ring Road, Bundoora, Vic. 3083, Australia.*

[3] *School of Applied Systems Biology, La Trobe University, Bundoora, Vic. 3083, Australia.*

[4] *Kihara Institute for Biological Research, Yokohama City University, Maioka 641-12, Yokohama 2440813, Japan*

[5] *International Maize and Wheat Improvement Center (CIMMYT), Km. 45 Carretera México-Veracruz, Colonia El Batán, Texcoco, Edo. de México, CP, 56130, Mexico*

[6]*Graham Centre for Agricultural Innovation (NSW Department of Primary Industries and Charles Sturt University), Wagga Wagga, NSW 2650, Australia*

[7]*Grains Research and Development Corporation, P.O. Box5367, Kingston, ACT 2604, Australia*

*Corresponding author email: Francis.Ogbonnaya@grdc.com.au

Abstract

Fungal diseases are among the most devastating biotic stresses and often cause significant losses in wheat production worldwide. A set of 173 synthetic hexaploid wheat (SHW) characterized for resistance against fungal pathogens that cause leaf, stem and yellow rusts, yellow leaf spot, *Septoria nodorum* and crown rot were used in genome wide association study (GWAS), using DArT and DArTSeq markers and detected quantitative trait loci (QTL) associated with disease resistance. Seventy four markers associated with 35 QTL were found to be significantly linked with disease resistances using a unified mixed model ($P=10^{-3}$ to 10^{-5}) of which 15 QTL originated from D genome. Six markers on 1BL, 3BS, 4BL, 6B and 6D conferred resistance to two diseases representing 10 of the 35 QTL. A further SHW set of 147 SHWs genotyped with DArT only validated 11 QTL detected in the previous 173 SHWs. We also confirmed the presence of the gene *Lr46/Yr29/Sr58* in our germplasm. In addition, gene-gene interactions between significantly associated loci and all loci across the genome revealed five significant interactions at FDR<0.05. Two significant leaf rust and one stem rust interactions were thought to be synergistic while another two QTL for yellow leaf spot involved antagonistic relations. To the best of our knowledge, this is the first GWAS for six fungal diseases using SHW. Identification of markers associated with disease resistance to one or more diseases represents an important source for pyramiding favorable alleles and introducing multiple disease resistance from SHW accessions into current elite wheat cultivars.

Keywords: *Linkage disequilibrium; multiple disease resistance; Synthetic hexaploid wheat; Genome-wide association study; Gene-gene interaction; genotyping by sequencing*

Introduction

Wheat is the world's third most important food crop that feeds 4.5 billion people in 95 developing countries (Braun et al. 2010). Although maintaining yield in wheat is determined by genetic potential, adequate control of biotic and abiotic stresses coupled with good management practices determine the actual yield achieved annually. Biotic stresses often cause significant challenges to increasing productivity, and have major implications for food security in many regions where wheat is the chief staple, particularly in developing countries where the costs of inputs are high. For example, the acquired virulence to the stem rust resistance gene *Sr31*, by the pathotype lineage designated as Ug99, and recent yellow rust epidemics, caused significant yield losses for wheat production in Ethiopia, Morocco, and Syria in 2010 (Solh et al. 2012). In the U.S., the estimate of cumulative disease losses for the 2011 wheat crop was 6.2% (Appel et al. 2011). Around 250 million dollars are spent each year in controlling foliar pathogens in Australia and the degree of protection afforded by fungicides matches that provided by genetic resistance (Oliver et al. 2011). Currently, identification and incorporation of resistance genes into commercially grown cultivars is the most cost-effective and environmentally safe means of controlling wheat diseases. In general, there are two kinds of resistance: qualitative or vertical and quantitative or horizontal resistance. Qualitative resistance is controlled by major genes which are race-specific, often not durable, and effective in seedling and adult plants. In contrast, quantitative resistance is controlled by minor genes that provide partial resistance and is predominantly effective in adult plants (Lagudah 2011).

In environments where plants are infected with multiple pathogens, multiple disease resistance (MDR) can contribute to maintaining wheat yield potential (Wissera et al. 2011). Evidence for multigenic resistance against wheat diseases goes back to the late nineteenth

century, when Farrer (1898) observed transgressive segregation of resistance against rusts. Although genes known to contribute to MDR in wheat are limited, durable genes which confer resistance to multiple fungal pathogens in wheat have been reported (Moore et al. 2015, Ogbonnaya 2011; Zwart et al. 2010; Lagudah et al. 2009, Spielmeyer et al. 2008; Ogbonnaya et al. 2008; Crossa et al. 2007).

The SHW have been reported to be a repository of novel genetic diversity for wheat improvement (Ogbonnaya 2011; Ogbonnaya et al. 2013). The SHW are a product of hybridization from their two progenitor species, the tetraploid, *Triticum turgidum* (AABB) and the diploid wild relative *Aegilops tauschii* (DD), and its synthesis is analogous to the final hybridization event that is postulated to have occurred in the evolution of common bread wheat from its progenitors (van Ginker and Ogbannaya 2007). Genetic diversity for resistance has been identified in SHW to a wide range of biotic stresses, including Hessian fly (Hatchett et al. 1981), cereal-cyst nematode (Eastwood et al. 1991), root-lesion nematode (Thompson 2008), *Septoria nodorum* blotch (Loughman et al. 2001), *Septoria tritici*, karnal bunt (Villareal et al. 1994), powdery mildew (Lutz et al. 1994), leaf rust (Assefa et al. 2000), stripe rust (Ma et al. 1995), stem rust (Marais et al. 1994), yellow leaf spot (Siedler et al. 1994; Tadesse et al. 2006), leaf blight (Mujeeb-Kazi et al. 2001a) and Fusarium head blight (Mujeeb-Kazi et al. 2001b). Recently, SHW were reported to possess resistance to multiple diseases (Ogbonnaya 2011; Zwart et al. 2010; Thompson 2008; Friesen and Faris 2004; Ogbonnaya et al. 2008; Xu et al. 2004). Further evidence for MDR genes existence in plants includes the detection of clusters of quantitative trait loci for different diseases and the identification of induced gene mutations that affect plant responses to infection with different pathogens (Wissera et al. 2011; and the references cited therein; Krattinger et al. 2009; Moore et al. 2015).

Identification of the genes that confer resistance to multiple diseases in wheat will facilitate and provide insight into the mechanisms that control MDR and allow for more effective deployment of resistance genes in the development of wheat varieties with durable resistance to multiple pathogens. Genome-wide association studies (GWAS), which relies on linkage disequilibrium (LD) between a genetic marker and a locus affecting a trait, were used to identify significant marker trait correlations in animal and plant genetics (Shirasu and Schulze-Lefert 2003; Neumann et al. 2011). In this approach, a collection of diverse accessions are phenotyped and genotyped to examine marker-trait association (Shirasu and Schulze-Lefert 2003; Flint-Garcia et al. 2003). Association mapping (AM) was first successfully used to identify alleles at loci contributing to susceptibility to human diseases (Goldstein et al. 2003). The AM is now being used in an increasing number of studies in wheat to complement previous bi-parental QTL studies (Breseghello and Sorrells 2006; Mulki et al. 2013; Joukhadar et al. 2013; Tadesse et al. 2014, 2015; Jighly et al. 2015b). Association mapping studies in combination with MDR gene interaction will provide a better understanding of disease resistance (Yu and Buckler 2006).

In this study, we aimed to (1) identify genomic regions containing MDR loci in SHW for resistances to six fungal pathogens: *Puccinia triticina* or leaf rust (Lr), *Puccinia graminis* f. sp. *tritici* or stem rust (Sr), *Puccinia striiformis* f. sp. *tritici* or yellow rust (Yr), *Pyrenophora tritici-repentis* or yellow leaf spots (YLS), *Parastagonospora (synonym: Septoria, Stagonospora, Phaeosphaeria) nodorum nodorum* glume and leaf blotch (SNG-SNL), and *Fusarium pseudograminearum* or crown rot (Cr) and (2) investigate the interaction between the different loci on disease expression to facilitate pyramiding of allele combinations across wheat genome with best performance.

Materials and Methods

121 Plant material and disease phenotyping

122 The SHW consisted of 320 lines from CIMMYT and Australia as part of the GRDC funded
123 synthetic wheat evaluation project (Table S1). Full details of the germplasm has been described
124 in Mulki et al. (2013). Details of one season phenotyping for the six diseases that included
125 standard Australian checks has also been previously described by Ogbonnaya et al. (2008).
126 Briefly, rusts were evaluated for adult resistance in three replications under field conditions
127 against the most commercially important pathotypes; *Puccinia graminis* f. sp. *tritici* (Pgt), 98–
128 1,2,3,5,6 (University of Sydney Plant Breeding Institute accession number 781219); *Puccinia*
129 *triticina* (Pt), 104–1,2,3,(6), (7), 11, 13 (accession number 200347) and 76–1,3,5,10,12
130 (accession number 990423); and *Puccinia striiformis* f. sp. *tritici* (Pst), 134 E16A+ (021510).
131 YLS was screened in four replications under controlled greenhouse conditions against three
132 isolates of *Pyrenophora tritici repentis* (isolate identification number 03–0148, 03–0152, 03–
133 0053) and the *Septoria tritici* pathotype 79.2.1A; while *Septoria nodorum* damage was evaluated
134 on both the glume (SNG) and the leaf (SNL) in three replications against the isolates WAC 4302,
135 WAC 4305, WAC 4306, and WAC 4309. All the disease reactions were scored using a scale
136 from 1 to 9 and were classified as susceptible "S" (1-2), moderately susceptible "MS" (3-4),
137 moderately resistant "MR" (5-6) and resistant "R" (7-9). Heritability of the studied traits were
138 inferred from the mixed model (Zhang et al. 2010).

139 Genotyping

140 The whole set (320 SHW) was genotyped with DArT markers; genomic DNA was extracted
141 from two week old seedlings using pooled leaf samples from five individual plants, frozen in
142 liquid nitrogen and stored at -80ºC before DNA extraction. DNA extraction was carried out

143 according to Ogbonnaya et al. (2001), after which 10 µl of a 100 ng µl-1 DNA of each sample
144 was sent to Triticarte Pty. Ltd. Australia (http://www.triticarte.com.au) as a commercial service
145 provider for whole genome scan using Diversity Arrays Technology (DArT) markers (White et
146 al. 2008). A subset of only 173 SHW were genotyped with DArTseq, a genotyping by
147 sequencing (GBS) approach. The full description of the DArTseq procedure was previously
148 given in (Sehgal et al. 2015). Only markers with minor allele frequency (MAF) >5% and missing
149 data <20% were selected for further analyses. The 173 SHW subset was used for the main
150 association test while the remaining 147 SHW cross validation set genotyped with only DArT
151 markers was used as to confirm the presence of some of the detected QTL as they are related to
152 the main set.

153 Statistical analysis

154 The genotype by trait analysis was used to establish a level of variability among the wheat
155 genotypes in response to different disease resistances in order to visualize the merit of genotypes
156 as well as interrelationships among traits. The methodology is similar to that used for multi-
157 environment trait genotype × environment interaction (GGE biplot) by including the genotypes
158 as entries and the diseases as testers. The GGE biplot model decomposes genotype effect (G)
159 plus genotype x environment (GE) effects though singular value decomposition into a number of
160 principal components. Thus, it removes the environment noise (Yan 2001). Although this
161 analysis is originally designed for GGE analysis, it can be applicable to any two-way data that
162 has the entry-by-tester structure. However, when using it to visualize genotype-by-trait data, the
163 trait units should be removed through standardization before applying biplot analyses (Yan and
164 Kang 2002). Such analysis is applicable to our data without any prior adjustments as all our
165 disease scores were scaled from 1 (susceptible) to 9 (resistant). However, since we are

comparing greenhouse and field experiments (heterogeneous testers), the model was scaled with the within trait standard error as recommended by (Yan and Kang 2002). Only SHWs with no missing phenotypes were included in this analysis.

Phylogenetic tree was drawn using neighbor joining algorithm implemented in DARwin software (Perrier 2006) using DArT markers for both the main and the validation sets. The R software (www.r-project.org) was used to plot the principal component analysis (PCA) using the DArT markers only while the kinship relations were plotted using the R package "heatmap" using DArT markers only.

The determination of linkage disequilibrium (LD) was described by measuring the R^2 values between markers as described by Hedrick (1987) and Weir (1996). LD statistics were calculated for each pair of markers per chromosome and across all chromosomes within each genome. The R^2 values were plotted against the genetic distance (cM) for each pair of markers within each chromosome and the second LOESS decay curves were fitted using the square root transformation (Breseghello and Sorrells 2006).

Genome association and prediction integrated tool (GAPIT) (Lipka et al. 2012) was used to perform association mapping analysis using the mixed linear model (MLM) that took into account population structure (Q) and kinship matrix (K) to control both Type-I error (Pritchard et al. 2000). The following equation was fitted in the model:

$$y = X\beta + Q\alpha + Z\mu + \varepsilon$$

Where y is the vector of phenotypes; X is a vector for the marker record relating individuals to the fixed marker effects β, which we are estimating; Q is fixed effect matrix (PCA) relating

187 individuals to the fixed effect regarding population structure vector α; Z is the incidence matrix
188 relating individuals to the random effect μ; and ε is a vector for the random residuals.

189 Marker alleles with P values ≤ 0.001 were declared to be significantly associated with a single
190 fungal disease resistance for the six diseases studied.

191 The map positions of DArTSeq markers were obtained from a consensus map of 64K markers
192 provided by DArT Pvt. Ltd., Australia. The databases http://cmap.cimmyt.org/cgi-bin/cmap;
193 https://ccg.murdoch.edu.au/cmap/ as well as the genetic maps of Detering et al. (2010), Jighly et
194 al. (2015a) and Lowe et al. (2011) were used to compare our QTL positions with previously
195 reported QTL.

196 The same datasets including genotyping, phenotyping and Q matrix were used to analyze
197 epistatic interactions between markers found to have significant main effects; and between
198 significant markers and other markers whether or not they were significant. A linear regression
199 model was used to calculate P values for pair-wise marker interactions including the Q matrix as
200 a covariate. The MDR gene interaction analysis was applied initially for each individual disease
201 and the significance threshold for the interactions analysis was estimated using false discovery
202 rate (FDR) ≤ 0.05 (Table 1) considering a total number of tested interactions for each disease
203 resistance = total number of markers × number of QTL for the trait (Benjamini and Hochberg
204 1995). For the significant interactions, a two sample t-test via 10,000 Monte-Carlo permutation
205 samples were applied to judge the significant differences between phenotypes for interacted
206 allelic combinations.

207 **Results**

208 Multiple fungal disease screening in SHW

All 320 SHW were screened against six fungal diseases (seven scores, *Septoria Nodorum* has two component scoring of glume (SNG) and leaf (SNL) blotches). Infection responses varied for each disease and ranged from susceptible to resistant (Figure S1). The full details of experiments and results for individual diseases have been reported previously (Ogbonnaya et al. 2008; Ogbonnaya 2011). Only the genotype AUS36217 showed moderately resistance response to all diseases while approximately 16.25% of the SHW possessed either resistant or moderately resistant reactions to at least five of the diseases evaluated in this study. The gene by trait analysis resulted in a good number of genotypes close to the position of the ideal genotype (Figure 1). The pseudo-heritability estimation inferred from the mixed model for the studied traits ranged from 22.4% for Yr to 69.4% Lr (Figure S2).

Marker coverage, genetic diversity and linkage disequilibrium

A total of 12,207 DArT (453) and DArTSeq (11,754) markers were polymorphic in the SHW panel, of which 6,176 were of known map position with an average of 294.1 markers per chromosome. One thousand five hundred and sixty six, 1,773 and 2,837 loci were mapped on the A, B and D genomes respectively, with an average distance of 1.38, 1.06 and 0.74 cM for the A, B and D genomes. Chromosome 4B and 1A had the least number of markers with only 131 while 7D had the highest number of markers (711 markers). The DArTSeq markers have advantages over the original DArT markers being of higher density, of co-dominant inheritance and possesses better D genome coverage. However, only 55 (12.1%) of the DArT markers were distributed on the seven D genome chromosomes. Table S2 and Figure S3 show the distribution of both DArT and DArTSeq markers across wheat genome while table S3 shows the full genotypic data of both sets.

231 The PCA analysis was run for the 320 SHW using 453 polymorphic DArT markers. The first
232 two principal components together explained about 21.3% of the total variation and the
233 germplasm were wide spreading on the two axes. Interestingly, the SHW genotyped with both
234 markers as well as the cross validation set with DArT markers clustered together except for
235 about 20 SHWs which belonged to the validation set (~13.5% of the validation population)
236 which clustered away from the main set (Figure 2). The phylogenetic tree and the kinship
237 analyses showed similar results to PCA (Figure S4, S5).

238 Plotting intra-chromosomal R^2 values for each pair of loci against their interval genetic distance,
239 indicated that the LD started to decrease below 0.22 after ~120 cM. However, when each
240 genome was considered separately, the A genome started to decay at 10 cM, B genome at 2 cM
241 while D genome was at 200 cM (Figure S6). Further, the D genome also exhibited higher intra- +
242 inter-chromosomal R^2 values with median value about 0.2 (Figure S7). Interestingly, LD
243 decayed faster for chromosomes 2D and 5D than the other D genome chromosomes at about 10
244 cM and 1 cM respectively (data not shown).

245 Association analysis of QTL for six disease resistance

246 Table 1 presents a summary of markers significantly associated with the various fungal diseases
247 evaluated. There were a total of 74 DArT and DArTSeq markers representing 35 QTL that were
248 associated with all disease resistances with R^2 values which ranged from 6 to 13.9%. The highest
249 R^2 value was 13.9% for the SNG associated marker *wPt-8262* on 5AL followed by *wPt-2858* on
250 2AL associated with Yr resistance with R^2 of 13.5%. The genotypes of A and B genome
251 associated marker for the genotypes with common durum parents were summarized in table S4
252 while figure S8 shows the Manhattan and QQ plots for all disease traits.

On an individual disease basis, five markers located in three genomic regions (2BL, 3BL and 7DL) were associated with Cr resistance with R^2 values which ranged between 8.2 and 9.3% while 31 markers representing five different genomic locations on 1BS, 2DL, 3D, 6DL and 7D were identified as being associated with resistance to YLS, with R^2 values which ranged between 6.7 and 11.4%. There were a number of chromosome regions associated with resistance to each of the three rusts. Nine markers on five genomic regions, 1BL, 1DS, 2DL, 6D and 7DL, were significantly linked to Lr resistance with R^2 values which ranged between 6.7 and 8.4%, while nine markers associated with Yr resistance were located on 1BL-1, 1BL-2, 2AL, 2BL, 3D and 6BL with R^2 values that ranged between 9.4 and 13.5%. Twelve markers were associated with Sr resistance on seven genomic regions: 1BL, 2DS-1, 2D-2, 3BS, 4BL, 6B and 6DL. The R^2 values ranged between 6 and 10.2%. Thirteen markers on eight genomic regions (2AL, 2BL, 2DL, 3BS, 4BL, 5AL, 6B and 7D) were linked with resistance to SNG with R^2 values that ranged from 6.2 to 13.9%. Similarly, one marker on 7B was linked with SNL with an R^2 value of 8.4%.

Of the 35 detected QTL, ten were detected with DArT markers only, two with the diagnostic marker for *Lr46*/*Yr29* and 22 with DArTSeq only; with only one common QTL for both marker types which is the QTL Sr05 on 4BL.

Validation of the AM analysis of QTL for six disease resistance based on DArT markers only

The validation set of 147 SHW genotyped with DArT only confirmed the multiple disease QTL *Lr46*/*Yr29* on 1BL as well as the SNG QTL on 3BS with the exact markers detected in the main set of 173 SHWs. Further, additional eight QTL were also confirmed with closely linked markers for Lr, Sr, SNG, SNL and YLS (Table 2). Five out of the 11 confirmed QTL were linked with

MDR resistance in the main set which are Lr01, SNG04, SNG05, Sr06 and Yr02. QTL found in the validation set only were reported in Table S5.

Multiple-fungal disease resistance loci

Out of the 35 detected QTL, three, 17 and 15 QTL were detected on A, B and D genomes respectively. Six markers on five wheat chromosomes (1BL, 3BS, 4BL, 6B and 6D) were significantly associated with resistance to two different fungal pathogens representing 10 out of the detected 35 QTL (Table 1). Figure S9 represents the allelic effects of the MDR markers. Only the marker *Lr46*/*Yr29* on 1BL was significantly associated with resistance to Yr and Lr in coupling while the other five markers were associated with the two diseases in repulsion. The markers *wPt-3921* on 3BS, *1094836* on 4BL and *1019982* on 6B were associated with Sr and SNG resistances while the markers *1126111* and *1126778* on 6D were associated with Lr and YLS.

On the chromosome arm bases, genomic regions on 1BL, 2DL and 7DL possessed the highest number of QTL with four. The 1BL arm contain two genomic regions that contain the *Lr46*/*Yr29* MDR QTL as well as Yr QTL (Yr01) which lies at genetic position of 162.3 cM and the Sr QTL Sr02 at 289 cM. The chromosome arm 2DL carries the unmapped QTL Sr03 and the QTL Lr03, YLS02 and SNG03 between the genetic positions 252.2 cM for YLS02 and 289.8 cM for SNG03. The 7DL arm possessed four QTL, Cr03, Lr05, SNG08 and YLS05. Unfortunately, the position of the 7DL QTL could not be precisely determined because of distance they spanned due to the large LD blocks. Another interesting region was found on chromosome arm 2BL between 163.8 and 179.5 cM carrying the QTL Cr01, SNG02 and Yr04. The long arm of chromosome 2A also carry the QTL SNG03 at 129.8 cM and Yr03 at 82.2 cM (Table 1).

Gene-gene Interaction

Significant interactions were detected for YLS, Sr and Lr only while no interactions were detected for the other disease resistances (Table 2). The Lr QTL on chromosome 6D showed two interactions with regions on 3BL and 6B, while the Sr QTL on 2DS showed a single interaction with the marker *1262585* on 2AS. Two interactions for the YLS resistance QTL on 6DL were detected with loci on 1DL and 6B. The R^2 values ranged from 15.9% for the YLS interaction 6DL/6B to 24.4% for the YLS interaction 6DL/1DL. Comparing the allele combination disease score for the interacted alleles showed that Lr and Sr interactions are synergistic while YLS interactions are antagonistic as the two Lr and the Sr resistance alleles showed high resistance response when combined with the interacted alleles of the markers *2280676*, *1208017* and *1262585*; respectively (Figure 3).

Discussion

This study describes the use of GWAS to identify loci underlying individual and multiple disease resistances, and their interaction in SHW. The SHWs consisted of 320 genotypes produced from interspecific crosses between 222 *Aegilops tauschii* accessions and 50 elite *Triticum turgidum* lines which were previously evaluated for several fungal and root diseases (Ogbonnaya et al. 2008; Ogbonnaya 2011; Mulki et al. 2013). The present study was carried out for six important fungal diseases. Only nine genotypes of the 320 SHW showed complete resistance to all diseases, however, about 20% of the SHW possessed either a resistant or moderately resistant reaction to two or more diseases.

Linkage disequilibrium

GWAS studies are highly dependent on LD decay determined by the interval genetic distance between loci, recombination rate and the number of generations since the most recent common ancestor (Mackay and Powell 2006). To the best of our knowledge, this germplasm has longer LD blocks than any other previously described wheat germplasm. However, when each genome was considered separately, we found that the extensive decay was on the D genome only (except for 2D and 5D) while the other two genomes have a comparable or lower LD decay than results from other studies in wheat. The longest ranges reported so far on wheat were estimated at between 30 and 40 cM (Crossa et al. 2007; Dreisigacker et al. 2008; Jighly et al. 2015b); while other studies reported a decay of about 1-2 cM (Tadesse et al. 2014; Tadesse et al. 2015). Compared to bi-parental mapping, GWAS can target more favorable loci and have better mapping resolution as it exploits more diversity but it is also limited in detecting rare variants and can have higher type I error due to population structure (Pritchard et al. 2000; Flint-Garcia et al 2003, 2005; Breseghello and Sorrells 2006). It has been reported by other authors that for genome wide association mapping, genetic materials characterized by high LD are preferable due to the reasonably low number of markers required to reveal a significant marker-trait association but materials with low LD and efficient number of markers can lead to higher mapping resolution (Flint-Garcia et al 2003; Maccaferri et al. 2006). However, such a long range LD in D genome (about 200 cM) will cause huge uncertainty on the accuracy of the QTL position. The high LD indicates the existence of narrow genetic diversity and high population structure of the *Aegilops tauschii* parents used to develop the SHW germplasm in this study. On the other hand, the marker coverage (one marker every 1.38 cM for A genome and one every 1.06 cM for B genome) in this study was sufficient for a whole-genome association scan in the SHW population.

340 Fungal disease resistance characterization in SHW

341 *Novel QTL detection in SHW*

342 The present study detected one QTL for each of Sr and YLS in genomic regions that have
343 not been previously reported for those diseases. Our analysis revealed two Sr QTL on 2D of
344 which one is located in the region of *Sr46* gene (Yu et al. 2015) while the other QTL is
345 associated with the SNP markers *1101415* and *1102301*. Despite the very long LD observed in
346 the D genome, the two SNP markers have no LD with the *Sr46*-associated markers indicating
347 that they may be associated with a novel Sr resistance gene. Similarly a haplotype block of eight
348 SNP markers on 6DL including the marker *1037337* which mapped at the position 169.1 cM
349 were linked to a novel YLS QTL of which the marker *1139583* had the lowest P value of $1.6\times10^{-}$
350 ^{4} with R^2 value of 8.8%. To the best of our knowledge, no YLS QTL has been mapped on 6DL
351 in the region of this QTL.

352 The present study confirmed the introduction of novel genes from *Ae. tauchii* to bread wheat
353 germplasm regardless the accurate positioning on the genome. This study can guide future
354 research to develop bi-parental populations with one synthetic parents possessing potentially
355 value with the reported novel genes or initiating synthetic germplasms with more diverse *Ae.*
356 *tauchii* resources for GWAS studies. *Previously reported disease resistance loci*

357 Five different QTL were detected for each of Lr and YLS. For both diseases, four out of
358 the five QTL were found in the D genome of which one was common but in repulsion on
359 chromosome 6D. All of the Lr D genome QTL identified in this study were found on regions
360 known to carry introduced leaf rust resistance genes from wild relatives. *Lr19* was introduced to
361 hexaploid wheat chromosome 7D from *Agropyron elongatum* (Gupta et al. 2006) and *Lr38*

362 originated from *Agropyron intermedium* (Mebrate et al. 2008). The QTL identified in the SHW
363 used in the present study may be homoeologous to *Lr19* and *Lr38*. The other two genes are *Lr39*
364 and *Lr42* which were found originally in *Aegilops tauschii* (Singh et al. 2004; Sun et al. 2010).
365 On the other hand, SHW has been extensively used to control YLS (Alam and Gustafson 1988;
366 Xu et al. 2004; Tadesse et al. 2006; Singh et al. 2006) and the chromosome 3D gene *Tsn3*,
367 detected in our germplasm, is one of the best examples (Tadesse et al. 2007). The other D
368 genome YLS QTL in the SHW germplasm were located on chromosomes 2DL and 7D and were
369 previously reported in hexaploid landrace germplasm through association mapping (Gurung et al.
370 2011). Further studies will be necessary to determine whether the SHW alleles associated with
371 resistance are similar to those of the wild species alleles in future QTL studies.

372 Although the SHW in this study were not tested against any of stem rust Ug99 isolates,
373 all the Sr QTL identified in this study were found on regions that carry Ug99 resistance except
374 for the untested novel QTL on chromosome 2DS (Yu et al. 2011, 2014; Guerrero-Chavez et al.
375 2015). The 1BL gene *Sr58* was reported to carry Ug99 resistance and was found in durum
376 germplasm (Herrera-Foessel et al. 2011). We also detected the gene *Sr46* on 2DS in the SHW
377 germplasm which was first reported in the *Aegilops tauschii* accession "CIae 25" (Yu et al.
378 2015); and a QTL in the regions of the gene *Sr37* which was previously reported in durum wheat
379 chromosome 4BL (Singh et al. 2013). Further evaluation of the SHW in Ug99 hotspots is
380 required to confirm Ug99 resistance in the SHW.

381 Only three QTL were reported to be associated with Cr resistance genes of which the
382 SNP marker *1384280* identified in the current study on 2BL QTL was previously reported in
383 durum wheat near the marker *wPt-9336* (Eberhard 2011). Poole et al. (2012) and Ma et al.

(2010) reported QTL in hexaploid wheat in two genomic regions on 3BL and 7DL QTL, respectively, consistent with the result from the current study.

Zegeye et al. (2014) detected a seedling Yr QTL on chromosome 3DL in an SHW germplasm through association mapping. However, it is difficult to determine whether the 3D QTL identified in this study is similar to the previously reported QTL since the two SNP markers (*100136169* and *1267912*) in the current study are unmapped and the LD started to decay after 200 cM for D genome in this germplasm. On the other hand, we identified a number of QTL in genomic regions that were previously found to carry Yr resistance in hexaploid wheat on chromosome regions 1BL, 2AL and 6BS (Basnet et al. 2014; Dedryver et al. 2009; Rosewarne et al. 2013; Santra et al. 2008). We also detected the presence of the *Yr29* gene which is found in both durum and hexaploid wheat (William et al. 2003; Herrera-Foessel et al. 2011). Similarly, all the SNG/SNL QTL identified in the current study were located on genomic regions previously reported to carry SNG/SNL QTL in bread wheat (Table 1) (Shankar et al. 2008; Czembor et al. 2003; Adhikari et al. 2011; Cockram et al. 2015; Schnurbusch et al. 2003; Kumar et al. 2010; Aguilar et al. 2005).

Detection of MDR loci

Using MLM we identified six markers on five different chromosomes (1BL, 3BS, 4BL, 6B and 6D) associated with MDR loci in SHW of which only the 1BL QTL showed coupling phase with the resistances associated with the well documented gene *Lr46*/*Yr29*. The 3BS, 4BL and 6B QTL showed associations with Sr and SNG while the 6D QTL was associated with Lr and YLS. Earlier studies reported the clustering of resistance genes in specific regions. Sukhwinder-Singh et al (2012) reported that it is not uncommon in wheat to find regions

406 inherited as MDR loci. They argued that these are typically due to absence of recombination
407 from alien chromosomal segments, such as the Yr, Sr and powdery resistances from rye
408 chromosome 1RS segment or the triple rust and nematode resistances from *Ae. ventricosa*
409 introgressed on wheat chromosome 2A. These introgressed segments were shown to carry
410 diverse and multiple gene clusters that encode nucleotide binding and leucine rich repeat
411 sequences, the most frequent class of plant disease resistance genes. However, the SHW have no
412 history of alien introgression to explain the MDR identified in this study which will make it
413 easier to pyramid the four MDR QTL we detected in repulsion. Previous reported isolated wheat
414 MDR genes with pleiotropic effects include Lr34 and Lr67 (Krattinger et al. 2009; Moore et al.
415 2015).

416 MDR can be a result of genes with pleiotropic effect, unlinked genes or a cluster of
417 resistance genes. The gene *Lr46/Yr29/Sr58/Pm39/Ltn2* on 1BL exhibits MDR (William et al.
418 2003) and it was previously reported in CIMMYT durum wheat (Herrera-Foessel et al. 2011). In
419 the present study, the resistance allele of *Lr46* diagnostic marker was associated with Lr and Yr
420 resistances. Although this marker was not associated with Sr resistance in this germplasm, the
421 SNP *1006460* showed a potential association with *Sr58* as it is physically close to the *Sr58*
422 linked marker *Xbarc80* (Yu et al. 2014). Further, both *Lr46*/*Yr29* and *Sr58* were detected in the
423 validation set. Additional MDR region was located on chromosome 2BL within 15.7 cM which
424 contain QTL for SNG02, Yr04 and Cr01. Interestingly, 46 SHWs carry the resistance alleles of
425 the three QTL together.

426 Although most of the markers in the present study were associated with a single disease
427 only, some of these were previously reported to be linked with different disease resistance genes
428 and stresses in previous studies using different germplasm. The marker *wPt-2757* on 3BS was

associated with fusarium head blight, Sr and grain yield under salinity conditions (Bhavani et al. 2011; Agnes et al. 2014; Genc et al. 2013). Similarly, the SNG/Sr associated marker *wPt-3921* (chromosome 3BS) was previously reported to be linked to fusarium head blight, Sr, and Yr (Bhavani et al. 2011; Agnes et al. 2014; Chen et al. 2012) while the 4BL marker *wPt-8543* associated with SNG was also associated with Sr and Yr in previous reports (Zwart et al. 2010; Letta et al. 2013).

Gene-gene interaction

The interaction between loci was investigated to elucidate the mechanism of disease resistance and provide evidence for epistasis or pleiotropy. Five significant interactions were identified that contributed to Lr, Sr and YLS in the SHW between one QTL for each of the previous diseases and one or two other genomic loci (Table 3). The two Lr interactions as well as the Sr interaction were synergistic as combination between the resistance and the significantly interacted alleles showed superior phenotypes (Figure 3); while the YLS interactions can be considered as antagonistic because only the susceptible alleles showed significant difference with both marker alleles interacting. However, studying those interactions in a larger population size will confirm their presence and can facilitate better understanding for their molecular basis. Synergistic interactions are favorable for breeding programs but they will need continued tracking for the presence of both alleles while antagonistic interactions will require eliminating genotypes carrying the negative interacted alleles during the breeding progress. Knowledge of these interactions will enhance genetic gains in deploying and breeding for MDR as a mean of controlling both biotrophic and necrotrophic pathogens.

Our study explored the possible role of polyploidy in MDR in wheat, having a genome with three homeologous sets of chromosomes, and suggests the existence of interactions between genes involved in various fungal disease resistances. Earlier, a comprehensive study (Segre et al. 2005) demonstrated systematic epistatic interactions using yeast as a model organism. The study emphasized the co-dependency of genes from various functional categories to establish a phenotypic difference. Specifically, the authors showed that epistatic interactions could be organized into a network formed by functional modules and that interactions between functional modules are more likely to occur than within modules. In our study, the module could be thought of as a biological pathway, and the interactions between the loci would imply cross-talk between these pathways. As underlined by Moore and White (2007), making biological interpretations from statistical models of epistasis is difficult to do for any method since we are trying to make inferences about biological processes at the cellular level in an individual from statistical summaries of variation in a population. Further investigations of these results could provide insight into understanding different resistance gene relationships as well as mechanisms that contribute to different resistance gene networks.

Conclusion

Molecular markers identifiably linked with multiple disease resistance genes will be particularly effective for breeding programs in order to facilitate and improve the selection for different disease resistance genes simultaneously. The ultimate aim of this study is to generate knowledge so that MDR can be effectively deployed for the development of wheat cultivars possessing durable multiple disease resistance. In this study, we identified markers associated with Sr, Yr, Lr, Cr, SNG, SNL and YLS of which some were associated with up to two diseases, some of

which were novel. This is the first association mapping study that reported markers associated with the resistance for six diseases together.

Competing interest

The authors declare that they have no competing interest.

Abbreviations

MDR, Multiple disease resistance; SHW, Synthetic Hexaploid Wheat; MLM, Mixed Linear Model; QTL, Quantitative Trait Loci; GWAS, Genome Wide Association Study; LD, Linkage Disequilibrium; Lr, Leaf Rust; Sr, Stem Rust; Yr, Yellow Rust; YLS, Yellow Leaf Spot; SNG, *Septoria Nodorum* Glume Blotch; SNL, *Septoria Nodorum* Leaf Blotch; Cr, Crown Rot; DArT, Diversity Arrays Technology.

Acknowledgment

The authors acknowledge financial support from the Grains Research and Development Corporation, International Centre for Agricultural Research in the Dry Areas (ICARDA), the International Maize and Wheat Improvement Centre (CIMMYT) and Department of Environment and Primary Industries, Victoria. They thank J. Wilson, M. S. McLean, S. P. Taylor, J. P. Thompson, H. S. Bariana, M. M. Shankar and A. Milgate for their assistance with disease phenotyping.

References

Adhikari TB, Jackson EW, Gurung S, Hansen JM, Bonman JM (2011) Association mapping of quantitative resistance to *Phaeosphaeria nodorum* in spring wheat landraces from the USDA National Small Grains Collection. Phytopathology 101(11):1301-1310

493 Ágnes SH, Szabolcs LK, Mónika V, László P, János P, Csaba L, Ákos M (2014) Differential influence of QTL
494 linked to Fusarium head blight, Fusarium-damaged kernel, deoxynivalenol contents and associated
495 morphological traits in a Frontana-derived wheat population. Euphytica 200(1):9-26

496 Aguilar V, Stamp P, Winzeler M, Winzeler H, Schachermayr G, Keller B et al (2005) Inheritance of field resistance
497 to *Stagonospora nodorum* leaf and glume blotch and correlations with other morphological traits in hexaploid
498 wheat (*Triticum aestivum* L.). Theor Appl Genet 111(2):325-336

499 Alam KB, Gustafson JP (1988) Tan-spot resistance screening of *Aegilops* species. Plant Breeding 100:112–118

500 Appel JA, DeWolf E, Bockus WW, Todd T (Preliminary 2011) Kansas wheat disease loss estimates. Kansas
501 cooperative plant disease survey report, August 18-2011

502 Assefa S, Fehrmann H (2000) Resistance to wheat leaf rust in *Aegilops tauschii* Coss and inheritance of resistance in
503 hexaploid wheat. Genet Resour Crop Evol 47:135–140

504 Basnet BR, Singh RP, Ibrahim AMH, Herrera-Foessel SA, Huerta-Espino J, Lan C, Rudd JC (2014)
505 Characterization of *Yr54* and other genes associated with adult plant resistance to yellow rust and leaf rust in
506 common wheat Quaiu 3. Mol breed 33(2):385-399

507 Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple
508 testing. J R Statist Soc B 57:289–300

509 Bhavani S, Singh RP, Argillier O, Huerta-Espino J, Singh S, Njau P, Brun S, Lacam S and Desmouceaux N (2011)
510 Mapping durable adult plant stem rust resistance to the race Ug99 group in six CIMMYT wheats. 2011 BGRI
511 Technical Workshop, pp 43–53

512 Braun HJ, Atlin G, Payne T (2010) Multi-location testing as a tool to identify plant response to global climate
513 change. Reynolds, CRP. (Ed.) Climate change and crop production, CABI, London, UK.

514 Breseghello F, Sorrells MS (2006) Association mapping of kernel size and milling quality in wheat (*Triticum*
515 *aestivum* L.) cultivars. Genetics 172:1165-1177

Chen J, Chu C, Souza EJ, Guttieri MJ, Chen X, Xu S, et al (2012) Genome-wide identification of QTL conferring high-temperature adult-plant (HTAP) resistance to stripe rust (*Puccinia striiformis* f. sp. *tritici*) in wheat. Mol breed 29(3):791-800

Cockram J, Scuderi A, Barber T, Furuki E, Gardner KA, Gosman N, Kowalczyk R, Phan HP, Rose GA, Tan KC, Oliver RP (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(11):2257-66

Crossa J, Burgueño J, Dreisigacker S, Vargas M, Herrera-Foessel SA, Lillemo M, Singh RP, Trethowan R, Warburton M, Franco J, Reynolds M, Crouch JH, Ortiz R (2007) Association analysis of historical bread wheat germplasm using additive genetic covariance of relatives and population structure. Genetics 177:1889–1913

Czembor PC, Arseniuk E, Czaplicki A, Song Q, Cregan PB, Ueng PP (2003) QTL mapping of partial resistance in winter wheat to *Stagonospora nodorum* blotch. Genome 46(4):546-554

Dedryver F, Paillard S, Mallard S, Robert O, Trottet M, Nègre S, Verplancke G, Jahier J (2009) Characterization of genetic components involved in durable resistance to stripe rust in the bread wheat 'Renan'. Phytopathology 99:968–973

Detering F, Hunter E, Uszynski G, Wenzl P, Andrzej K (2010) A consensus genetic map of wheat: ordering 5,000 Wheat DArT markers. 20th ITMI & 2nd WGC Workshop, 1–5 September, Beijing

Dreisigacker S, Kishii M, Lage J, Warburton M (2008) Use of synthetic hexaploid wheat to increase diversity for CIMMYT bread wheat improvement. Aust J Agric Res 59:413–420

Eastwood RF, Lagudah ES, Appels R, Hannah M, Kollmorgen JF (1991) *Triticum tauschii*: a novel source of resistance to cereal cyst nematode (*Heterodera avenae*). Aust J Agric Res 42:69–77

Eberhard FS (2011) Molecular marker assisted selection for crown rot resistance in *Triticum turgidum* ssp. durum (Doctoral dissertation, University of Southern Queensland)

539 Faris JD, Friesen TL (2005) Identification of quantitative trait loci for race-nonspecific resistance to tan spot in
540 wheat. Theor Appl Genet 111:386-392

541 Farrer W (1898) The making and improvement of wheats for Australian conditions. AgricGaz NSW 9:131–168

542 Flint-Garcia SA, Thornsberry JM, Buckler ES (2003) Structure of linkage disequilibrium in plants. Annu Rev Plant
543 Biol 54:357-374

544 Flint-Garcia SA, Thuillet AC, Yu J, Pressoir G, Romero SM, Mitchell SE, Doebley J, Kresovich S, Goodman MM
545 and Buckler ES (2005) Maize association population: a high-resolution platform for quantitative trait locus
546 dissection. Plant Journal 44:1054–1064

547 Friesen TL, Faris JD (2004) Molecular mapping of resistance to *Pyrenophora tritici-repentis* race 5 and sensitivity
548 to *PtrToxB* in wheat. Theor Appl Genet 109:464-471

549 Genc Y, Oldach K, Gogel B, Wallwork H, McDonald GK, Smith AB (2013) Quantitative trait loci for agronomic
550 and physiological traits for a bread wheat population grown in environments with a range of salinity levels. Mol
551 breed 32(1):39-59

552 Goldstein DB, Tate SK, Sisodiya SM (2003) Pharmacogenetics goes genomics. Nat Rev Genet 4:937-947

553 Guerrero-Chavez R, Glover KD, Rouse MN, Gonzalez-Hernandez JL (2015) Mapping of two loci conferring
554 resistance to wheat stem rust pathogen races TTKSK (Ug99) and TRTTF in the elite hard red spring wheat line
555 SD4279. Mol Breed 35(1):1-10

556 Gupta SK, Charpe A, Prabhu KV, Haque QMR (2006) Identification and validation of molecular markers linked to
557 the leaf rust resistance gene *Lr19* in wheat. Theor Appl Genet 113(6):1027-1036

558 Gurung S, Mamidi S, Bonman JM, Jackson EW, del Rio LE, Acevedo M, Mergoum M, Adhikari TB (2011)
559 Identification of novel genomic regions associated with resistance to *Pyrenophora tritici-repentis* races 1 and 5
560 in spring wheat landraces using association analysis. Theor Appl Genet 123:1029–1041

561 Gurung S, Mamidi S, Bonman JM, Xiong M, Brown-Guedira G, Adhikari TB (2014) Genome-wide association
562 study reveals novel quantitative trait Loci associated with resistance to multiple leaf spot diseases of spring
563 wheat. PLoS ONE 9(9):e108179

564 Hatchett JH, Martin TJ, Livers RW (1981) Expression and inheritance of resistance to Hessian fly in synthetic
565 hexaploid wheats derived from *Triticum tauschii* (Coss) Schmal. Crop Sci 21:731–734

566 Hedrick PW (1987) Gametic disequilibrium measures: proceed with caution. Genetics 117:331-374

567 Herrera-Foessel SA, Singh RP, Huerta-Espino J, Salazar VC, Lagudah ES (2011) First report of slow rusting gene
568 *Lr46* in durum wheat. Borlaug Global Rust Initiative, June 13-16, 2011 Technical Workshop, St Paul,
569 Minnesota, USA pp191

570 Jighly A, Joukhadar R, Alagu M (2015a) SimpleMap: A Pipeline to Streamline High-Density Linkage Map
571 Construction. The Plant Genome 8(2). DOI:10.3835/plantgenome2014.09.0056

572 Jighly A, Oyiga BC, Makdis F, Nazari K, Youssef O, Tadesse W, Abdalla O, Ogbonnaya FC (2015b) Genome-wide
573 DArT and SNP scan for QTL associated with resistance to stripe rust (*Puccinia striiformis* f. sp. *tritici*) in elite
574 ICARDA wheat (*Triticum aestivum* L.) germplasm. Theor Appl Genet 128:1277–1295

575 Joukhadar R, El-Bouhssini M, Jighly A, Ogbonnaya FC (2013) Genomic regions associated with resistance to five
576 major pests in wheat. Mol Breed 32:943–960

577 Krattinger SG, Lagudah ES, Spielmeyer W et al (2009) A putative ABC transporter confers durable resistance to
578 multiple fungal pathogens in wheat. Science 323:1360–1363

579 Kumar U, Joshi AK, Kumar S, Chand R, Röder MS (2010) Quantitative trait loci for resistance to spot blotch caused
580 by *Bipolaris sorokiniana* in wheat (*T. aestivum* L.) lines 'Ning 8201'and 'Chirya 3'. Mol breed 26(3):477-491

581 Lagudah ES (2011) Molecular genetics of race non-specific rust resistance in wheat. Euphytica 179:81–91

582 Lagudah ES, Krattinger SG, Herrera-Foessel S, Singh RP, Huerta-Espinoso J, Spielmeyer W, Brown-Guedira G,
583 Selter LL, Keller B (2009) Gene-specific markers for the wheat gene Lr34/Yr18/Pm38 which confers resistance
584 to multiple fungal pathogens. Theor Appl Genet 119: 889-898

585 Letta T, Maccaferri M, Badebo A, Ammar K, Ricci A, Crossa J, Tuberosa R (2013) Searching for novel sources of
586 field resistance to Ug99 and Ethiopian stem rust races in durum wheat via association mapping. Theor Appl
587 Genet 126(5):1237-1256

588 Lipka AE, Tian F, Wang Q, Peiffer J, Li M, Bradbury PJ et al (2012) GAPIT: genome association and prediction
589 integrated tool. Bioinformatics 28(18):2397-2399

590 Loughman R, Lagudah ES, Trottet M, Wilson RE, Mathews A (2001) *Septoria nodorum* blotch resistance in
591 *Aegilops tauschii* and its expression in synthetic amphiploids. Aust J Agric Res 52:1393–1402

592 Lowe I, Jankuloski L, Chao S, Chen X, See D, Dubcovsky J (2011) Mapping and validation of QTL which confer
593 partial resistance to broadly virulent post-2000 North American races of stripe rust in hexaploid wheat. Theor
594 Appl Genet 123(1):143-157

595 Lutz J, Hsam SLK, Limpert E, Zeller FJ (1994) Powdery mildew resistance in *Aegilops tauschii* Coss. and synthetic
596 hexaploid wheats. Genet Resour Crop Evol 41:151–158

597 Ma H, Singh RP, Mujeeb-KaziA (1995) Resistance to stripe rust in *Triticum turgidum*, *T. tauschii* and their
598 synthetic hexaploids. Euphytica 82:117–124

599 Ma J, Li HB, Zhang CY, Yang XM, Liu YX, Yan GJ, Liu CJ (2010) Identification and validation of a major QTL
600 conferring crown rot resistance in hexaploid wheat. Theor Appl Genet 120(6):1119-1128

601 Maccaferri M, Sanguineti MC, Natoli E, Araus-Ortega JL, Bensalem M et al (2006) A panel of elite accessions of
602 durum wheat (*Triticum durum* Desf.) suitable for association mapping studies. Plant Genet Resour 4:79–85

603 Mackay I, Powell W (2006) Methods for linkage disequilibrium mapping in crops. Trends Plant Sci 12:57–63

604 Marais GF, Potgieter GF, Roux HS (1994) An assessment of the variation for stem rust resistance in the progeny of
605 a cross involving the *Triticum* species *aestivum*, *turgidum* and *tauschii*. S Afr J Plant Soil 11:15–19

606 Mebrate SA, Oerke EC, Dehne HW, Pillen K (2008) Mapping of the leaf rust resistance gene Lr38 on wheat
607 chromosome arm 6DL using SSR markers. Euphytica 162(3):457-466

608 Moore JH, White BC (2007) Tuning Relief for genome-wide genetic analysis. In: Marchiori E, Moore JH,
609 Rajapakse JC. Lecture Notes in Computer Science Volume 4447. New York: Springer. Pp. 166-175

610 Moore JW, Herrera-Foessel S, Lan C, Schnippenkoetter W, Ayliffe M, Huerta-Espino J, Lillemo M, Viccars L,
611 Milne R, Periyannan S, Kong X (2015) A recently evolved hexose transporter variant confers resistance to
612 multiple pathogens in wheat. Nature genetics 47:1494–1498

613 Mujeeb-Kazi A, Cano S, Rosas V, Cortes A, Delgado R (2001a) Registration of five synthetic hexaploid wheat and
614 seven bread wheat lines resistant to wheat spot blotch. Crop Sci 41:1653–1654

615 Mujeeb-Kazi A, Delgado R, Juarez L, Cano S (2001b) Scab resistance (Type II: spread) in synthetic hexaploid
616 germplasm. Ann Wheat Newsl 47:118–120

617 Mulki MA, Jighly A, Ye G, Emebiri LC, Moody D, Ansari O, Ogbonnaya FC (2013) Association mapping for
618 soilborne pathogen resistance in synthetic hexaploid wheat. Mol Breed 3:299-311

619 Neumann K, Kobiljski B, Denčić S, Varshney RK, Börner A (2011) Genome-wide association mapping: a case
620 study in bread wheat (*Triticum aestivum* L.). Mol Breed 27:37–58

621 Ogbonnaya FC (2011) Development, management and utilization of synthetic hexaploid in wheat improvement. In:
622 Bonjean AP, Angus WJ, van Ginkel M. The World Wheat Book – A history of Wheat Breeding Volume 2.
623 Lavoisier, Paris, France. pp. 823-843

624 Ogbonnaya FC, Abdalla O, Mujeeb-Kazi A, AG Kazi, Xu Steven, Gosman N, Lagudah ES, Bonnett D, Sorells ME,
625 Tsujimoto H (2013) Synthetic hexaploids: Harnessing species of primary gene pool for wheat improvement.
626 Plant Breeding Reviews 37:35-122

627 Ogbonnaya FC, Imtiaz M, Bariana HS, McLean M, Shankar M, Hollaway GJ, Trethowan R, Lagudah ES, van
628 Ginkel M (2008) Mining synthetic hexaploids for multiple disease resistance to improve wheat. Aust J Agric
629 Res 59:421-431

630 Ogbonnaya FC, Seah S, Delibes A, Jahier J, Lopez-Brana I, Eastwood RF, Lagudah ES (2001) Molecular-genetic
631 characterisation of a new nematode resistance gene in wheat. Theor Appl Genet 102:623–629

632 Oliver RP, Tucker M, Rybak K, Antoni E, Lichtenzveig J (2011) Managing fungicide resistance in broad acre
633 cropping in Australia. Research update report in GRDC Australia.
634 (http://www.grdc.com.au/director/events/researchupdates?item_id=C13F2CCD0DF0838ED195B4C05D522FD
635 5&pageNumber=1)

636 Perrier X, Jacquemoud-Collet JP: DARwin software. 2006, http://darwin.cirad.fr/

637 Poole GJ, Smiley RW, Paulitz TC, Walker CA, Carter AH, See DR, Garland-Campbell K (2012) Identification of
638 quantitative trait loci (QTL) for resistance to Fusarium crown rot (*Fusarium pseudograminearum*) in multiple
639 assay environments in the Pacific Northwestern US. Theor Appl Genet 125(1):91-107

640 Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data.
641 Genetics 155:945-959

642 Rosewarne GM, Herrera‐Foessel SA, Singh RP, Huerta‐Espino J, Lan CX, He ZH (2013) Quantitative trait loci of
643 stripe rust resistance in wheat. Theor Appl Genet 126(10):2427-2449

644 Santra DK, Chen XM, Santra M, Campbell KG, Kidwell KK (2008) Identification and mapping QTL for high-
645 temperature adult-plant resistance to stripe rust in winter wheat (*Triticum aestivum* L.) cultivar 'Stephens'.
646 Theor Appl Genet 117:793–802

647 Schnurbusch T, Paillard S, Fossati D, Messmer M, Schachermayr G, Winzeler M, Keller B (2003) Detection of
648 QTLs for Stagonospora glume blotch resistance in Swiss winter wheat. Theor Appl Genet 107(7):1226-1234

649 Segrè D, DeLuna A, Church GM, Kishony R (2005) Modular epistasis in yeast metabolism. Nat Genet 37:77-83

650 Sehgal D, Vikram P, Sansaloni CP, Ortiz C, Saint Pierre C, Payne T et al (2015) Exploring and Mobilizing the Gene
651 Bank Biodiversity for Wheat Improvement. PLoS ONE 10(7):e0132112

652 Shankar M, Walker E, Golzar H, Loughman R, Wilson RE, Francki MG (2008) Quantitative trait loci for seedling
653 and adult plant resistance to *Stagonospora nodorum* in wheat. Phytopathology 98(8):886-893

654 Shirasu K, Schulze-Lefert P (2003) Complex formation, promiscuity and multi-functionality: protein interactions in
655 disease-resistance pathways. Trends in Plant Science 8(6):252-258

Siedler H, Obst A, Hsam SLK, Zeller FJ (1994) Evaluation for resistance to *Pyrenophora tritici-repentis* in *Aegilops tauschii* Coss. and synthetic hexaploid amphiploids. Genet Resour Crop Evol 41:27–34

Singh A, Pandey MP, Singh AK, Knox RE, Ammar K, Clarke JM, Clarke F, Singh RP, Pozniak CJ, DePauw RM, McCallum B, Cuthbert RD, Randhawa HS, Fetch T (2013) Identification and mapping of leaf, stem and stripe rust resistance QTL and their interactions in durum wheat. Mol Breed 31:405–418

Singh PK, Mergoum M, Ali S, Adhikari TB, Elias EM, Hughes GR (2006) Identification of new sources of resistance to tan spot, *Stagonospora nodorum* blotch, and *Septoria tritici* blotch of wheat. Crop science 46(5):2047-2053

Singh S, Franks CD, Huang L, Brown-Guedira GL, Marshall DS, Gill BS, Fritz A (2004) *Lr41*, *Lr39*, and a leaf rust resistance gene from *Aegilops cylindrica* may be allelic and are located on wheat chromosome 2DS. Theor Appl Genet 108(4):586-591

Solh M, Nazari K, Tadesse W, Wellings CR (2012) The growing threat of stripe rust worldwide. Paper presented at: Borlaug Global Rust Initiative (BGRI) conference, Beijing, China. 1–4 Sept. 2012

Spielmeyer W, Singh RP, McFadden H, Wellings CR, Huerta-Espino J, Kong X, Appels R, Lagudah ES (2008) Fine scale genetic and physical mapping using interstitial deletion mutants of *Lr34*/*Yr18*: a disease resistance locus effective against multiple pathogens in wheat. Theor Appl Genet 116:481–490

Sukhwinder-Singh, Hernandez MV, Crossa J, Singh PK, Bains NS, Singh K, Sharma I (2012) Multi-Trait and Multi-Environment QTL Analysis for Resistance to Wheat Diseases. PLoS ONE 7(6):e38008

Sun X, Bai G, Carver BF, Bowden R (2010) Molecular Mapping of Wheat Leaf Rust Resistance Gene. Crop science 50(1):59-66

Tadesse W, Hsam SL, Wenzel G, Zeller FJ (2006) Identification and Monosomic Analysis of Tan Spot Resistance Genes in Synthetic Wheat Lines (L.× Coss.). Crop science 46(3):1212-1217

Tadesse W, Hsam SLK, Zeller FJ (2007) Evaluation of common wheat cultivars for tan spot resistance and chromosomal location of a resistance gene in the cultivar 'Salamouni'. Plant Breed 125:318–322

680 Tadesse W, Ogbonnaya FC, Jighly A, Nazari K, Rajaram S, Baum M (2014) Association mapping of resistance to
681 yellow rust in winter wheat cultivars and elite genotypes. Crop Science 54(2):607-616

682 Tadesse W, Ogbonnaya FC, Jighly A, Sanchez-Garcia M, Sohail Q, Rajaram S, Baum M (2015) Genome-Wide
683 Association Mapping of Yield and Grain Quality Traits in Winter Wheat Genotypes. PLoS ONE
684 10(10):e0141339.

685 Thompson JP (2008) Resistance to root-lesion nematodes (*Pratylenchusthornei* and *P. neglectus*) in synthetic
686 hexpaloid wheats and their durum and *Aegilops tauschii* parents. Aust J Agric Res 59:432–446

687 van Ginkel M, Ogbonnaya FC (2007) Novel genetic diversity from synthetic wheats in breeding cultivars for
688 changing production conditions. Field Crops Research 104:86–94

689 Weir BS (1996) Genetic data analysis II: methods for discrete populations genetic data. Sinauer Associates,
690 Sunderland

691 Villareal RL, Mujeeb-Kazi A, Fuentes-Davila G, Rajaram S, Toro ED (1994) Resistance to karnal bunt (*Tilletia*
692 *indica* Mitra) in synthetic hexaploid wheats derived from *Triticum turgidum* × *T. tauschii*. Plant
693 breeding 112(1):63-69

694 White J, Law JR, Mackay I, Chalmers KJ, Smith JSC, Kilian A, Powell W (2008) The genetic diversity of UK, US
695 and Australian cultivars of *Triticum aestivum* measured by DArT markers and considered by genome. Theor
696 Appl Genet 116:439-453

697 William M, Singh RP, Huerta-Espino J, Islas SO, Hoisington D (2003) Molecular marker mapping of leaf rust
698 resistance gene *Lr46* and its association with stripe rust resistance gene *Yr29* in wheat. Phytopathology 93:153-
699 159

700 Wissera RJ, Kolkmanb JM, Patzoldta ME, Hollandc JB, Yud J, Krakowskyc M, Nelsonb RJ, Balint-Kurtie PJ
701 (2011) Multivariate analysis of maize disease resistances suggests a pleiotropic genetic basis and implicates a
702 GST gene. Proc Natl Acad Sci USA 108(18):7339-7344

703 Xu SS, Friesen TL, Mujeeb-Kazi A (2004) Seedling resistance to tan spot and *stagonospora nodorum* blotch in
704 synthetic hexaploid wheats. Crop Sci 44:2238-2245

705 Yan W (2001) GGEBiplot - a Windows application for graphical analysis of multi-environment trial data and other
706 types of two-way data, Agron J 93:1111–1118Yan W, Kang MS (2002) Cultivar Evaluation Based on Multiple
707 Traits. In: GGE biplot analysis: A graphical tool for breeders, geneticists, and agronomists. CRC press.

708 Yu G, Zhang Q, Friesen TL, Rouse MN, Jin Y, Zhong S et al (2015) Identification and mapping of Sr46 from
709 *Aegilops tauschii* accession CIae 25 conferring resistance to race TTKSK (Ug99) of wheat stem rust
710 pathogen. Theor Appl Genet 128(3):431-443

711 Yu J, Buckler ES (2006) Genetic association mapping and genome organization of maize. Curr Opin Biotechnol
712 17:155–160

713 Yu LX, Barbier H, Rouse MN, Singh S, Singh RP, Bhavani S et al (2014) A consensus map for Ug99 stem rust
714 resistance loci in wheat. Theor Appl Genet 127(7):1561-1581

715 Yu LX, Lorenz A, Rutkoski J, Singh RP, Bhavani S, Huerta-Espino J, Sorrells ME (2011) Association mapping and
716 gene-gene interaction for stem rust resistance in spring wheat germplasm. Theor Appl Genet 123:1257–1268

717 Zegeye H, Rasheed A, Makdis F, Badebo A, Ogbonnaya FC (2014) Genome-wide association mapping for seedling
718 and adult plant resistance to stripe rust in synthetic hexaploid wheat. PLoS ONE 9(8):e105593.

719 Zhang Z, Ersoz E, Lai CQ, Todhunter RJ, Tiwari HK, Gore MA, Bradbury PJ, Yu J, Arnett DK, Ordovas JM
720 Buckler ES (2010) Mixed linear model approach adapted for genome-wide association studies. Nature genetics
721 42(4):355-360

722 Zwart RS, Thompson JP, Milgate AW, Bansal UK, Williamson PM, Raman H, Bariana HS (2010) QTL mapping of
723 multiple foliar disease and root-lesion nematode resistances in wheat. Mol Breed 26:107–124

724 Table 1. List of significant markers that are associated with six fungal disease resistances and previously reported genes and QTL in
725 the regions of QTL identified in this study.

Disease	QTL	Marker	Chr[a]	Position	P	MAF[b]	R^2	FDR[c]	Add[d]	Gene/Marker	Reference
Cr	Cr01	*1384280*	2BL	179.5	4.3E-04	0.31	9.2	0.95	0.6	*wPt-9336*	Eberhard 2011
Cr	Cr02	*983467*	3BL	130.3	8.7E-04	0.25	8.2	0.95	0.6	*wPt-5390*	Poole et al. 2012
Cr	Cr03	*3022945*	7DL	265.4	1.0E-03	0.15	8.0	0.95	-0.7	*wPt-3462*	Ma et al. 2010
Cr	Cr03	*1365344*	7DL*	-	8.8E-04	0.25	8.2	0.95	0.6	*wPt-3462*	Ma et al. 2010
Cr	Cr03	*3021273*	7DL*	-	4.0E-04	0.43	9.3	0.95	0.5	*wPt-3462*	Ma et al. 2010
Lr	Lr01	*Lr46/Yr29* [1]	1BL	-	5.8E-04	0.28	8.4	0.95	-1.7	*Lr46*	William et al. 2003
Lr	Lr02	*985475*	1DS	47.8	8.6E-04	0.13	6.7	0.95	-1.2	*Lr42*	Sun et al. 2010
Lr	Lr03	*1106131*	2DL	257.2	8.1E-04	0.27	6.7	0.95	-1.5	*Lr39*	Singh et al. 2004
Lr	Lr03	*1097758*	2DL*	-	3.1E-04	0.25	7.8	0.95	-1.1	*Lr39*	Singh et al. 2004
Lr	Lr04	*1126111* [2]	6D*	-	1.0E-03	0.25	4.9	0.95	1.1	*Lr38*	Mebrate et al. 2008
Lr	Lr04	*1126778* [2]	6D*	-	9.1E-04	0.24	5.5	0.95	1.1	*Lr38*	Mebrate et al. 2008
Lr	Lr04	*3027151*	6D*	-	3.0E-04	0.20	7.9	0.95	1.6	*Lr38*	Mebrate et al. 2008
Lr	Lr05	*1047131*	7DL	198.2	2.8E-04	0.31	8.0	0.95	1.3	*Lr19*	Gupta et al. 2006
Lr	Lr05	*2337373*	7DL*	-	8.8E-04	0.27	6.6	0.95	1.3	*Lr19*	Gupta et al. 2006
SNG	SNG01	*wPt-1657*	2AL	129.8	3.1E-05	0.20	13.4	0.19	0.8	*QSng.daw-2A*	Shankar et al. 2008
SNG	SNG02	*1107710*	2BL	163.8	8.1E-04	0.30	8.5	0.81	1.2	*XU36894*	Czembor et al. 2003
SNG	SNG03	*wPt-7825*	2DL	289.8	4.6E-05	0.14	12.8	0.19	0.9	*wPt-665102*	Adhikari et al. 2011
SNG	SNG04	*wPt-8079*	3BS	18.1	7.7E-04	0.16	8.6	0.81	0.7	*Xbarc147*	Czembor et al. 2003
SNG	SNG04	*wPt-2757*	3BS	26.3	3.3E-04	0.20	9.8	0.63	0.7	*Xbarc147*	Czembor et al. 2003
SNG	SNG04	*wPt-3921* [3]	3BS	27.9	8.3E-04	0.17	7.8	0.81	0.7	*Xbarc147*	Czembor et al. 2003
SNG	SNG05	*1094836* [4]	4BL	78.2	1.0E-03	0.45	6.2	0.91	0.7	*QSng.daw-4B*	Shankar et al. 2008
SNG	SNG06	*wPt-8262*	5AL	233.0	2.3E-05	0.16	13.9	0.19	0.9	*QSnn.niab-5A.1*	Cockram et al. 2015
SNG	SNG07	*1019982* [5]	6B*	-	9.7E-04	0.06	8.2	0.81	1.5	*QSng.sfr-6BL*	Schnurbusch et al. 2003
SNG	SNG08	*1263913*	7DS	44.6	1.5E-04	0.35	11.0	0.42	2.0	*QSb.bhu-7D*	Kumar et al. 2010
SNG	SNG08	*1233921*	7DL	224.4	3.6E-04	0.32	9.7	0.63	-2.0	*QSb.bhu-7D*	Kumar et al. 2010

SNG	SNG08	*1216888*	7DL*	-	1.7E-04	0.32	10.8	0.42	-1.9	*QSb.bhu-7D*	Kumar et al. 2010
SNG	SNG08	*1227840*	7DL*	-	7.0E-04	0.31	8.7	0.81	-1.1	*QSb.bhu-7D*	Kumar et al. 2010
SNL	SNL01	*1208964*	7B*	-	1.4E-03	0.35	8.4	1	0.9	*QSnl.eth-7B3*	Aguilar et al. 2005
Sr	Sr01	*1006460*	1BL	289.0	2.4E-04	0.48	9.7	0.81	-0.9	*Sr58*	Yu et al. 2014
Sr	Sr02	*1088175*	2DS	36.1	3.3E-04	0.34	9.3	0.81	-1.1	*Sr46*	Yu et al. 2015
Sr	Sr02	*2247181*	2DS	40.8	7.7E-04	0.42	8.1	0.98	-1.2	*Sr46*	Yu et al. 2015
Sr	Sr02	*1109593*	2DS*	-	1.8E-04	0.38	10.1	0.81	-1.0	*Sr46*	Yu et al. 2015
Sr	Sr03	*1101415*	2DL*	-	4.0E-04	0.37	9.0	0.81	2.2	-	Novel
Sr	Sr03	*1102301*	2D*	-	5.0E-04	0.36	8.5	0.88	1.8	-	Novel
Sr	Sr04	*wPt-3921* [3]	3BS	27.9	1.1E-03	0.19	7.5	0.98	-0.7	*wPt-3921*	Yu et al. 2014
Sr	Sr05	*1094836* [4]	4BL	78.2	1.1E-03	0.44	7.7	0.98	-0.9	*Sr37*	Yu et al. 2014
Sr	Sr05	*wPt-8543*	4BL	98.0	4.6E-04	0.21	8.8	0.81	-0.8	*Sr37*	Yu et al. 2014
Sr	Sr06	*1019982* [5]	6B*	-	1.1E-03	0.07	6.0	0.98	-1.3	*wPt-5333*	Yu et al. 2011
Sr	Sr07	*983699*	6DL	190.9	3.8E-04	0.38	9.1	0.81	-0.8	*Sr29*	Yu et al. 2014
Sr	Sr07	*2255204*	6DL	194.0	1.7E-04	0.25	10.2	0.81	-1.2	*Sr29*	Yu et al. 2014
YLS	YLS01	*wPt-2706*	1BS	109.6	5.0E-04	0.34	7.5	0.29	-0.6	*QTs.fcu-1BS*	Faris & Friesen 2005
YLS	YLS02	*3034128*	2DL	252.2	5.0E-04	0.06	7.5	0.29	-1.2	*wPt-664805*	Gurung et al. 2011
YLS	YLS02	*2242263*	2DL	254.3	5.6E-04	0.07	7.4	0.3	-1.2	*wPt-664805*	Gurung et al. 2011
YLS	YLS02	*1385233*	2DL	292.4	7.4E-04	0.06	7.0	0.35	-1.2	*wPt-664805*	Gurung et al. 2011
YLS	YLS02	*1052396*	2DL*	-	2.1E-05	0.06	11.4	0.15	-1.4	*wPt-664805*	Gurung et al. 2011
YLS	YLS02	*1101263*	2DL*	-	6.9E-04	0.07	7.1	0.34	-1.1	*wPt-664805*	Gurung et al. 2011
YLS	YLS02	*1072100*	2DL*	-	4.6E-04	0.07	7.6	0.29	-1.2	*wPt-664805*	Gurung et al. 2011
YLS	YLS02	*1092634*	2DL*	-	1.2E-04	0.05	9.3	0.2	-1.4	*wPt-664805*	Gurung et al. 2011
YLS	YLS02	*1097383*	2DL*	-	3.1E-04	0.06	8.0	0.29	-1.2	*wPt-664805*	Gurung et al. 2011
YLS	YLS02	*1100277*	2DL*	-	1.2E-04	0.05	9.3	0.2	-1.4	*wPt-664805*	Gurung et al. 2011
YLS	YLS02	*1100904*	2DL*	-	5.0E-04	0.06	7.5	0.29	-1.2	*wPt-664805*	Gurung et al. 2011
YLS	YLS02	*1118682*	2DL*	-	1.2E-04	0.05	9.3	0.2	-1.4	*wPt-664805*	Gurung et al. 2011
YLS	YLS02	*1135085*	2DL*	-	5.6E-04	0.06	7.3	0.3	-1.2	*wPt-664805*	Gurung et al. 2011
YLS	YLS02	*1144738*	2DL*	-	3.7E-05	0.06	10.7	0.15	-1.5	*wPt-664805*	Gurung et al. 2011
YLS	YLS02	*1319101*	2DL*	-	5.0E-04	0.06	7.5	0.29	-1.2	*wPt-664805*	Gurung et al. 2011

YLS	YLS02	*2241933*	2DL*	-	5.0E-04	0.06	7.5	0.29	-1.2	*wPt-664805*	Gurung et al. 2011
YLS	YLS02	*2242411*	2DL*	-	5.0E-04	0.06	7.5	0.29	-1.2	*wPt-664805*	Gurung et al. 2011
YLS	YLS03	*1116422*	3DS	8.3	2.2E-04	0.14	8.5	0.26	-0.9	*tsn3*	Tadesse et al. 2007
YLS	YLS03	*1122499*	3D	97.7	4.4E-05	0.29	10.4	0.15	1.1	*tsn3*	Tadesse et al. 2007
YLS	YLS03	*1089159*	3DL	170.1	7.1E-04	0.26	7.1	0.34	-0.8	*tsn3*	Tadesse et al. 2007
YLS	YLS03	*3020470*	3DL	270.5	4.9E-05	0.30	10.3	0.15	1.0	*tsn3*	Tadesse et al. 2007
YLS	YLS03	*3222137*	3DL*	-	4.5E-04	0.37	7.6	0.29	-1.6	*tsn3*	Tadesse et al. 2007
YLS	YLS04	*1037337*	6DL	169.1	7.9E-04	0.13	6.9	0.36	0.8	-	Novel
YLS	YLS04	*100018632*	6DL*	-	9.4E-04	0.26	6.7	0.4	-0.7	-	Novel
YLS	YLS04	*1114521*	6DL*	-	3.7E-04	0.20	7.9	0.29	0.8	-	Novel
YLS	YLS04	*1126111* [2]	6DL*	-	8.3E-03	0.24	5.0	0.74	-0.6	-	Novel
YLS	YLS04	*1126778* [2]	6DL*	-	7.7E-04	0.23	5.5	0.61	-0.7	-	Novel
YLS	YLS04	*1139583*	6DL*	-	1.6E-04	0.37	8.8	0.25	0.7	-	Novel
YLS	YLS04	*1233591*	6DL*	-	2.2E-04	0.15	8.5	0.26	0.8	-	Novel
YLS	YLS04	*2249359*	6DL*	-	2.4E-04	0.30	8.4	0.26	1.0	-	Novel
YLS	YLS05	*1054897*	7DL*	-	9.3E-04	0.21	6.7	0.4	-0.7	*wPt-730876*	Gurung et al. 2011
Yr	Yr01	*988333*	1BL	162.3	6.8E-04	0.41	10.4	1	0.9	*QYr.tam-1B*	Basnet et al. 2014
Yr	Yr01	*1093720*	1BL*	-	5.7E-04	0.35	10.7	1	1.0	*QYr.tam-1B*	Basnet et al. 2014
Yr	Yr02	*LR46/Yr29* [1]	1BL	-	4.4E-04	0.28	11.1	0.95	-1.5	*Yr29*	William et al. 2003
Yr	Yr03	*wPt-1615*	2AL	212.0	1.2E-04	0.35	13.5	0.76	0.8	*QRYr2A.2*	Dedryver et al. 2009
Yr	Yr03	*wPt-2858*	2AL	212.0	1.2E-04	0.35	13.5	0.76	0.8	*QRYr2A.2*	Dedryver et al. 2009
Yr	Yr04	*wPt-8776*	2BL	167.7	5.6E-04	0.25	10.7	1	0.8	-	Novel
Yr	Yr05	*100136169*	3D*	-	5.5E-04	0.36	10.8	1	-0.7	-	Zegeye et al. 2014
Yr	Yr05	*1267912*	3D*	-	4.5E-04	0.24	11.2	1	-1.2	-	Zegeye et al. 2014
Yr	Yr06	*wPt-8153*	6BS	25.8	9.2E-04	0.32	9.4	1	0.7	*QRYr6B.1*	Santra et al. 2008

[a] Chr: Chromosome; [b] MAF: minor allele frequency; [c] FDR: false discovery rate; [d] Add: Additive effect; * Chromosomes determined by linkage disequilibrium with other markers; Numbers from [1-5] account for markers with multiple associations.

729 Table 2: List of validated QTL and their associated markers.

Disease	**QTL**	**Marker**	**Chr[a]**	**P**	**MAF[b]**	**R^2**	**MM[c]**
Lr	Lr01	*LR46*	1BL	3.8E-03	0.22	4.2	*LR46* *
Lr	Lr05	*tPt-4614*	7DL	1.9E-03	0.48	7	*1047131*
SNG	SNG04	*wPt-3921*	3BS	3.5E-03	0.33	5.6	*wPt-3921* *
SNG	SNG04	*wPt-8079*	3BS	1.9E-03	0.48	7	*wPt-8079*
SNG	SNG04	*wPt-2757*	3BS	2.1E-03	0.49	6.7	*wPt-2757*
SNG	SNG05	*wPt-7412*	4BL	5.0E-03	0.45	6.7	*1094836* *
SNL	SNL01	*wPt-5069*	7B	5.0E-03	0.2	7.6	*1208964*
Sr	Sr01	*wPt-6690*	1BL	3.1E-03	0.48	5.1	*1006460*
Sr	Sr06	*wPt-1113*	6B	2.4E-03	0.39	5.6	*1019982* *
Sr	Sr07	*wPt-2518*	6DL	2.4E-03	0.44	5.6	*2255204*
YLS	YLS01	*wPt-9524*	1BS	4.5E-03	0.14	5.6	*wPt-2706*
YLS	YLS02	*wPt-2644*	2DL	4.5E-03	0.41	5.6	*1052396*
Yr	Yr02	*LR46*	1BL	3.1E-03	0.4	4.5	*LR46* *

730

731 [a] Chr: Chromosome

732 [b] MAF: minor allele frequency

733 [c] MM: main set marker

734 * Account for markers with multiple associations.

735

736

737 Table 3 Gene–gene interactions for the studied disease resistances.

738

Disease	ID	QTL	Marker1	R/S[a]	Chr[b]	Marker2	Alleles	Chr[b]	Pos[c]	R^2	P	FDR
Lr	GG01	Lr04	*1126778*	A/G	6D*	*2280676*	A/G	3BL*	-	21.9	9.1E-08	8.2E-07
Lr	GG01	Lr04	*1126778*	A/G	6D*	*3222491*	C/G	3BL	264	16.7	3.2E-07	2.5E-06
Lr	GG01	Lr04	*1126778*	A/G	6D*	*1E+08*	A/G	3BL*	-	21.3	3.1E-07	1.6E-06
Lr	GG02	Lr04	*1126778*	A/G	6D*	*1208017*	A/G	6B*	-	16.3	5.4E-07	3.3E-06
Lr	GG02	Lr04	*3027151*	A/C	6D*	*2277702*	C/T	6B*	-	16.4	5.7E-07	4.1E-06
Sr	GG03	Sr03	*1101415*	A/G	2DS*	*1262585*	A/G	2AS	16	18.2	4.8E-07	1.2E-06
Sr	GG03	Sr03	*1102301*	T/C	2DS*	*1262585*	A/G	2AS	16	19.2	1.7E-07	5.9E-07
YLS	GG04	YLS04	*1139583*	T/C	6DL*	*1225863*	C/T	1DL	217	24.4	6.6E-07	2.5E-06
YLS	GG05	YLS04	*1126778*	G/A	6DL*	*1862984*	C/G	6B*	-	15.9	3.3E-07	8.2E-07

739

740 [a] R/S: resistance/susceptible alleles

741 [b] Chr: Chromosome

742 [c] Pos: Position on the linkage map (cM)

743

FIGURE LEGENDS

Figure 1 Gene-by-trait bi-plot of the reaction of SHWs to the six studied diseases, *Septoria Nodorum* has two scores (SNG and SNL). The arrow in the middle of the circles represents the position of the ideal genotype. Both PC1 and PC2 explained about 42.75% of the total variation. Green names represent SHWs while blue names represents traits.

Figure 2 Principal component analysis of the studied 320 SHWs. The red crosses represent the validation set while the blue squares represent the main set. The first two principal components explained together about 21.3% of the total variation.

Figure 3 Average disease score (the allelic effect) for each allele combination of the interactions a) GG01; b) GG02; c) GG03; d) GG04; and e) GG05. Significant differences were estimated via permuted t-test and the stars indicate levels of significance, "*" = $P < 0.05$; "**" = $P < 0.01$; *** = $P < 0.001$; "-" = not significant. For interactions detected with multiple markers in table 3, the highest P value only was presented here. X-axis is the allele combination between the resistance (R) and the susceptible (S) alleles with marker alleles that interacted.

Supporting Information

Table S1 List of the 320 synthetic hexaploid wheat genotypes used in this study, their pedigrees and their phenotypes scored from 1 (susceptible) to 9 (resistant).

Table S2 The number of mapped DArT and DArTSeq markers on each wheat chromosome

Table S3 The full genotypic data for both main and validation sets

Table S4 Genotypes of the associated marker for the main set

Table S5 QTL detected in the validation panel only

Figure S1 Results of the response of 320 SHWs to each of the six diseases evaluated. YLS = yellow leaf spot, Cr = crown rot, Lr = leaf rust, Sr = stem rust, Yr = yellow rust, SNL = *Stagonospora nodorum* leaf blotch, SNG = *Stagonospora nodorum* glume blotch. S = susceptible, MS = moderately susceptible, MR = moderately resistant, and R = resistant

Figure S2 Pseudo-heritability estimation inferred from the mixed model for the studied traits

Figure S3 Map position of both DArT (red) and DArTSeq (black) markers on wheat genome

Figure S4 Phylogenetic tree of the 320 SHWs, red genotypes represent the main set while blue genotypes represent the validation set

Figure S5 Kinship relations for the 320 SHWs

Figure S6 Scatter plot for the genetic distance against R^2 value for each pair of markers on the same chromosome (LD decay) for a) whole genome; b) genome A; c) genome B; d) genome D. Red lines represent the LOESS second degree smoothing while the blue horizontal lines represents the R^2 cut off 0.2

Figure S7 Inter-chromosomal R^2 values for each pair of markers for each genome

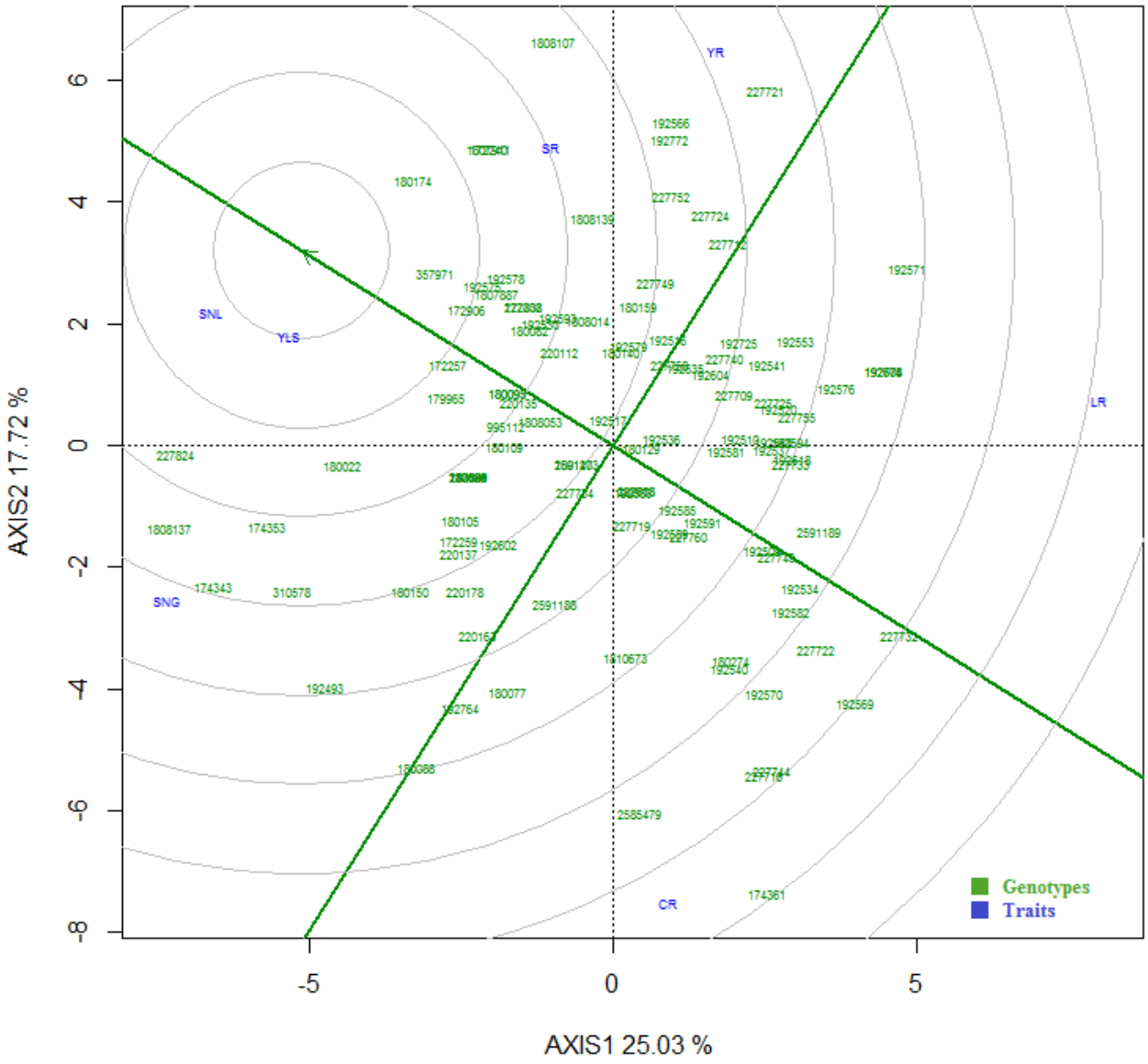
Figure S8 Manhattan and QQ plots for studied traits. Cr = crown rot, Lr = leaf rust, Sr = stem rust, Yr = yellow rust, SNL = *Stagonospora nodorum* leaf blotch, SNG = *Stagonospora nodorum*

782 glume blotch and YLS = yellow leaf spot. Chromosomes were numbered starting from the
783 homoeologous chromosome group one to seven with within group order of A, B and D genome,
784 respectively. Chromosome 22 represents the unmapped markers.

785 Figure S9 The average disease score (the allelic effect) for the alleles of the markers with
786 multiple associations. For the 6D QTL, we used only the marker *1126778*.

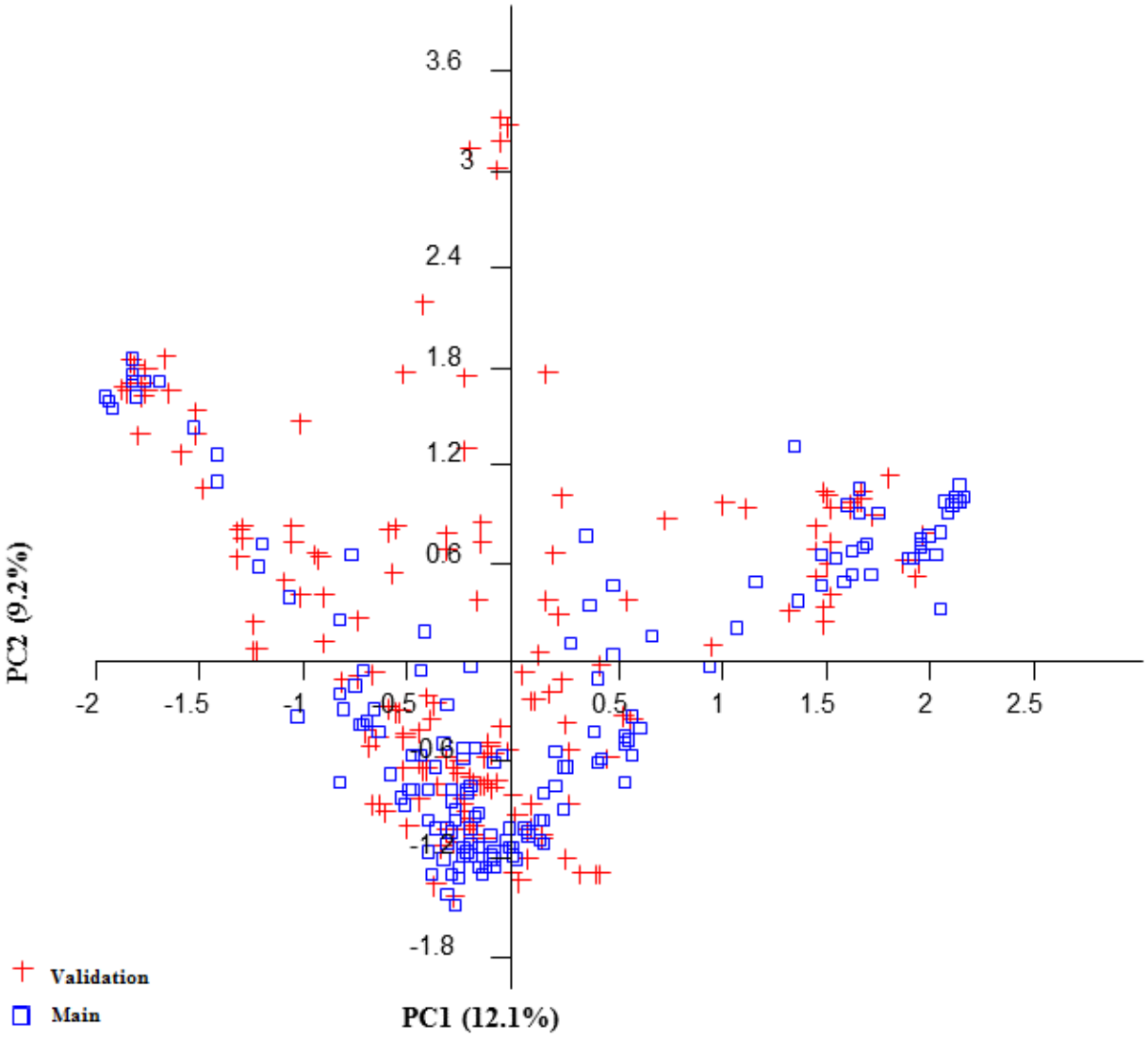
787

788


AXIS2 17.72 %
AXIS1 25.03 %
6
4
2
0
-2
-4
-6
-8
-5
0
5
YR
SR
SNL
YLS
SNG
LR
CR
1808107
227721
192566
192772
180174
227752
227724
1808139
227732
192571
357971
192578
227749
172906
180159
1808014
192536
192725
192553
220112
227740
192541
172257
192604
227709
192576
179965
220135
227795
995112
1808053
227824
180022
180105
1808137
174353
172259
192602
220137
227719
192591
2591189
174343
310578
180150
220178
192534
2591188
192582
220161
227722
227732
1810673
192493
180077
192570
192569
192764
180088
227744
2585479
174361
Genotypes
Traits

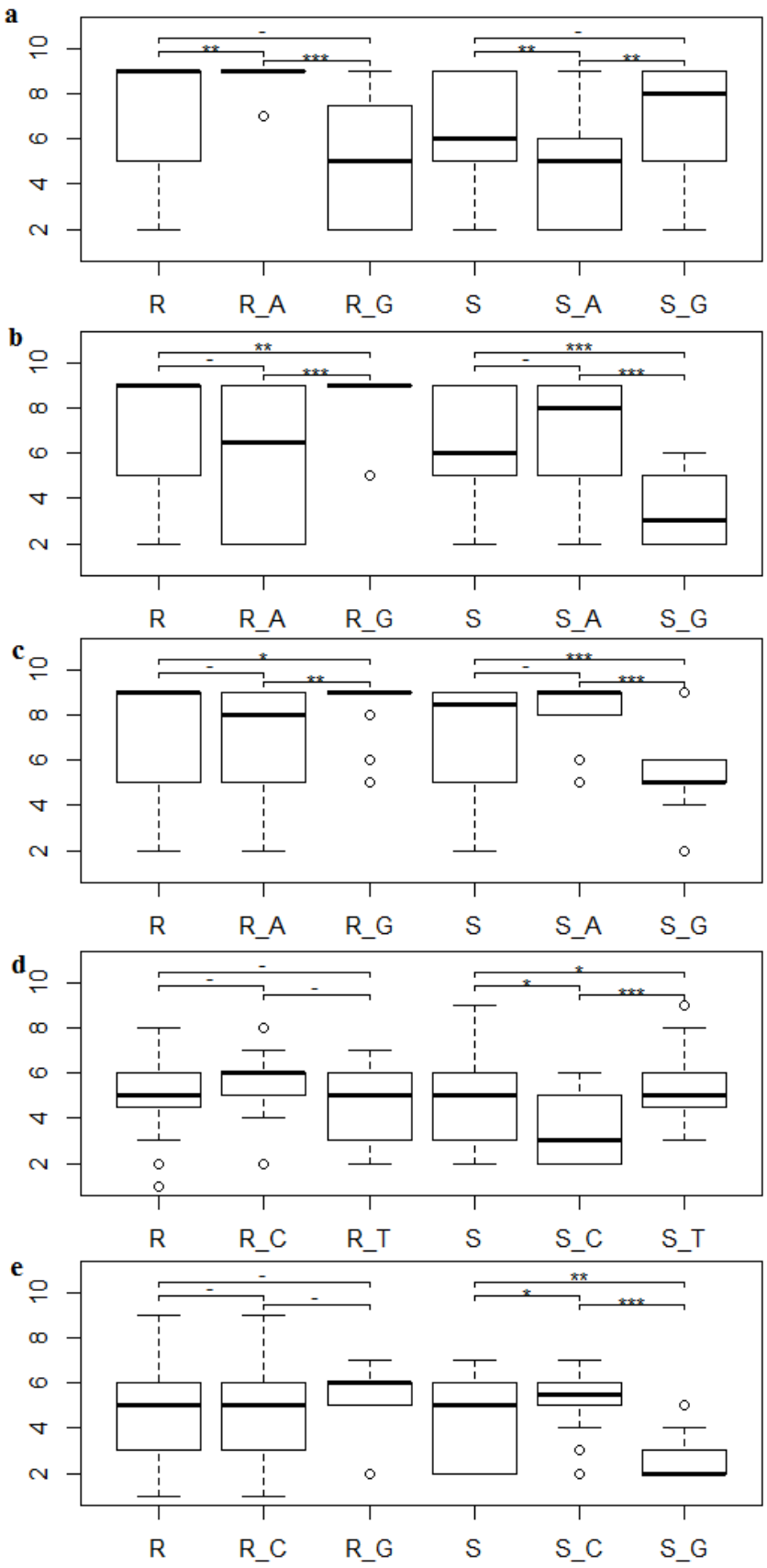
789

790


3.6
3
2.4
1.8
1.2
0.6
-1.8
-2
-1.5
-1
0.5
1
1.5
2
2.5
PC2 (9.2%)
PC1 (12.1%)
Validation
Main

791

792



793