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Genetic variability for resistance to fungal pathogens in bread wheat

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Abstract: Sustainable global wheat production requires wheat varieties, that are sufficiently resistant to the main wheat diseases. The economically important fungal pathogens worldwide include powdery mildew (PM), yellow rust (YR), leaf rust (LR) and blotch causing pathogens including Septoria nodorum blotch (SNB) and Septoria tritici blotch (STB). Here, we present the evaluation of winter wheat varieties of diverse origin against the prevalent local populations of PM, YR, LR, STB and SNB under natural infection conditions through image-based phenotyping in two consecutive years (2019 and 2020). We found several varieties to be resistant against multiple diseases. Following the association mapping, we obtained a total of 206 marker trait associations for all the parameters scored which were condensed to 79 quantitative trait loci (QTLs) (eight QTLs for PM, 25 QTLs for LR, 11 QTLs for YR, 19 QTLs for SNB and eight QTLs for STB) based on the linkage disequilibrium among the molecular markers. The known genes present at these QTLs are discussed in detail. The varieties resistant to multiple diseases, identified with the QTLs and molecular markers can be considered as elite raw material for future wheat breeding.

Keywords: association mapping; breeding; disease resistance; leaf blotch; powdery mildew; rusts; wheat

Wheat meets 19 and 21% of the human calorie and protein requirements, respectively, and grows on 216 million hectares of global area with an annual production of 765 million tonnes (FaoStat 2019, http://www.fao.org/faostat). The constant increase in the global population is always a threat to sustainable wheat production and global food security (Arif et al. 2022) as wheat is the staple food of ~ 40% of the global population (Li et al. 2018). This scenario dictates the world to utilise all the available resources to sustain and increase the current wheat yield (Hassan et al. 2020), which has shown to be stagnant in many parts of the world since the 1970s (Ray et al. 2013). However, this drive to increase the yield on a sustainable basis is challenging. Climate change and an amalgam of biotic and abiotic stresses are impediments to increasing the wheat production (Figueroa et al. 2018).

Global wheat grains are in danger due to the reduced genetic diversity that has shaped the emergence of various diseases (Figueroa et al. 2018). Wheat is affected by a number of pests, pathogens and nematodes. Among the 200 pathogenic microbes known to attack wheat, fungal pathogens like rusts (*Puccinia* ssp.), septoria leaf blotch (*Septoria* spp.), powdery mildew (PM) and *Fusarium* species are ranked among the top ten in molecular plant pathology (Dean et al. 2012). Moreover, the economically important fungal pathogens worldwide include *Blumeria graminis* DC Speer f.sp. *tritici* [the causal

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agent of powdery mildew (PM)], *Puccinia striiformis* Westendorp [the causal agent of yellow rust (YR)], *P. recondita* Desmazières [the causal agent of leaf rust (LR)], *Parastagonospora nodorum* Berkeley [the causal agent of Septoria nodorum blotch (SNB)] and *Zymoseptoria tritici* Berkeley & Curtis [the causal agent of Septoria tritici blotch (STB)].

Wheat rusts have been known to hamper wheat production ever since its domestication (Roelfs 1992). The rust fungi are obligate parasites that are completely dependent on the nutritional resources obtained from living host cells (Duplessis et al. 2012). There are three wheat rusts that include stem (caused by *P. graminis* f.sp. *tritici*), yellow and leaf rust (McIntosh et al. 1995). In addition to rusts, wheat is also host to many blotch diseases that include STB, SNB and tan spot (Figueroa et al. 2018). STB is considered as one of the principal leaf diseases of wheat in temperate growing regions (Fones & Gurr 2015), whereas recent years have witnessed a rise in its prevalence (Abdullah et al. 2020). SNB was a constant threat in Australia. The prevalence of SNB, however, is also a constant threat in Europe including France and northern Europe (Figueroa et al. 2018). Another important disease with a larger prevalence in a cool and maritime climate is PM which is caused by *Blumeria graminis* f.sp. *tritici* (Marone et al. 2013).

The purpose of the present research was to evaluate winter wheat varieties originating from diverse locations against the prevalent local populations of stripe and leaf rusts, STB and SNB and PM under natural infection conditions. SNB was measured on either the glumes (SNBG) or leaves (SNBL) or both (SNB). Another objective was to associate the known single nucleotide polymorphism (SNP) markers with the disease response using the association mapping (AM) tool.

MATERIAL AND METHODS

Plant material. The plant material used in this investigation was composed of a panel of 94 hexaploid wheat accessions (Table S1 in the Electronic Supplementary Material (ESM)) that were originally selected from a very large set of 2 500 accessions (Novi Sad Core Collection, Institute of Field and Vegetable Crops, Serbia) after repeated phenotyping for a plethora of traits and determination of the genetic diversity (Kobiljski et al. 2002). The selected wheat panel is representative of 21 countries across five continents. Furthermore, the population has been phenotyped and reported for seed longevity related traits (Arif et al. 2012a; Arif & Börner 2020), dormancy and pre-harvest sprouting traits (Arif et al. 2012b) and insect resistance (Arif et al. 2022) which indicates that the population carried enough genetic diversity that can be exploited for the identification of disease resistance genes.

Experimental design. The experiments were conducted in two years 2018–2019 (S1) and 2019–2020 (S2) at the Plant Breeding and Acclimatization Institute, National Research Institute in Radzikow (NRIR) (latitude = 52.214 N and longitude = 20.642 E), Poland under field conditions. The field experiment was composed of two blocks in each experimental season (S1 and S2) where the genotypes were randomly assigned to each block. Hence, each block carried 47 entries. In addition, the variety Durin was also planted in each block that served as the control. Each genotype was planted in the form of two plots of 1 m^2 with 2 m of fallow land kept between the two plots.

Disease scoring. Image-based disease phenotyping was carried out during the course of this investigation in both S1 and S2 which enabled the multidimensional characterisation of the host-pathogen interactions in our germplasm. For the disease phenotyping of cereals, a number of scales have been developed. In our study, for the purpose to describe the infection types and to quantify the disease severity and prevalence, Arabic numerals (1, 2, 3, 4, 5, 6, 7, 8, 9) were used, where 1 means a highly susceptible and 9 means a highly resistant disease response, as follows:

1 – Highly susceptible: Severe infection on all the leaves; spike also infected to some degree. [Spike infection is scored on a modified scale based on the percentage of the total area covered; the percentage figure follows the numerical leaf infection score and is separated by a slash (/)].
2 – Susceptible: Severe lesions on the lower and middle leaves; a moderate to severe infection of the upper third of plant; flag leaf infected in amounts more than a trace;
3 – Susceptible to moderately susceptible: Severe lesions on the lower and middle leaves with the infection extending to the leaf below the flag leaf, or with a trace infection on the flag leaf.
4 – Moderately susceptible: Severe infected plants in amounts more than an infection of the lower leaves; a moderate to light infection extending only to the middle of the plant.
5 – Moderately susceptible to moderately resistant: Severely infected in amounts more than

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an infection of the lower leaves; a moderate to light infection extending only to the middle of the plant.

6 – Moderately resistant: A moderate infection of the lower leaves with a scattered to light infection.

7 – Resistant: A light infection of the lower third of plant; the lowermost leaves are infected at moderate to severe levels.

8 – Resistant: Scattered lesions on the second set of leaves with the first leaves lightly infected.

9 – Highly resistant: A few isolated lesions on the lowest leaves only.

The evaluated panel of wheat varieties showed a differential response to each disease. We classified the germplasm into four resistance/susceptibility classes as follows: 1 – susceptible [disease score (DS) < μ – ð (mean – standard deviation)]; 2 – medium susceptible (DS > μ – ð), 3 – medium resistant (DS < μ + ð) and 4 – resistant (DS > μ + ð). Among the tested set of varieties, Durin was classified as resistant among the varieties which constitute a resistance group of varieties to each of the diseases in question (Table S3 in the ESM).

Statistical and association analyses. All the statistical analyses were conducted in R (R Studio, Ver. 2021.09.0+35) through various packages. For example, the overlaid histograms were constructed using "ggplot2" package. A three-way analysis of variance (ANOVA) considering the genotypes, blocks and season effects was also performed in R. The correlation analysis was carried out using the package "Factoextra".

For the purpose of genotyping, an optimised and reduced version of the 90 K iSELECT SNP-chip resulting in a 15 K Infinium SNP array (Wang et al. 2014) was convened. Data of 11 139 SNPs available from the public domain of Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany (IPK) (http://dx.doi.org/10.5447/IPK/2017/4) and reported in (Arif & Börner 2020; Arif et al. 2022) were used to find the linked markers associated with the disease resistance.

Before the formal association mapping (AM), the genotypic data were subjected to a population structure analysis using a subset of 241 evenly spaced SNPs which was achieved using the STRUCTURE software (Ver. 2.3.4) (Pritchard et al. 2000). The analysis revealed the presence of three subgroups in our panel. Further details are provided in (Arif & Börner 2020). TASSEL (Ver. 5.2.43) (Bradbury et al. 2007) software was employed where a mixed linear model (MLM) (Yu et al. 2006) was chosen, which takes the population structure (calculated from STRUCTURE (Ver. 2.3.4)) and kinship (calculated from TASSEL (Ver. 5.2.43)) into account. By default, markers that gave a *P*-value of 0.001 (– log10 value of 3) for a given trait were claimed as a significant association. The same criterion was chosen in a previous report (Dababat et al. 2021) to detect marker trait associations (MTAs) linked to the nematode resistance (biotic stress) in another association mapping panel of wheat. The MTAs were visualised using the CMplot package in R.

RESULTS

Descriptive natural disease variation. Overall, there was less disease (more resistance) in S1 as compared to S2. The PM score in S1 and S2 was 7.18 ± 1.64 and 7.01 ± 0.70, respectively. The YR and LR values were determined in both S1 (YR = 8.72 ± 1.12; LR = 8.39 ± 1.49) and S2 (YR = 7.58 ± 0.39; LR = 8.77 ± 0.39) (Figure 1, Table S2 in the ESM). The STB scores were 8.52 ± 1.40 and 7.14 ± 0.29 in S1 and S2, respectively, whereas the SNB scores were 6.02 ± 0.88 and 5.20 ± 0.44 in S1 and S2, respectively. Between the SNBL and SNBG, the SNBL scores were higher (more resistance). For example, the SNBG scores in S1 and S2 were 6.26 ± 1.18 and 3.89 ± 0.44, respectively. Likewise, the SNBL scores were 5.77 ± 1.19 and 5.33 ± 0.44 in S1 and S2, respectively. The ANOVA did not reveal any significant genotypic differences within the germplasm for all the diseases except for YR. On the other hand, significant differences were imposed by the season/year on the disease at a *P*-value < 0.001 for all the diseases except for PM. There were no significant differences observed for the blocks, genotype × year, genotype × block, blocks × year and genotype × block × year interactions. Among the various diseases, the highest correlation was between SNB and SNBG (R^2 = 0.75) and SNBL (R^2 = 0.77). Furthermore, there was a correlation (R^2 = 0.47) between SNB and STB. In addition, PM and LR were also in a medium correlation (R^2 = 0.47). A low correlation was observed between the two rusts (R^2 = 0.24) (Figure 2).

Among the evaluated set, 18 varieties were classified as susceptible, and 18 varieties were assessed as resistant to PM. All the other varieties were medium susceptible or medium resistant. To YR, 14 varieties were susceptible and 73 were considered to be resistant. To LR, 6 varieties were susceptible, 26 medium susceptible and 60 appeared resistant. It should be indicated that none of the tested wheat varieties

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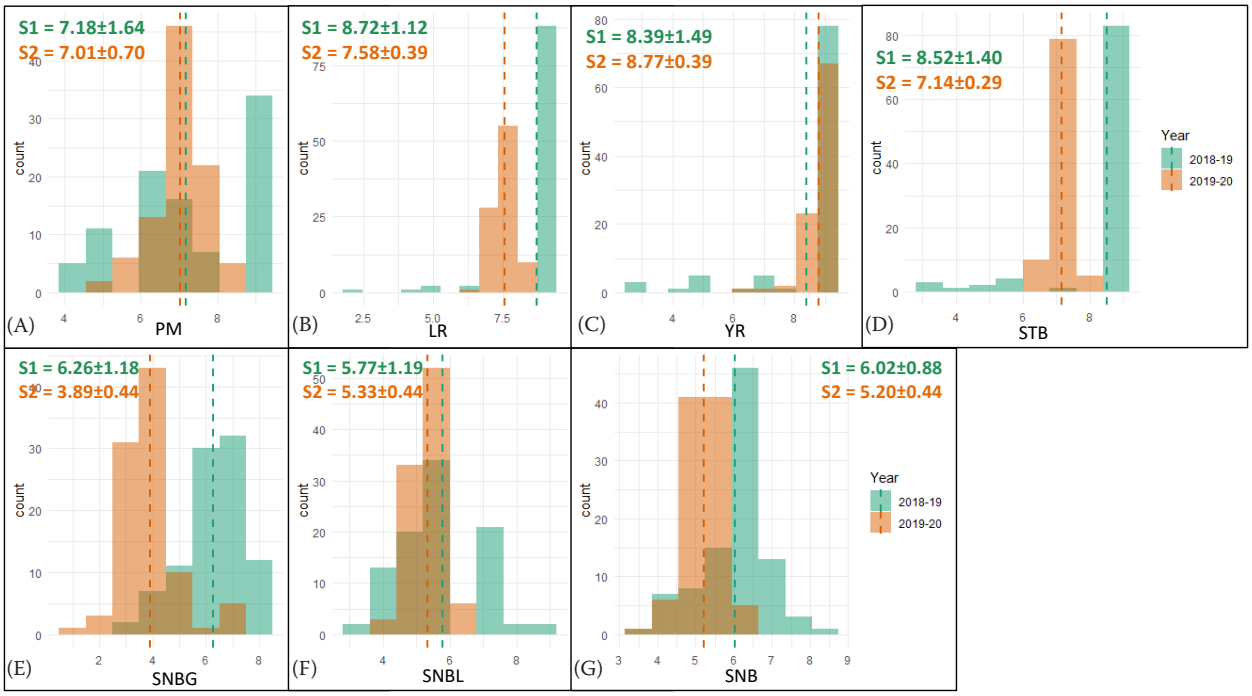

Figure 1. Frequency distribution of powdery mildew (PM) (A), leaf rust (LR) (B), yellow rust (YR) (C), Septoria tritici blotch (STB) (D) and *Parastagonospora nodorum* blotch on the glumes (SNBG) or leaves (SNBL) or both (SNB) (E–G) in the 2019 (S1, green) and 2020 (S2, brown) cropping seasons

The thick dashed lines indicate the means of the respective traits, whose values are provided as a text with the standard deviation

were classified as moderately resistant to both the yellow and leaf rusts. To SNBL and SNBG, approximately equal numbers of tested wheat varieties were classified as susceptible, moderately susceptible and moderately resistant. However, a substantially larger number of varieties were shown to be resistant on the leaves than on the heads. While considering a simultaneous evaluation to the SNB of the set of wheat varieties on the leaves and heads, the largest numbers of varieties were shown to be moderately

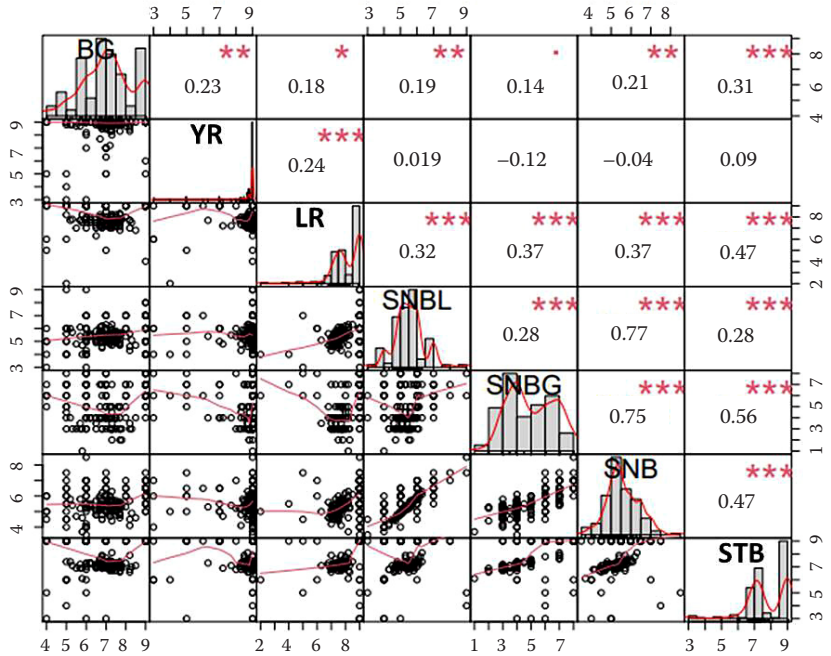

Figure 2. Correlation among the mean of the various disease scores in the germplasm

BG – powdery mildew; YR – yellow rust; LR – leaf rust; SNBL – *Parastagonospora nodorum* blotch on the leaves;, SNBG – *Parastagonospora nodorum* blotch on the glumes; SNB – Septoria nodorum blotch; STB – Septoria tritici blotch

*, **, ***significant at P = 0.05, 0.01 and 0.001

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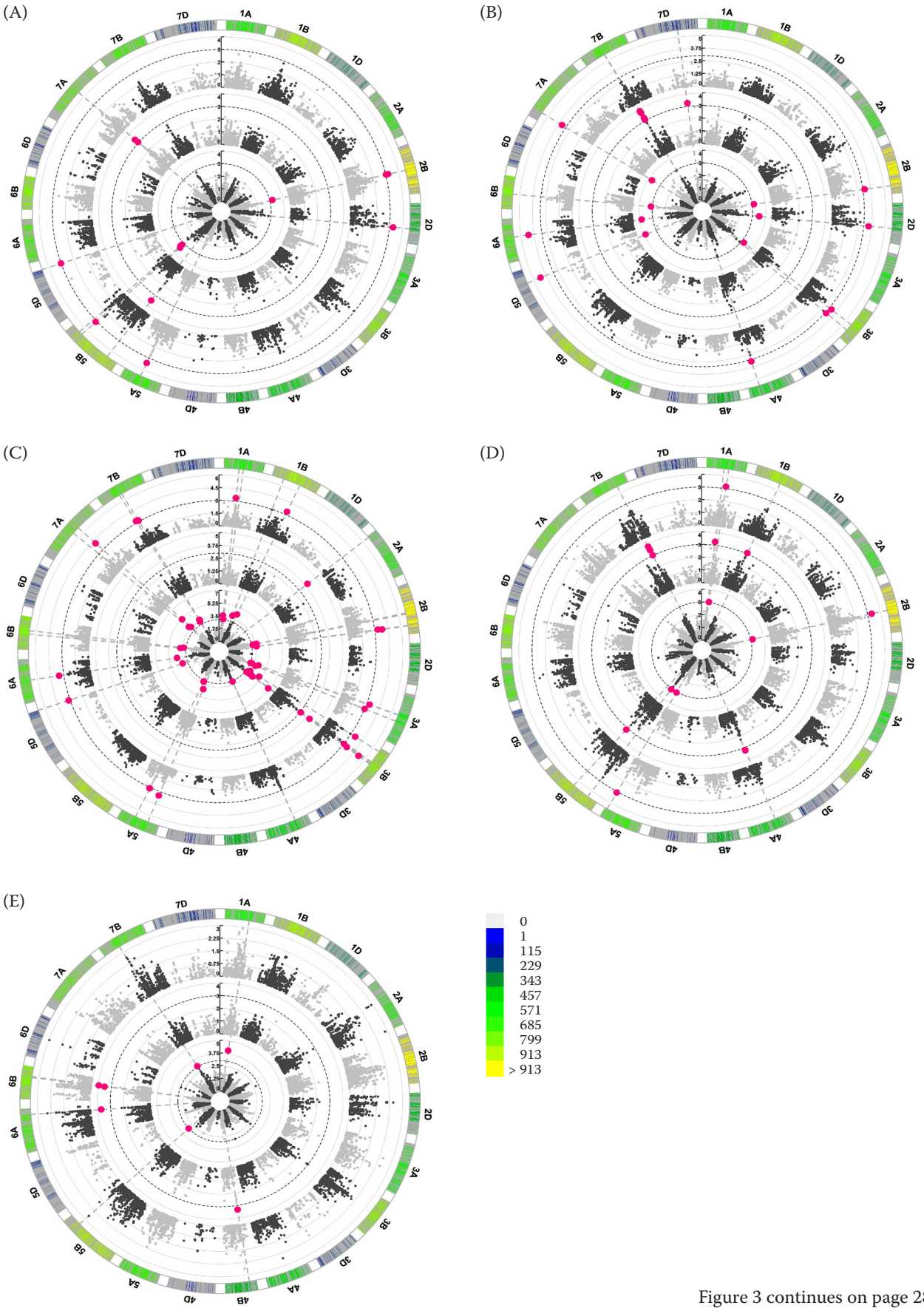

Figure 3 continues on page 28

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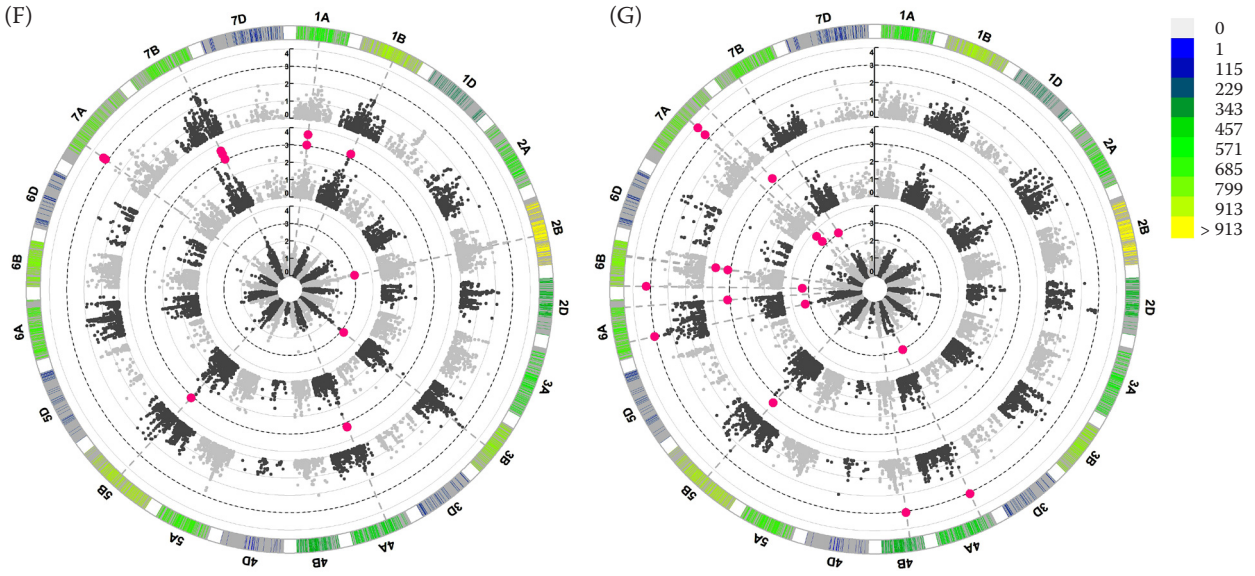

Figure 3. Genome-wide scan of powdery mildew (PM) (A), yellow rust (YR) (B), leaf rust (LR) (C), SNB (D), *Parastagonospora nodorum* blotch on the glumes (SNBG) (E), or leaves (SNBL) (F) and both (SNB) (G) of the 2019 (S1, inner circle) and 2020 (S2, second circle) infections and mean values (outer circle) in the form of circular Manhattan plots, where the chromosomes are plotted at the outermost circle, where the thin dotted black line indicates a significance level at a *P*-value < 0.001 (–log10 = 3 or more) beyond which an association is counted as a true association (highlighted pink dots)
The scale between chromosome 7D and 1A indicates the LOD (logarithm of the odds) threshold; the coloured boxes outside of the bottom right corner indicate the SNP density across the genome, where blue to yellow indicates less dense to dense

susceptible or moderately resistant. On the other hand, 77 (81.9%) varieties appeared to be moderately resistant against STB. The rest of the varieties were classified as resistant, moderately susceptible, and STB susceptible.

Association analyses. To capture the maximum variation and get an estimate of the genetic variance of the disease within the germplasm, we used the individual disease scores of S1 (2019) and S2 (2020) and the mean values to perform the association analysis. We obtained a total of 206 MTAs for a total of seven parameters (PM, YR, LR, SNB, SNBG, SNBL and STB) belonging to five diseases (Table S4 in the ESM). Of them, 13 were detected for PM (four and three MTAs in S1 and S2 and six MTAs with the mean value). For YR, we detected a total of 25 MTAs where seven, 10 and eight MTAs were with the corresponding S1, S2 and mean values. On the other hand, 86 MTAs were observed for LR where 60 were in S1, three were in S2 and 23 MTAs were detected for the mean value. A total of 29, seven and 25 MTAs were detected for the SNB, SNBG and SNBL, respectively, where the majority were detected in S2. For the STB, eight, five and eight MTAs were discovered, respectively, for the S1, S2 and mean disease scores (Figure 3).

The distribution of these MTAs also varied in the wheat genome. For example, the highest number of MTAs were detected on the group 7 chromosomes (66 MTAs) followed by the group 3 chromosomes (42 MTAs). A total of 24 and 22 MTAs were present on the group 5 and group 6 chromosomes, respectively. Group 2 carried 21 MTAs whereas 18 MTAs were present on the group 1 chromosomes. The least number of MTAs were carried by the group 4 chromosomes (13 MTAs).

DISCUSSION

Wheat diseases are responsible for considerable losses to sustainable wheat production (Chen 2005; Milus et al. 2009). Even if the yield is sustained, the quality of the grain produced is compromised (Dimmock & Gooding 2002). In spite of many fungicides available, developing resistant varieties has been proven as an environmentally sustainable means for reducing losses due to this disease (Line 2002; Chen 2005). Plant breeders rely on superior combinations of alleles residing in a genotype resistant to multiple diseases/stresses. In our germplasm, there were 22 varieties that showed absolute resistance against

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at least two diseases. From them, 13 varieties (Bankuty 1205, BCD 1302/83, Centurk, Mena, Peking, PKB Krupna, Purd/Loras, Sofija, Tom Thumb, Triple dirk "S", Vireo "S", ZG1011 and ZGK238/82) were resistant to three diseases. Likewise, seven varieties were completely resistant to four diseases (Donska polup, Lr 12, Mironovska 808, Norin 10, NS 66/92, Purdue 39120 and Purdue 5392). In addition, two varieties (Capelle Desprez and TJN 990-15) were resistant against five diseases. The varieties resistant to multiple diseases can be an excellent resource to breed future climate smart disease resistant cultivars. The varieties classified as resistant (Table S3 in the ESM) provide valuable raw material to induce genetic disease resistance in wheat.

To look for the genetic architecture of disease resistance in the germplasm, a total of 206 MTAs were detected (Figure 3, Table S4 in the ESM). Using the strategy of our previous reports (Dababat et al. 2021; Arif et al. 2022), we condensed these MTAs to 79 QTLs based on the linkage disequilibrium among the markers for the purpose of discussion. Among these 79 QTLs, there were eight QTLs exclusively linked with the PM distributed on chromosomes 2B, 2D, 5A, 5B (three QTLs), 5D and 7A where the QTLs on chromosomes 2B (*Q.BG.NRIR-2B*) and 5B (*Q.BG.NRIR-5B.2*) were detected in S1 and with the mean scores (Table S5 in the ESM). The variation explained by the PM QTLs ranged from 13–16.5%. Many PM associated QTLs have been well characterised in wheat (Kang et al. 2020b) on all the chromosomes except chromosome 3D. Our QTLs on chromosome 2B (*Q.PM.NRIR-2B*) at ~ 92 cM mirrored a recently reported locus in three different experiments (Mohler & Stadlmeier 2019) where three candidate genes were reported, *viz*. *TraesCS2B02G536100*, *TraesCS-2B02G536200*, *TraesCS2B02G536300*. The gene associated with peak marker (*TraesCS2B02G536100*) was encoding for a sulfate transporter. Pathogen feeding is known to be under the influence of transporters (Lapin & Van den Ackerveken 2013). They also act as cleansing agents for detrimental compounds (Hwang et al. 2016). The importance of transporters is also evident from the fact that a well-known pathogen resistance gene *Lr34*/*Yr18*/*Pm38*/*Sr57* in wheat is a mutational altered ATP-binding cassette transporter (Krattinger et al. 2009). In our case, the SNP (*wsnp_Ex_c27867_37030229*) of QTL *Q.PM.NRIR-2B* encoded for *cytoplasmic like leucine-tRNA ligase* whose function is to inhibit the fungal protein synthesis via leucyl-transfer RNA (Wolverton & Wu 2019). The SNP of 5B QTL *Q.PM.NRIR-5B.2* did not yield any blast hits. Several QTLs associated with PM, however, have been reported at ~ 50 ± 10 cM on chromosome 5B (Kang et al. 2020a) including the *Pm16* gene. Likewise, the other two QTLs were located in regions previously known to carry resistance for PM (Kang et al. 2020a).

There were 25 exclusive QTLs detected for LR on chromosomes 1A (two QTLs), 1B (two QTLs), 2A, 2B (three QTLs), 3A (two QTLs), 3B (four QTLs), 4A, 5A (two QTLs), 5D, 6A, 6B (two QTLs), 6D, 7A (two QTLs), 7B (two QTLs) where 14 QTLs (*Q.LR.NRIR-1A.1*, *Q.LR.NRIR-1B.2*, *Q.LR.NRIR-2B.3*, *Q.LR.NRIR-3A.1*, *Q.LR.NRIR-3A.2*, *Q.LR.NRIR-3B.1*, *Q.LR.NRIR-3B.3*, *Q.LR.NRIR-3B.4*, *Q.LR.NRIR-5A.1*, *Q.LR.NRIR-5A.2*, *Q.LR.NRIR-5D*, *Q.LR.NRIR-7A.2*, *Q.LR.NRIR-7B.1* and *Q.LR.NRIR-7B.2*) were detected in either S1 or S2 and with the mean scores (Table S6 in the ESM). The highest number of *Lr* genes is known to be residing on the group 2 chromosomes (66 QTLs) where at least 31 QTLs have been mapped to chromosome 2B (Pinto da Silva et al. 2018). In addition, seven *Lr* resistance genes are known to be located on chromosome 2B (McIntosh et al. 2008) including three race specific APR genes (*Lr13*, *Lr35*, *Lr48*). *Lr35* is located near the centromere, whereas the other two are located on chromosome 2BS. Seven *Lr* genes are mapped to chromosomes of group 3 where five genes (*Lr24*, *Lr27*, *Lr32*, *Lr63* and *Lr66*) are effective at all the growth stages (McIntosh et al. 2008). In addition, 17 QTLs have also been reported on chromosome 3B to be associated with LR indicating the importance of chromosome 3B in the LR resistance. Our study identified six QTLs (two on chromosome 3A and four on 3B) on the group three chromosomes. In addition, several leaf rust resistance QTLs (which could represent minor genes) in our varieties could also be one reason that 60 varieties were resistant to LR.

Eleven QTLs in our germplasm could be detected for YR resistance on chromosomes 2B, 2D, 3B (two QTLs), 4A, 5D, 6A, 6B, 7A, 7B and 7D (Table S7 in ESM) where six QTLs (*Q.Yr.NRIR-2B*, *Q.Yr.NRIR-2D*, *Q.Yr.NRIR-3B.1*, *Q.Yr.NRIR-5D*, *Q.Yr.NRIR-6A* and *Q.Yr.NRIR-7A*) were detected in S1 and with the mean scores. Numerous *Yr* resistance genes [*Yr5* (Sui et al. 2009), *Yr7* (Zhang et al. 2013; Feng et al. 2015), *Yr43* (Feng et al. 2015), *Yr44* (Sui et al. 2009; Cheng & Chen 2010), *Yr53* (Feng et al. 2015) and *Yr72* (Chhetri 2015)] are present on chromosome 2BL. The same is the case with the other chromosomes. Hence, the 73 cultivars resistant to Yr could

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be due to the presence of many resistant loci in our germplasm.

The MTAs associated with SNB, SNBG and SNBL were condensed to 19 QTLs on chromosomes 1A (three QTLs), 1B, 2B (two QTLs), 3B, 4A, 4B, 5A, 5B (three QTLs), 6A, 6B (two QTLs), 7A and 7B (two QTLs) (Table S8 in ESM). In this case, the three QTLs of SNBL on chromosomes 1A, 2B and 5A (*Q.SNBL.NRIR-1A*, *Q.SNBL.NRIR-2B* and *Q.SNBL.NRIR-5A*) were detected with the mean values as well. On the other hand, four QTLs (*Q.SNB/SNBL.NRIR-1A*, *Q.SNB/SNBL.NRIR-1B*, *Q.SNB/SNBL.NRIR-4A*, *Q.SNB/SNBL.NRIR-5B* and *Q.SNB/SNBL.NRIR-7B*) carried MTAs for both SNBL and SNB. Previously, QTLs for SNB resistance have been reported on chromosomes 1B, group 2 and 5 chromosomes, 3B, 4B, 6A and 7B (Francki 2013) where the most consistent QTL was detected on chromosome 5B conferring seedling resistance. More recently, chromosomes 1A, 5A, and 5B were reported to carry five QTLs for SNB resistance (Singh et al. 2019). Hence, our varieties carried most of the known loci of SNB resistance due to which a substantial number of varieties were declared resistant against SNB.

Finally, the MTAs linked with STB were condensed to 11 QTLs on chromosomes 4A, 4B, 5B 6A (two QTLs), 6B (three QTLs), 7A (two QTLs and 7B (Table S9 in the ESM). Here, chromosomes 4A (*Q.STB.NRIR-4A*), 6A (*Q.STB.NRIR-6A.1*), 6B (*Q.STB.NRIR-6B.1*) and 7A (*Q.STB.NRIR-7A.1*) carried QTLs with MTAs detected in S1 and with mean scores. Exploiting the "NIAB Elite MAGIC" population for STB resistance under natural infection conditions, 25 QTLs were reported, where 10 QTLs on chromosomes 1B (two QTLs), 1D, 2A, 3D,4A, 4D, 6A, 6B, and 6D were potentially novel (Riaz et al. 2020). It is also known that *Stb11*, *Stb6* and *Stb7* are located on chromosomes 1BS, 3AS and 4AL, respectively (Chartrain et al. 2005a). Another gene, *Stb12* has been mapped to chromosome 4AL where single sequence repeat (SSR) markers were linked with the reported gene (Chartrain et al. 2005b). On the other hand, our 4A QTL (*Q.STB.NRIR-4A*) was detected at ~ 29.86 which could be on a short arm of 4A. All the above-mentioned studies were conducted on constructed populations using various marker systems; hence, we cannot conclusively state what genes are present in our germplasm at the various QTLs being reported. The resistant varieties and the QTLs present in them provide excellent resources to develop future climate smart and target oriented wheat varieties.

All in all, our study exploited a winter wheat collection to look for resistance against five major wheat diseases. Several varieties were found to be resistant against multiple diseases (Table S3 in the ESM). For example, we found 15 varieties completely resistant to at least three diseases. Among them, one single variety, *viz*. Capelle Despreż, was found to be resistant to four diseases including YR, LR, SNB and STB. In addition, Brigand, Lr 12, Mina, PKB Krupna, TJB 990-15, Purdue 5392, Mironovska 808 and Norin 10 were completely resistant to YR, LR and SNB /SNBG/ SNBL. On the other hand, there were four varieties, *viz*. NS 22/92, NS 66/92, Vireo S, and ŻGK 238/82, which were completely resistant to PM, YR, LR and SNB/SNBG/SNBL. Peking 11 was found resistant to PM, LR and SNB, whereas Read Coat was resistant to PM, YR, and SNB. In addition, we located several QTLs present in the germplasm. The varieties resistant to multiple diseases, identified with the QTLs and molecular markers can be considered as elite raw material for future wheat breeding.

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