



# Genome-wide association analyses of leaf rust resistance in cultivated emmer wheat

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## Abstract

**Key message** Fifteen and eleven loci, with most loci being novel, were identified to associate with seedling and adult resistances, respectively, to the durum-specific races of leaf rust pathogen in cultivated emmer.

**Abstract** Leaf rust, caused by *Puccinia triticina* (*Pt*), constantly threatens durum (*Triticum turgidum* ssp. *durum*) and bread wheat (*Triticum aestivum*) production worldwide. A *Pt* race BBBQD detected in California in 2009 poses a potential threat to durum production in North America because resistance source to this race is rare in durum germplasm. To find new resistance sources, we assessed a panel of 180 cultivated emmer wheat (*Triticum turgidum* ssp. *dicoccum*) accessions for seedling resistance to BBBQD and for adult resistance to a mixture of durum-specific races BBBQJ, CCMSS, and MCDSS in the field, and genotyped the panel using genotype-by-sequencing (GBS) and the 9 K SNP (Single Nucleotide Polymorphism) Infinium array. The results showed 24 and nine accessions consistently exhibited seedling and adult resistance, respectively, with two accessions providing resistance at both stages. We performed genome-wide association studies using 46,383 GBS and 4,331 9 K SNP markers and identified 15 quantitative trait loci (QTL) for seedling resistance located mostly on chromosomes 2B and 6B, and 11 QTL for adult resistance on 2B, 3B and 6A. Of these QTL, one might be associated with leaf rust resistance (*Lr*) gene *Lr53*, and two with the QTL previously reported in durum or hexaploid wheat. The remaining QTL are potentially associated with new *Lr* genes. Further linkage analysis and gene cloning are necessary to identify the causal genes underlying these QTL. The emmer accessions with high levels of resistance will be useful for developing mapping populations and adapted durum germplasm and varieties with resistance to the durum-specific races.

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## Introduction

Wheat (*Triticum aestivum* L.,  $2n = 6x = 42$ ) leaf (brown) rust, caused by the fungus *Puccinia triticina* Eriks. (*Pt*), is one of the three most important rust diseases of wheat (Anikster et al. 1997). Although leaf rust is less damaging than stem rust and stripe rust, it causes higher annual yield

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losses due to its frequent and widespread occurrence in most of the wheat-growing areas around the world (Chai et al. 2020). Among the wheat diseases, leaf rust is now ranked as the most devastating disease, affecting 94.4% of global wheat production and causing 3.25% yield loss (a value of \$5.6 billion) annually at the global level (Savary et al. 2019; Chai et al. 2020). In some hotspots such as Indo-Gangetic Plain and Mainland China, annual yield losses were estimated at 4.25% and 4.38%, respectively (Savary et al. 2019). Although leaf rust caused the lowest yield loss annually in North America (0.54%) compared to other hotspots investigated by Savary et al. (2019), its regular outbreaks also had a major impact on wheat production in this region (Aboukhaddour et al. 2020; Kolmer et al. 2022). For instance, the losses caused by leaf rust in Canada reached up to 10% of total yield per year between 2000 and 2009 (Huerta-Espino et al. 2011). In the United States, a leaf rust outbreak in 2007 caused yield losses up to 14% in Kansas (Kolmer et al. 2009). A recent estimation indicated that leaf rust caused yield losses at 8% in Oklahoma and 3% in Texas and Kansas in 2019 (Kolmer et al. 2022).

Leaf rust will be a continuous threat to global wheat production. Chai et al. (2020) estimated that leaf rust could cause annual grain losses of 8.6–18.3 million metric tons, which are equivalent to economic values of \$1.5–\$3.3 billion, projected from 2000 to 2050. To minimize the occurrence and spread of leaf rust disease, the wheat community has placed a major effort on the utilization of host plant resistance. For rust diseases, resistance has been broadly classified at seedling and adult stages. Seedling resistance is pathogen race-specific and is controlled by single resistance genes with major effects that confer a hypersensitive response to prevent the spread of infection (Ellis et al. 2014; Mondal et al. 2016). In contrast, adult plant resistance is mostly race non-specific and effective at later stages of plant growth, and it is often controlled by multiple genes with minor and additive effects and found to be more durable (Zhang et al. 2021). To date, 81 leaf rust resistance (*Lr*) genes have been identified from common wheat and its wild and cultivated relatives (McIntosh et al. 2013, 2014, 2016, 2018, 2020; Xu et al. 2022). Most of the reported *Lr* genes confer race-specific resistance, except for *Lr34* (Singh et al. 2012), *Lr46* (Singh et al. 2013), *Lr67* (Herrera-Foessel et al. 2014) and *Lr68* (Herrera-Foessel et al. 2012). It has been commonly accepted that combinations of certain race-specific and race-non-specific resistance genes can enhance the level and durability of resistance against different *Pt* races at both seedling and adult stages. Thus far, six *Lr* genes have been cloned, including *Lr1* (Cloutier et al. 2007), *Lr10* (Feuillet et al. 2003), *Lr21* (Huang et al. 2003), *Lr22a* (Thind et al. 2017), *Lr34* (Krattinger et al. 2009) and *Lr67* (Moore et al. 2015).

The *Pt* pathogen displays high genetic diversity, with new virulent races constantly emerging and evolving in nature (Huerta-Espino 1992; Kolmer 2005; Omara et al. 2021; Komer et al. 2022). For example, *Pt* race BBG/BN, detected in northwestern Mexico in 2001 with virulence to *Lr72*, overcame the resistance of widely adapted durum wheat [*T. turgidum* ssp. *durum* (Desf.) Husn.,  $2n=4x=28$ , AABB] cultivars from the CIMMYT (International Maize and Wheat Improvement Center) breeding program, which had been effective for more than 25 years (Singh et al. 2004; Aoun et al. 2016). This resulted in severe epidemics of leaf rust in durum wheat between 2000 and 2003, causing heavy yield loss worth \$32 million (Singh et al. 2004). In addition, a new variant, BBG/BP, with virulence to *Lr12*, *Lr27* and *Lr31*, was detected in durum cultivars between 2007 and 2008 (Huerta-Espino et al. 2008). In the US, a highly virulent race similar to BBG/BN, designated as BBBQD, was detected in durum fields of California in 2009 and Kansas in 2013 (Kolmer 2013, 2015). This poses a threat to the major durum-producing regions of the US such as North Dakota, which produces 58% of the total US durum wheat (Aoun et al. 2016). Therefore, it is necessary to identify resistance genes against race BBBQD among the existing wheat gene pools.

Cultivated emmer wheat [*T. turgidum* ssp. *dicoccum* (Schrank ex Schübler) Thell.,  $2n=4x=28$ , AABB] is one of the oldest domesticated crops, and an important genetic resource for the improvement of durum and bread wheat. Several breeding programs have utilized cultivated emmer to develop wheat cultivars with good resistance to rust and other fungal diseases (Zaharieva et al. 2010; Sharma et al. 2019). Wild emmer wheat [*T. turgidum* ssp. *dicoccoides* (Körn. ex Asch. and Graebner) Thell.], the wild progenitor of cultivated emmer, served as the source of *Lr53* and *Lr64* for leaf rust resistance (Marais et al. 2005; McIntosh et al. 2013). However, the presence of *Lr* genes in the germplasm collection of cultivated emmer has not been explored. Therefore, we conducted this study with aims to find sources of resistance to the durum-specific *Pt* races in a collection of cultivated emmer accessions and to identify quantitative trait loci (QTL) contributing to leaf rust resistance.

## Materials and methods

### Plant materials

A diverse panel of 180 cultivated emmer wheat accessions, originating from 32 countries and covering 5 continents, was used in this study (Table S1). These cultivated emmer wheat accessions are currently maintained at the National Small Grains Collection (NSGC, Aberdeen, ID), a component of the National Plant Germplasm System (NPGS) of the United

States Department of Agriculture–Agriculture Research Service (USDA-ARS). The detailed source information was retrieved from USDA-ARS GRIN-Global (<https://npgsw.eb.ars-grin.gov/gringlobal/search>).

## Evaluation of seedling resistance

Leaf rust resistance was evaluated at both seedling and adult stages. At the seedling stage, the 180 emmer accessions were evaluated for their reactions to *Pt* race BBBQD in the greenhouse. BBBQD is virulent to *Lr10*, *Lr39*, *LrB* and it is avirulent to *Lr1*, *Lr2a*, *Lr2c*, *Lr3*, *Lr3ka*, *Lr9*, *Lr11*, *Lr14a*, *Lr16*, *Lr17*, *Lr18*, *Lr21*, *Lr24*, *Lr26*, *Lr28*, *Lr30*, and *Lr42* (Table 1). All the seedlings were screened at the North Dakota Agricultural Experiment Station (AES) Research Greenhouse Complex, Fargo, ND in 2013 and 2014 with three replicates each. Five seeds per accession were planted in a single cell in 50-cell trays containing sunshine mix #1 (Sungro Horticulture Distribution Inc., Quincy, MI, USA) and slow-release commercial fertilizer Osmocote 15-9-12, N-P-K (Everris NA Inc., OH, USA) in a rust-free greenhouse set to 22 °C/18 °C (day/night) with 16-h photoperiod. The leaf rust susceptible durum line RL6089 was included in each tray as the susceptible check. After seedling emergence, foliar fertilizer, Peat Lite 20-20-20, was applied once a week. Seedlings at the two-leaf stage were inoculated with fresh rust spores suspended in Soltrol-170 solvent (Phillips Petroleum, Bartlesville, OK, USA) at a rate of 0.01 g/mL and then left to air dry. After inoculation, plants were placed in a dark dew chamber for 16–18 h at 20 °C and were then relocated to the greenhouse. About 12–14 days after inoculation, the infection types (ITs) were scored using a 0–4 scale (McIntosh et al. 1995), where IT with 0 represents no visible sign or symptom, 1 represents small uredinia with necrosis, 2 represents small to medium sized uredinia with green islands and surrounded by necrosis or chlorosis, 3 represents medium sized uredinia with or without chlorosis, and 4 represents large uredinia without chlorosis. Two additional signs “-” and “+” indicate smaller and larger uredinia, respectively, for each basic level. For genome-wide association study (GWAS), the IT scores were converted to a linearized 0–9 scale (referred as infection response, IR)

using the method described by Zhang et al. (2014). The conversion was performed in R using the scripts for ‘seedling rust score conversion’ provided by Gao et al. (2016) ([https://github.com/umngao/rust\\_scores\\_conversion](https://github.com/umngao/rust_scores_conversion)). The mean IR scores of six replicates were used for marker-trait association analysis. Genotypes with IR scores of 1–3 were considered as resistant, 4–6 as moderately resistant, and 7–9 as susceptible.

## Evaluation of adult plant resistance

Leaf rust resistance at the adult stage was evaluated in a field nursery of the Debre Zeit Research Center, Ethiopian Institute of Agricultural Research, Ethiopia. The nursery site was located at 08° 46' N, 39° 00' E, and 1,900 m in elevation in Debre Zeit, Ethiopia. The evaluation experiments for the emmer panel were conducted with one replicate at the main-season (June-to-October growing season) of 2013 and two replicates at the off-season (December-to-April growing season) of 2014. The accessions were planted in single 1-m row plots and were exposed to natural *Pt* inoculum collected from the nursery. Common wheat variety ‘Thatcher’ was used as the susceptible check. The races of *Pt* population present in the collected spores were identified as BBBQJ, CCMSS and MCDSS (Table 1) by the USDA-ARS Cereal Disease Laboratory (St. Paul, MN).

The leaf rust reactions of each accession were scored as disease severity (DS) and infection response (IR) at the soft dough stage when the disease was fully developed and the symptoms had not been affected by host senescence (Newcomb et al. 2013). DS was represented as the percentage of tissue infected, and it was determined based on the modified Cobb Scale (Peterson et al. 1948). IR was represented as ‘R’ for resistant, ‘MR’ for moderately resistant, ‘MS’ for moderately susceptible, and ‘S’ for susceptible. ‘RMR’, ‘MRMS’, or ‘MSS’ represented mixed responses, with the predominant response being listed first (Newcomb et al. 2013). The IR evaluations were converted to a numeric 0–1 scale (Roelfs et al. 1992), and genotypes with the range of 0–0.3 were considered resistant, 0.4–0.6 as moderately resistant, and 0.7–1 as susceptible. The mean of DS and IR were multiplied to obtain the coefficient of infection (COI) (Yu et al.

**Table 1** Virulence/avirulence profile of *Puccinia triticina* (*Pt*) races<sup>a</sup>

Races	Virulent on genes	Avirulent on genes
BBBQD	<i>Lr10</i> , <i>39</i> , <i>B</i>	<i>Lr1</i> , <i>2a</i> , <i>2c</i> , <i>3</i> , <i>3ka</i> , <i>9</i> , <i>11</i> , <i>14a</i> , <i>16</i> , <i>17</i> , <i>18</i> , <i>21</i> , <i>24</i> , <i>26</i> , <i>28</i> , <i>30</i> , <i>42</i>
MCDSS	<i>Lr1</i> , <i>3</i> , <i>10</i> , <i>14a</i> , <i>17</i> , <i>21</i> , <i>26</i> , <i>28</i> , <i>39</i> , <i>B</i>	<i>Lr2a</i> , <i>2c</i> , <i>3ka</i> , <i>9</i> , <i>11</i> , <i>16</i> , <i>18</i> , <i>24</i> , <i>30</i> , <i>42</i>
BBBQJ	<i>Lr10</i> , <i>14b</i> , <i>20</i> , <i>39</i> , <i>B</i>	<i>Lr1</i> , <i>2a</i> , <i>2c</i> , <i>3a</i> , <i>3ka</i> , <i>3bg</i> , <i>9</i> , <i>11</i> , <i>14a</i> , <i>16</i> , <i>17</i> , <i>18</i> , <i>24</i> , <i>26</i> , <i>28</i> , <i>30</i>
CCMSS	<i>Lr3</i> , <i>3ka</i> , <i>10</i> , <i>13bg</i> , <i>14a</i> , <i>14b</i> , <i>20</i> , <i>26</i> , <i>30</i> , <i>B</i>	<i>Lr1</i> , <i>2a</i> , <i>2c</i> , <i>9</i> , <i>11</i> , <i>16</i> , <i>17</i> , <i>18</i> , <i>24</i> , <i>42</i>

<sup>a</sup>Virulence/avirulence profile of *Pt* races BBBQD and MCDSS were adapted from Aoun et al. (2016), BBBQJ and CCMSS were adapted from Aoun et al. (2017), and Kolmer and Acevedo et al. (2016)

2012), which was used for the association analysis for adult plant resistance. These phenotypic data of field studies were automatically converted to three numerical measures (DS, IR and COI) using the *R* scripts for ‘field rust score conversion’ provided by Gao et al. (2016) ([https://github.com/umngao/rust\\_scores\\_conversion](https://github.com/umngao/rust_scores_conversion)).

Welch’s *t*-test was performed for multiple replicates of each growing seasons and between 2013 and 2014 replicates at both seedling and adult stages using the `ggbetweenstats()` function in *R* package, ‘`ggstatsplot`’ (Patil 2021). Because no significant difference ( $p > 0.05$ ) was observed among replicates for each growing season and between yearly replicates, the average of all replicates for seedlings and adult plants were taken and used for further analysis. The broad-sense heritability of traits was performed using the *R* package ‘`variability`’ with `gen.var()` function (Popat et al. 2020). Pearson’s correlation between different disease traits of seedlings and adult plants was calculated using the ‘`corrplot`’ *R* package (Wei and Simko 2021).

### Plant genotyping

The emmer panel was genotyped using the genotyping-by-sequencing (GBS) method as described in Poland et al. (2012) and Liu et al. (2020) and the wheat 9 K SNP Infinium iSelect array (Cavanagh et al. 2013) as per manufacturer’s guidelines (Illumina, San Diego, CA). For the GBS dataset, SNP discovery genotype calling was performed using the TASSEL-GBS pipeline (Glaubitz et al. 2014) with Bowtie2 alignment (Langmead and Salzberg 2012) to the IWGSC Chinese Spring RefSeq v1.0 reference genome (IWGSC 2018). SNP markers aligned on D genome were removed for further population structure and association analysis. The LD *k*-nearest neighbor (LD KNNi) imputation for missing genotypes (Money et al. 2015) and SNP filtering were performed using TASSEL 5.0 (Bradbury et al. 2007). SNP markers with > 5% minor allele frequency (MAF), genotypes with < 10% missing SNP calls, and markers with < 20% missingness were retained and used in GWAS analysis.

### Population structure and principal component analyses

The population structure of the emmer panel was analyzed by randomly selecting 5000 SNPs from the GBS data. The genotype file was converted to population genetic data (PGD) format using PGDSpider 2.1.1.5 (Lischer and Excoffier 2012). The population structure was analyzed using STRUCTURE 2.3.4 (Pritchard et al. 2000), with both the burn-in period and Markov Chain Monte Carlo (MCMC) reps after burn-in set at 10,000. The constant alpha value was achieved over this period of time. Three iterations were performed for each simulated value of *K*, ranging from 1 to 10. The optimal *K* values for the

population and subpopulation structures of the emmer panel were determined by structure harvester (<http://taylor0.biology.ucla.edu/structureHarvester/>) (Earl and vonHoldt 2012), using the log probability of the data [LnP(D)] and delta K ( $\Delta K$ ) based on the rate of change in [LnP(D)] between successive *K* values (Evanno et al. 2005). These population/subpopulation structures were also reflected as clusters in the principal component (PC) analyses performed using the *R* packages, ‘GAPIT3’ (Wang and Zhang 2021) and ‘stats’ with `prcomp` function (Team 2013). The number of PCs was chosen based on the scree plot. PCA plots were generated using the ‘`ggplot2`’ package in *R* (Wickham 2016).

### Marker-trait associations and gene annotations

GWAS analyses were conducted to identify significant markers associated with seedling and adult plant resistance to leaf rust. These involve association of filtered SNPs of either the GBS or the 9 K SNP array with seedling (IR) or adult plant (COI) responses of the 180 emmer accessions. Four statistical models were implemented for GWAS analysis in GAPIT3 (Wang and Zhang 2021) with population structure and kinship accounted for. These included the single-locus mixed linear model (MLM) (Zhang et al. 2010), and the three higher-power, multi-locus models multiple-locus MLM (MLMM), Fixed and random model circulating probability unification (FarmCPU) (Liu et al. 2016), and Bayesian-information and linkage-disequilibrium iteratively nested keyway (Blink) (Huang et al. 2018). Phenotypic variances explained ( $R^2$ , % PVE) by the individual SNPs were derived from the MLM model. The *Q-Q* plots were used to evaluate the best fit model for seedling and adult plant resistance using both genotypic datasets. Based on the *Q-Q* plots, the significance threshold for marker-trait associations was set at  $p \leq 0.0001$  [ $-\log_{10}(p) \geq 4$ ]. Manhattan plots were generated using the ‘`CMplot`’ package in *R* (<https://github.com/YinLiLin/CMplot>) (Yin et al. 2021).

Annotation for the associated markers for seedling and adult plant resistance were assigned based on the closest high-confidence annotated gene identified near the SNP physical location using the *T. aestivum* IWGSC RefSeq v1.0 reference genome (IWGSC 2018). To identify novel resistance genes in the cultivated emmer panel, the physical positions of significant SNPs were compared to the markers associated with known *Lr* genes, which were previously mapped to the IWGSC RefSeq v1.0 by two earlier studies (Fatima et al. 2020; Zhang et al. 2021).

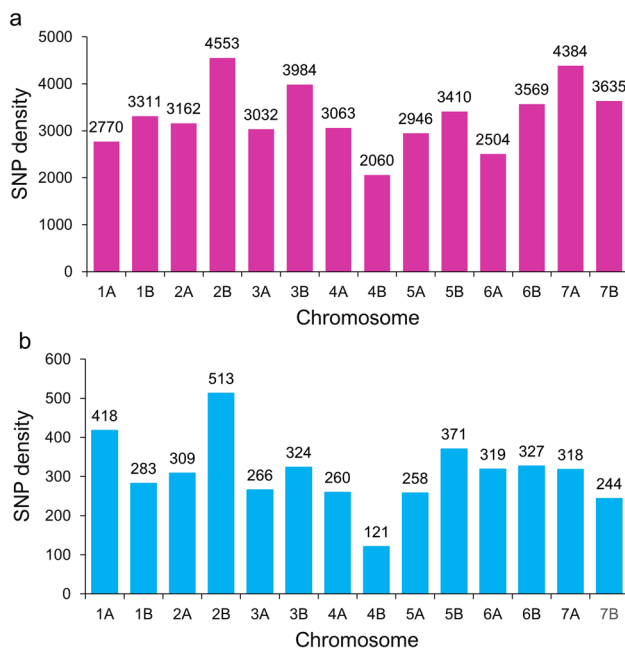


## Results

### Distribution and genotyping of the emmer panel

A diverse panel of 180 cultivated emmer accessions from USDA-ARS National Small Grains Collection were evaluated to identify loci associated with leaf rust resistance in the emmer population. The emmer accessions were originally collected from 32 different countries around the world, with the majority from Ethiopia, Spain, Russia, India and Serbia (Table S1).

The emmer panel was genotyped by GBS and the 9 K SNP Infinium array, resulting in 66,215 and 5911 polymorphic SNPs, respectively. After LD KNNi imputation and filtration, 46,383 SNPs from the GBS dataset (Table S2) and 4331 SNPs from the 9 K SNP array (Table S3) were retained with >5% minor allele frequency (MAF) and <20% missingness. The distribution of the filtered SNPs on the 14 chromosomes ranged between 2060 and 4553 SNPs from the GBS dataset (Fig. 1a) and 121–513 SNPs from the 9 K SNP array (Fig. 1b). In both cases, chromosome 2B was the most densely populated with SNPs and 4B was the least.



**Fig. 1** Chromosomal distribution of filtered SNPs on the A and B genomes of cultivated emmer using **a** GBS dataset and **b** 9 K SNP array based on the alignment to the IWGSC Chinese Spring RefSeq v1.0 (IWGSC 2018) and the consensus wheat SNP map in Cavanagh et al. (2013), respectively

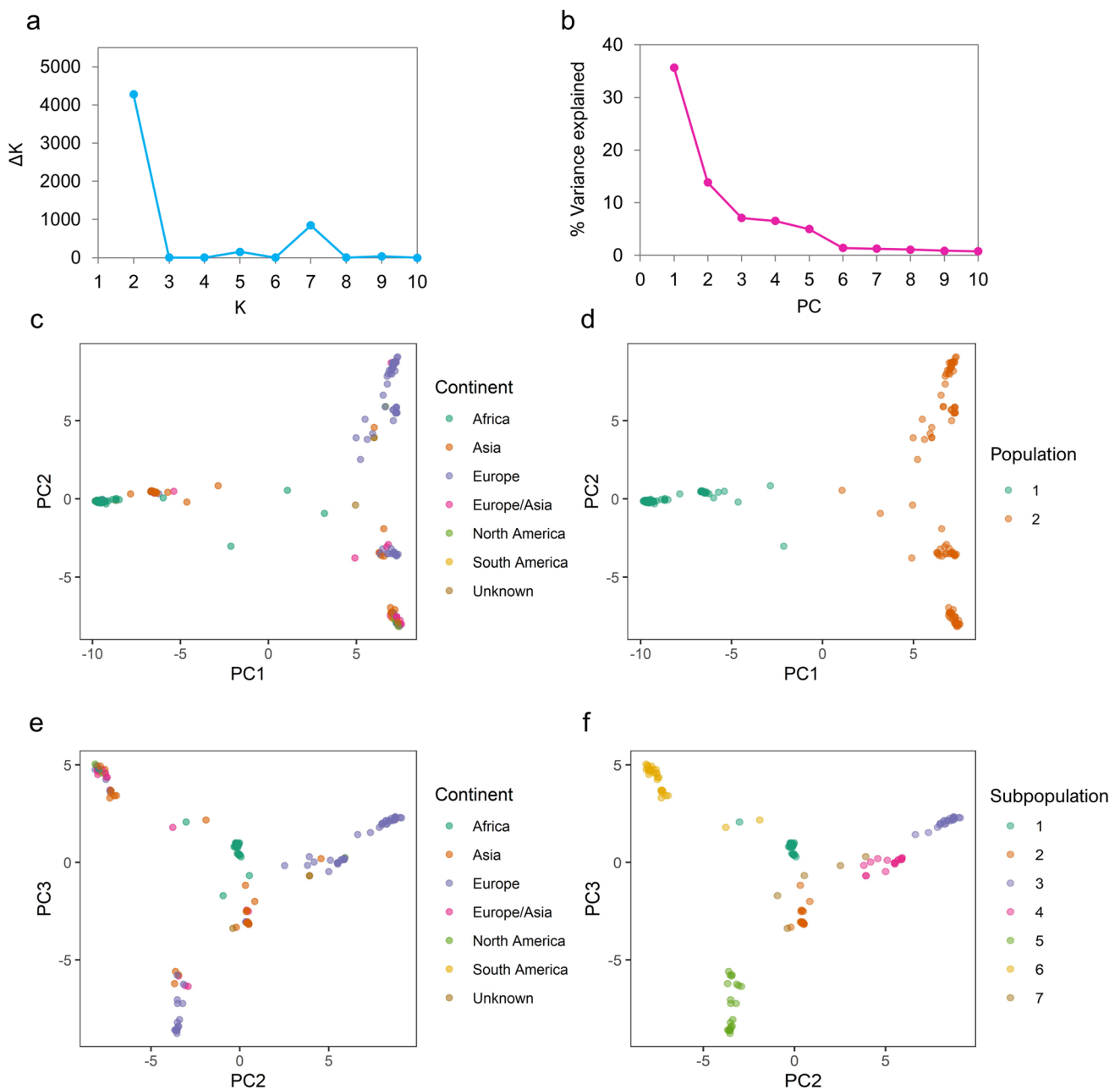
### Population structure and principal component analyses

To determine the population structure of emmer panel, 5000 SNPs from the GBS dataset were randomly selected and analyzed in STRUCTURE 2.3.4 (Pritchard et al. 2000). Distinct peaks were observed at  $K=2$  and  $K=7$ , suggesting two major populations and seven subpopulations in the emmer panel (Fig. 2a). The population clusters based on the optimal  $K$  values were also assessed in principal component analyses. The scree plot revealed the first three PCs to explain most of the genetic variation (57%) present in the emmer panel (Fig. 2b). At  $K=2$ , the first two PCs revealed the majority of emmer accessions from Africa were clustered in one group, whereas Europe, North and South America in a separate group (Fig. 2c, d). Emmer accessions from Asia were divided into both groups (Fig. 2c, d), with India and Iran as the two major countries that clustered with Africa and Europe, respectively (Table S1). At  $K=7$ , the second and third PCs clustered the emmer accessions from Ethiopia (Africa), India (Asia) and Spain (Europe) into the respective Subpopulation 1, 2 and 3 (Fig. 2e, f, Table S1). The formation of other clusters was based on a close geographic proximity. For example, most of the emmer accessions from Former Yugoslavia and Serbia (Asia and Europe) were clustered into Subpopulation 5, and Iran and Georgia (Asia and Europe) into Subpopulation 6. Emmer accessions belonging to the rest of European countries were clustered into Subpopulation 4, whereas no specific cluster was observed for Subpopulation 7.

### Phenotyping of the emmer panel

The emmer panel was evaluated for leaf rust responses at seedling and adult stages. The raw phenotypic data and calculated infection response (IR) values at the seedling stage and coefficient of infection (COI) values at the adult stage of all emmer accessions are presented in Table S4. The infection response against *Pt* race BBBQD at the seedling stage in greenhouse had a mean of 6.3 based on the 0–9 scale (Table 2). In the field nurseries, adult plants inoculated with the mixture of *Pt* races BBBQJ, CCMSS and MCDSS had mean leaf rust responses of 11.2, 0.7 and 9.1% for DS, IR and COI, respectively (Table 2). These mean values were derived from both 2013 and 2014 replicates. No significant differences were observed between the yearly replicates for seedlings ( $p=0.294$ ) and adult plants ( $p=0.054$ ) based on Welch's  $t$ -test (Fig. S1).

Significant phenotypic variations were observed for all the leaf rust responses at both seedling and adult stages (Fig. 3). However, IR at both stages were skewed more toward susceptibility (Fig. 3a, b), whereas DS was skewed toward low severity (Fig. 3c). COI represented the product



**Fig. 2** Population structure analysis of the emmer panel based on 5000 randomly selected SNPs from the GBS dataset. **a** The plot shows delta  $K$  ( $\Delta K$ ) for different number of populations ( $K$ ) assumed in the analysis. **b** The scree plot displaying the percent variance

explained by the principal component (PC) analyses. The first two PCs clustering the emmer panel based on **c** continents and **d**  $K=2$  populations. The second and third PCs clustering the emmer panel based on **e** continents and **f**  $K=7$  subpopulations

of IR and DS (Fig. 3d); thus, these traits were positively correlated. A stronger correlation was detected between COI and DS (0.92) than COI and IR (0.45) (Fig. 3e). The leaf rust responses of seedlings and adult plants exhibited positive, but weak correlations.

With regards to leaf rust responses, 16% of the emmer accessions were resistant, 16% were moderately resistant, and 68% were susceptible at the seedling stage (Fig. 3f). At

the adult stage, 2%, 31% and 66% of emmer accessions were resistant, moderately resistant, and susceptible, respectively. As evident, the majority of emmer accessions were susceptible at both stages. However, more accessions were found to be resistant at the seedling stage and moderately resistant at the adult stage. Among 180 accessions, 24 showed consistent resistance to *Pt* race BBBQD at seedling stage across two test experiments (Table 3) and nine had consistent adult

**Table 2** Descriptive statistical analyses of the emmer wheat panel in response to leaf rust at the seedling and adult stages

Stages	Seedling	Adult		
		DS	IR	COI
Accessions	180	180	175	175
Minimum	0.7	0	0.2	0.7
Maximum	8.7	46.7	1	41.3
Mean	6.3	11.2	0.7	9.1
Standard deviation	2.2	9.8	0.2	8.7
Broad-sense Heritability ( $H^2$ )	0.83	0.45	0.48	0.42

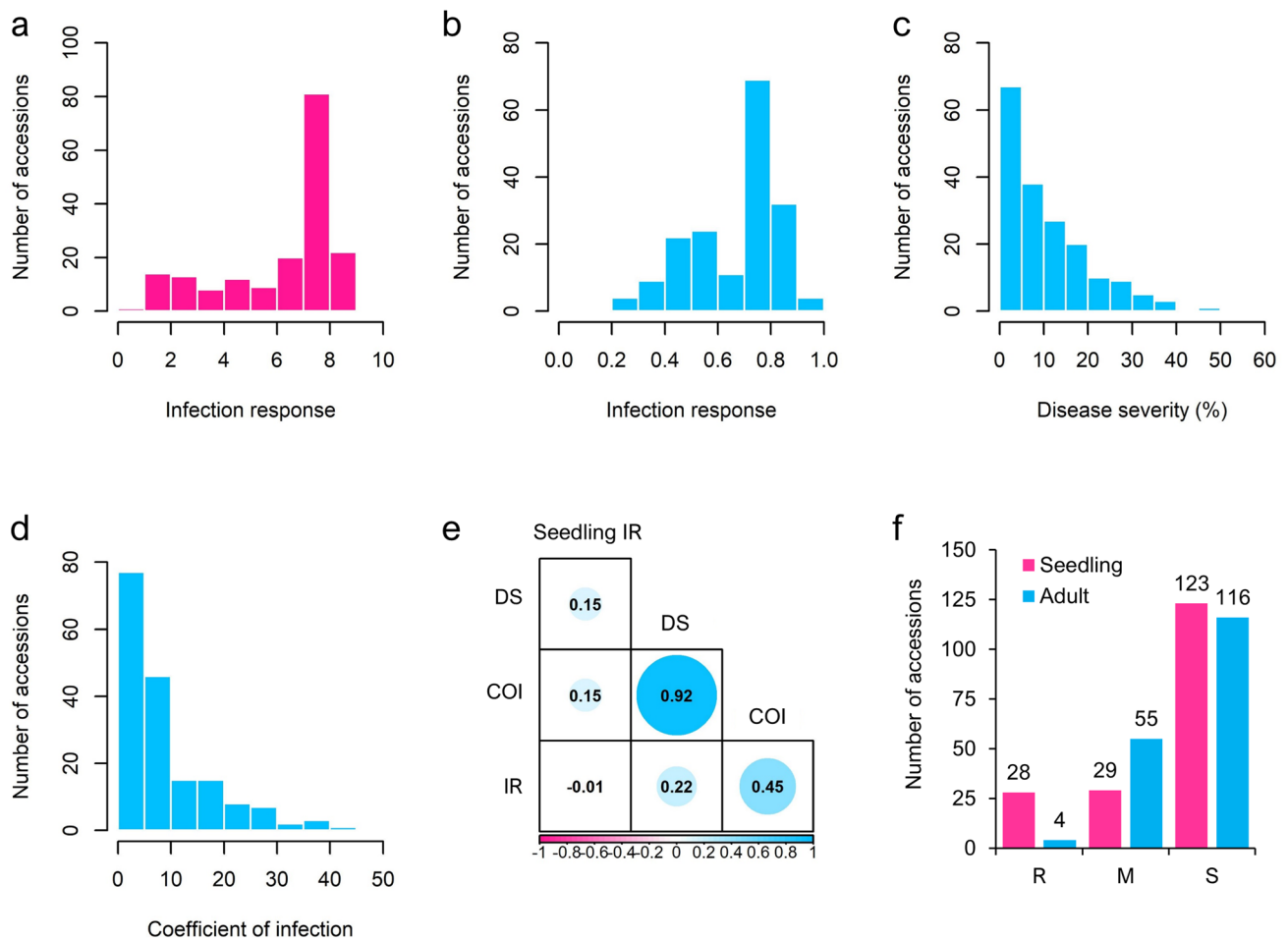
DS disease severity, IR infection response, and COI coefficient of infection

resistance in the field nursery in 2013 and 2014 (Table 4). Two accessions (PI 94674–1 and PI 94738–1) showed consistent resistance among replicates at both stages, whereas 79 accessions were susceptible at both stages (Table S4).

### Marker-trait associations

Four statistical models were primarily implemented for GWAS analyses to find marker-trait associations for seedling and adult plant resistance using both the GBS dataset (Table S5-S6) and the 9 K SNP array (Table S7, S8). The assessment of Q-Q plots revealed FarmCPU to be the best fit model for the GBS dataset (Fig. S2, S3) and MLMM for the 9 K SNP array (Fig. S3, S4). Using the GBS dataset, 13 SNPs were identified to significantly ( $p < 0.0001$ ) associate with seedling resistance (Fig. 4a) and 15 SNPs with adult plant resistance (Fig. 4b). Using the 9 K SNP array, 9 SNPs were found to significantly ( $p < 0.0001$ ) associate with seedling resistance (Fig. 5a) and 2 SNPs with adult plant resistance (Fig. 5b). No overlap in significant SNPs were observed between the seedling and adult plant resistance as well as between the GBS and 9 K SNP array datasets.

We further performed the Welch’s *t*-test to retain only the associated SNPs that displayed a significant phenotypic



**Fig. 3** Leaf rust responses of the emmer panel. Histograms of **a** infection response (IR) at the seedling stage; **b** IR, **c** disease severity (DS) and **d** coefficient of infection (COI) at the adult stage; **e** Correlation

between different leaf rust responses at the seedling and adult stages. **f** The susceptibility of emmer accessions at the seedling and adult stages based on linearized infection responses (**a**, **b**)

**Table 3** Cultivated emmer wheat accessions with consistent resistance to *Puccinia triticina* (*Pt*) race BBBQD at the seedling stage in greenhouse (GH) and their adult plant responses to a mixed *Pt* races in a field nursery in Debre Zeit, Ethiopia

Entry No.	Accession No <sup>a</sup>	IT GH <sup>b</sup>						Average IR	DS/IR Field <sup>c</sup>			Average COI
		2013			2014				2013	2014		
		1	2	3	1	2	3			1	2	
6	CItr 14916-1	13	-	1	;1	;1	1	1.9	5MS	5S/20MS	TMS	5.0
7	CItr 14917-1	13	1	1	;1	;1	;1	1.7	10MRMS	TRMS	TMS	2.6
12	PI 94621-1	12 <sup>-</sup>	-	-	;1	1	1	2.3	10MS	30RMR	5MR	6.0
25	PI 94674-1	2	-	2 <sup>-</sup>	1;	;	;1	2.2	0	30R	10RMR	4.4
28	PI 94738-1	1 <sup>+2-</sup>	21	1 <sup>-</sup>	2	2 <sup>-</sup>	;	2.9	0	20MR/5RMR	5MR	3.7
29	PI 94747-1	1 <sup>+2</sup>	21;	2	1	1 <sup>+</sup>	;	2.8	5MS	30RMR	TMR	4.3
41	PI 194042-1	21	21	11 <sup>+</sup>	1	1	1 <sup>+</sup>	2.9	30MS	1MS	5MS	9.6
44	PI 197485-1	22 <sup>-</sup>	1;	22 <sup>+</sup>	;	;	-	2.3	TMR	5S	5MS	3.3
50	PI 244341-1	2	21	-	;1	1	-	2.9	10MS	30MSS	20MS	16.7
52	PI 254167-1	2	21	-	1;	;13 <sup>-</sup>	;1	2.7	TMS	0	0	1.6
54	PI 254190-1	-	21	1	;	1;	1	1.9	5MS	20RMR	0	4.7
56	PI 273981-1	;	1 <sup>+2</sup>	1;	1	1	1	1.8	0	5MS	TMS	2.8
57	PI 275996-1	1	1 <sup>+</sup>	;1	1	1	1	1.9	0	0	TMS	1.6
64	PI 349046-1	12	-	1	;1	;	-	1.4	5MS	20RMR	5RMR	3.6
83	CItr 14639	12	12	12	;1 <sup>+</sup>	1	1 <sup>+</sup>	2.5	5MS	15MSS	5MS	7.0
84	CItr 14751	1	1	1	;	1	-	1.6	TMS	30MSS	10MSS	12.1
106	PI 193878	11 <sup>+</sup>	1	1	1	1;	1	1.9	TMS	20MSS	20MS	11.7
123	PI 275999	2 <sup>-</sup>	-	2 <sup>-</sup>	;1	1;	;1	2.1	5MR	TMS	TMS	1.7
160	PI 361833	21	1;	;1	;1	1	;	1.4	5MS	TMS	5MS	3.2
169	PI 384318	1;	1	;1 <sup>-</sup>	1	1	;1	1.4	0	20MS	20MS	16.0
173	PI 479957	21	1	1	1	1;	;	1.9	TMS	10MS	20MRMS	6.7
174	PI 479965	21;	1;	21	1	1;	-	2.3	TMS	60MSS	40MSMR	26.9
175	PI 480307	1	-	1	1	1	-	2.0	0	40MS	30MSS	29.1
177	PI 480313	1	1;	13	-	;	;1	1.6	0	20MS	10MS	12.0

<sup>a</sup>Accession numbers (USDA-ARS National Small Grains Collection, Aberdeen, Idaho) followed by “-1” (e.g., CItr 14916-1) indicated a single plant selection. <sup>b</sup>Infection type (IT) follows 0, ;, 1, 2, 3, and 4 scales described in McIntosh et al. (1995), where they represents no visible sign or symptom (0), small yellow flecks (;), small uredinia with necrosis (1), small to medium sized uredinia with green islands and surrounded by necrosis or chlorosis (2), medium sized uredinia with or without chlorosis (3), and large uredinia without chlorosis (4). The signs “-” and “+” indicate smaller and larger uredinia, respectively. For IT combinations, order indicates predominant types, e.g., IT 34 is predominantly IT 3 with decreasing amounts of IT 4. ITs 0-2 are considered resistant, and 3-4 are considered susceptible. <sup>c</sup>Adult plant reactions were scored as disease severity (DS) and infection response (IR). DS was represented as the percentage of tissue infected. IR was represented as ‘R’ for resistant, ‘MR’ for moderately resistant, ‘MS’ for moderately susceptible, and ‘S’ for susceptible. ‘RMR’, ‘MRMS’, or ‘MSS’ represented mixed responses, with the predominant response being listed first

difference ( $p \leq 0.05$ ) between the alternative alleles. This resulted in a total of 15 QTL for seedling resistance using both genotypic datasets, which were found on chromosomes 2A, 2B, 3B, 4A, 5A, 6B, 7A and 7B (Table 5). Among these, *w SNP\_Ex\_c5072\_9006666* on chromosome 4A and *w SNP\_Ex\_c1996\_3754394* on 2B contributed the most to the phenotypic variance (> 15%). These markers were found near the genomic regions of genes encoding DNA methyltransferase and cytochrome P450. The remaining loci have minor (< 5%) to moderate (< 10%) effects on the phenotypic variance. On the other hand, 11 QTL were identified for adult plant resistance that display

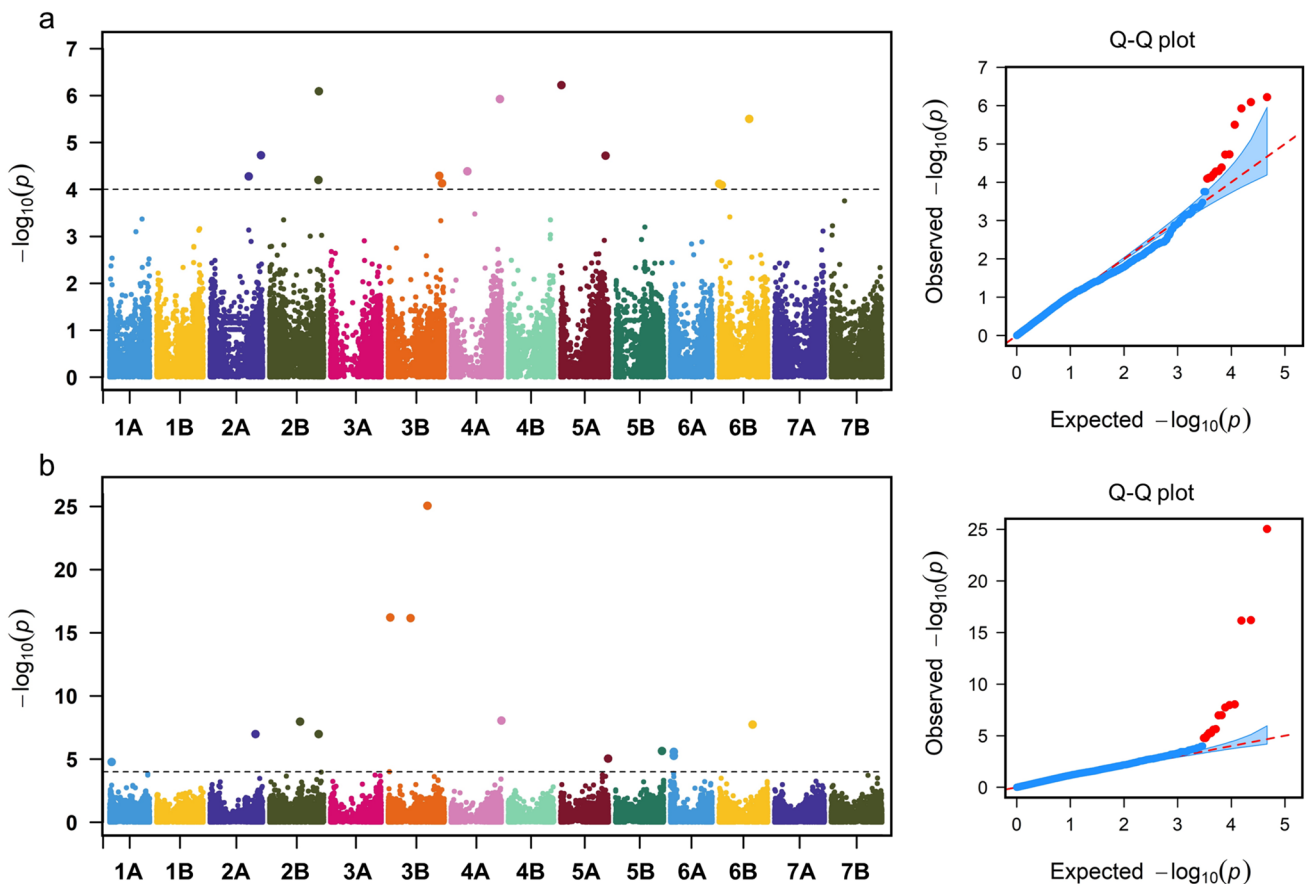
significant marker-trait associations between the alternative alleles (Table 5). These QTL were located on chromosomes 1A, 2B, 3B, 4A, 5B, 6A and 6B, and the majority of them exhibited minor effects on the phenotypic variance. Markers on chromosome 3B such as *S3B\_330595912* and *S3B\_583036386* displayed moderate effects and mapped near genes that encode protein MIZU-KUSSEI 1 and RING/U-box superfamily protein, respectively.



**Table 4** Cultivated emmer wheat accessions with consistent adult plant resistance to mixed races of *Puccinia triticina* (*Pt*) in a field nursery (Debre Zeit, Ethiopia) and their seedling reactions to *Pt* race BBBQD<sup>a</sup>

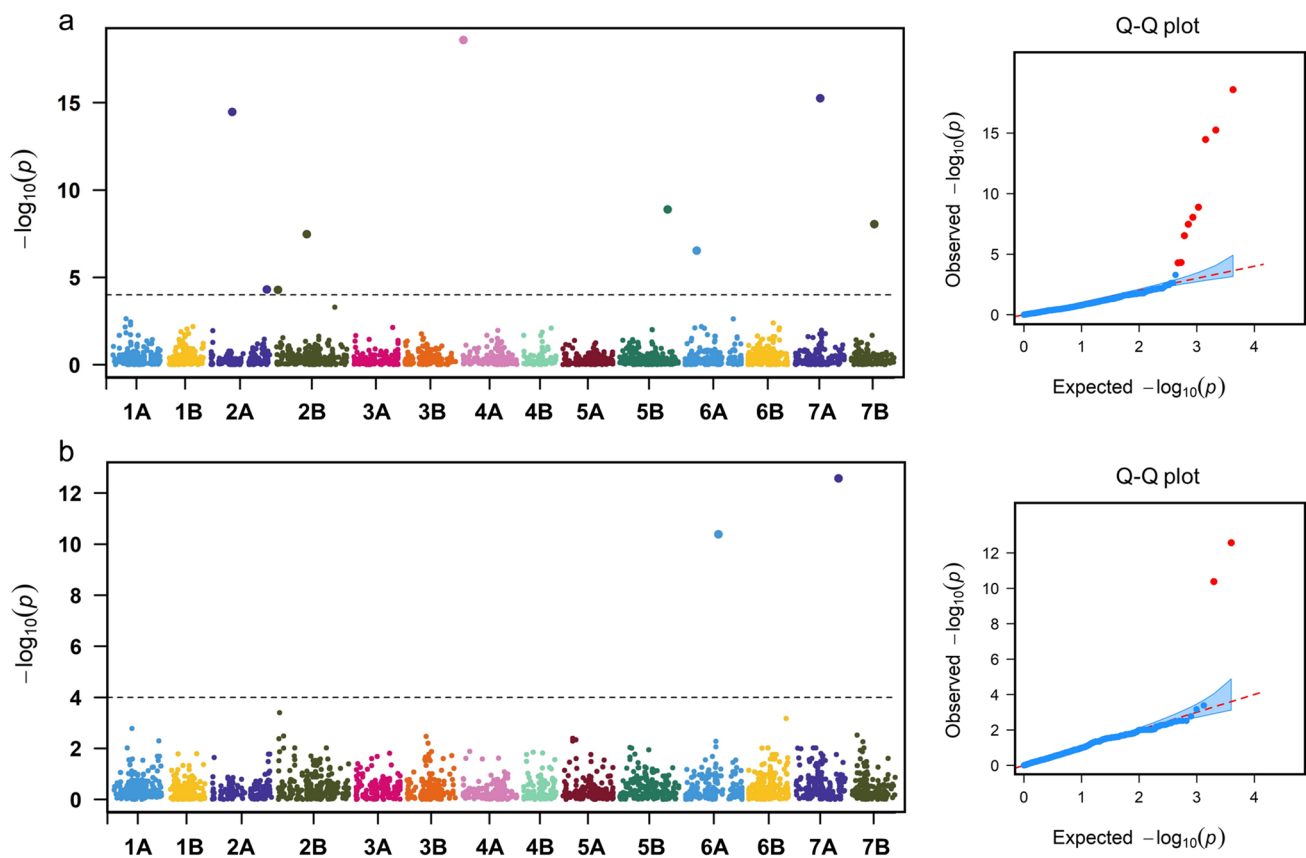
Entry No.	Accession No.	IT GH						Average IR	DS/IR Field			Average COI	
		2013			2014				2013	2014			
		1	2	3	1	2	3			1	2		
9	PI 41024-1	3	32	32	3	3	3	7.7	10MR	10MR	5MR/5MS	3.6	
11	PI 94616-1	3	3	–	3	3	3	8.0	10MR	15RMR	20MR	5.4	
13	PI 94625-1	32	4	–	3	3	3	8.0	15MR	15MR	5MRMS	4.9	
20	PI 94648-1	3	3	3 <sup>+</sup>	3	3	3	8.2	10MSMR	5R	TMR	2.8	
23	PI 94666-1	32	13	3	1	;	1	;	3.6	10MRMS	20MR	30MR	8.4
24	PI 94673-1	32 <sup>+</sup>	3 <sup>-</sup>	3	3	3	3	7.7	10MSMR	20MRMS	15MR	7.8	
30	PI 101971-1	3 <sup>+</sup> 2	3	2 <sup>+</sup> 3	3	3	3	7.7	0	20RMR	5RMR	3.4	
31	PI 133134-1	32	32 <sup>+</sup>	22 <sup>+</sup>	1	2 <sup>+</sup>	3	5.9	0	5RMR	0	1.4	
98	PI 94668	3	3	3	3	3 <sup>+</sup>	3	8.2	10MR	15RMR	5MR	3.4	

<sup>a</sup>See footnotes in Table 3



**Fig. 4** Manhattan plots displaying SNPs from the GBS dataset that are significantly associated with **a** infection response of cultivated emmer at the seedling stage, and **b** coefficient of infection at the adult stage, based on the FarmCPU model. The significance threshold was

set at  $[-\log_{10}(p) \geq 4]$  (dashed line). The y-axis indicates  $-\log_{10}(p)$  for GBS markers. The x-axis indicates the physical distribution of all the GBS markers on the 14 chromosomes of cultivated emmer based on the alignment to the IWGSC RefSeq v1.0 (IWGSC 2018)



**Fig. 5** Manhattan plots displaying SNPs from the 9 K SNP array that are significantly associated with **a** infection response of cultivated emmer at the seedling stage, and **b** coefficient of infection at the adult stage, based on the MLM model. The significance threshold was

set at  $[-\log_{10}(p) \geq 4]$  (dashed line). The y-axis indicates  $-\log_{10}(p)$  for SNP array markers. The x-axis indicates the positions of all the SNP array markers on the 14 chromosomes of cultivated emmer based on the consensus wheat SNP map in Cavanagh et al. (2013)

## Discussion

Constant evolution of *P. tritricina* population frequently renders the deployed *Lr* genes ineffective, necessitating new sources of leaf rust resistance. In the primary gene pool of wheat, cultivated emmer is an excellent source of useful genes for improving modern wheat germplasm. Resistance genes for stem rust such as *Sr2/Yr30* and *Sr13* were originally derived from cultivated emmer, which are now widely deployed in modern durum and/or bread wheat cultivars (McFadden 1930; Williams and Gough 1965; Zaharieva et al. 2010; Sharma et al. 2019). Stripe/yellow rust resistance genes such as *Yr15*, *Yr35*, *Yr36*, and *YrH52*, and leaf rust resistance genes such as *Lr53* and *Lr64* were discovered in the wild progenitor of cultivated emmer, and successfully transferred to modern wheat cultivars (Gerchter–Amitai et al. 1989; Peng et al. 1999; Uauy et al. 2005; Fu et al. 2009; Dadkhodaie et al. 2011; McIntosh et al. 2013). Despite their importance as resistance sources for rust diseases, the large collections of cultivated emmer have not been explored for leaf rust resistance.

The study herein evaluated the diverse panel of 180 cultivated emmer against the highly virulent *Pt* race BBBQD at the seedling stage and mixed races BBBQJ, CCMSS and MCDSS at the adult stage in the nursery field. Among these accessions, 24 (13.3%) had consistent seedling resistance to *Pt* race BBBQD at seedling stage and nine (5.0%) had consistent adult resistance. In addition, approximately 31 (17.2%) accessions had variable expressions for reactions to BBBQD at seedling stage among the replications in the two experiments, with most of the plants in these accessions having resistant reactions. This result indicated that cultivated emmer is good source for resistance to leaf rust. Aoun et al. (2016) evaluated 496 durum wheat accessions for resistance to the durum wheat-specific races including BBBQD, BBBQJ, and MCDSS used in this study. They found that only 4.9% (24) of 496 durum accessions showed resistance to BBBQD, indicating that source of resistance to this race is rare in durum germplasm. Therefore, the cultivated emmer accessions with resistance to BBBQD may carry novel *Lr* genes that could be used to improve modern durum crops for resistance to this newly emerged *Pt* race. In addition,

**Table 5** QTL of the cultivated emmer panel that are significantly ( $p < 0.0001$ ) associated with the seedling and adult plant resistance to leaf rust

QTL	SNP	Chr <sup>a</sup>	Position (Mb)	Marker code	Position (cM)	R <sup>2</sup>	WT	Alleles	R allele	Known <i>Lr</i> gene	Gene annotation
<i>Seedling resistance</i>											
<i>S-QLr2A.1</i>	wspn_Ex_c22862_32074455	2A	165.85	IWA2758	104	8.5	*	A/G	G		Serine/threonine-protein kinase
<i>S-QLr2A.2</i>	S2A_572053459	2A	572.05			4.7	**	C/T	T		MADS-box transcription factor
<i>S-QLr2B.1</i>	wspn_Ex_c1996_3754394	2B	1.33	IWA2482	8.3	16.2	**	C/T	T		Cytochrome P450
<i>S-QLr2B.2</i>	wspn_Ex_c5239_9272511	2B	102.98	IWA4135	65.2	5.3	*	T/C	T		Potassium transporter
<i>S-QLr2B.3</i>	S2B_728575970	2B	728.58			6.3	**	T/A	A		Subtilisin-like protease
<i>S-QLr3B.1</i>	S3B_757206389	3B	757.21			7.3	*	C/T	T		Uncharacterized
<i>S-QLr3B.2</i>	S3B_798205726	3B	798.21			4.2	*	A/G	G		F-box domain containing protein
<i>S-QLr4A.1</i>	S4A_722795420	4A	722.8			9.8	****	T/A	A	<i>Lr28 (Aegilops speltoides)</i>	30S ribosomal protein S8
<i>S-QLr4A.2</i>	wspn_Ex_c5072_9006666	4A	742.57	IWA4083	173.6	19.1	****	G/A	G		DNA methyltransferase
<i>S-QLr5A</i>	S5A_10890513	5A	10.89			9.6	****	G/T	T		GTPase family protein
<i>S-QLr6B.1</i>	S6B_2651834	6B	2.65			3.3	*	G/A	A		Mitochondrial metalloendopeptidase
<i>S-QLr6B.2</i>	S6B_40137103	6B	40.14			5.4	****	C/T	T	<i>Lr53 (T. dicoccoides)</i>	F-box family protein (LRR)
<i>S-QLr6B.3</i>	S6B_446331726	6B	446.33			6.0	****	C/T	T		Aldehyde dehydrogenase
<i>S-QLr7A</i>	wspn_Ku_c10202_16937059	7A	581.35	IWA6376	133.5	7.0	*	T/C	T		Reduced growth phenotype protein1
<i>S-QLr7B</i>	wspn_Ku_c854_1768346	7B	644.44	IWA7330	132.9	6.4	**	C/T	C		Uncharacterized
<i>Adult plant resistance</i>											
<i>A-QLr1A</i>	S1A_30014733	1A	30.01			1.0	****	T/C	T		CsAtPRS
	S1A_30014746	1A	30.01			1.0	****	C/T	C		
<i>A-QLr2B.1</i>	S2B_451416943	2B	451.42			4.3	****	C/T	C		Ser/Thr protein kinase
<i>A-QLr2B.2</i>	S2B_725576212	2B	725.58			0.0	***	C/T	C		Squamosa promoter-binding-like protein
<i>A-QLr3B.1</i>	S3B_32712061	3B	32.71			1.1	****	C/T	C		Calcium-binding EF hand protein
<i>A-QLr3B.2</i>	S3B_330595912	3B	330.6			9.7	****	G/C	G		Protein MIZU-KUSSEI 1
<i>A-QLr3B.3</i>	S3B_583036386	3B	583.04			8.3	****	C/T	C		RING/U-box superfamily protein
<i>A-QLr4A</i>	S4A_744305546	4A	744.31			2.8	***	G/T	G		Methyltransferase-like protein
<i>A-QLr5B</i>	S5B_689895163	5B	689.9			5.2	****	C/T	C	<i>Lr18 (T. timopheevi)</i>	Pentatricopeptide repeat protein
<i>A-QLr6A.1</i>	S6A_50118325	6A	50.12			2.7	****	G/C	C		Peroxidase
<i>A-QLr6A.2</i>	S6A_50356132	6A	50.36			2.8	****	C/G	C		Cytochrome P450
	S6A_50356134	6A	50.36			2.8	****	C/G	C		
<i>A-QLr6B</i>	S6B_497892791	6B	497.89			1.9	**	C/T	T		F-box protein-like (LRR)

<sup>a</sup>Chr, Chromosome, R<sup>2</sup>, percent phenotypic variance explained (PVE), R allele, resistant allele, WT, Welch's t-test with a significance level of \*, \*\*, \*\*\* and \*\*\*\* corresponding to  $p \leq 0.05$ , 0.01, 0.001, and 0.0001, respectively

a few of emmer wheat accessions from Africa with adult resistance to mixed races BBBQJ, CCMSS and MCDSS may also have unique *Lr* genes. To detect the genomic regions harboring the *Lr* genes, we performed GWAS analyses on the emmer panel and identified few overlapping loci with previously reported *Lr* genes or QTL in addition to finding new loci.

Seedling resistance is considered effective at all stages of plant growth, and it is often race-specific and controlled by major effect resistance genes (Ellis et al. 2014; Zetzsche et al. 2019). In the cultivated emmer panel, we identified 15 QTL that contributed to seedling resistance, of which, 2 QTL were found near the marker regions of two known *Lr* genes such as *Lr28* and *Lr53* (Table 5, S5). The moderate effect *S-QTr4A.1* was mapped to the similar genomic regions where the markers (*barc343-wmc219*) associated with *Lr28* were earlier detected (McIntosh and Te 1982; Sohail et al. 2014). However, *Lr28* was reported to originate from chromosome 7S#2 of *Aegilops speltoides*, and translocated to chromosome 4AL of bread wheat (Naik et al. 1998). Therefore, finding *Lr28* in the cultivated emmer genome might be unlikely and their shared genomic regions might have occurred by chance. On the other hand, a moderate effect QTL, *S-QTr6B.2*, was found < 1 Mb upstream of the *Lr53*-associated marker, *CFD1* (Dadhodaie et al. 2011; McIntosh et al. 2013). These QTL of cultivated emmer may represent the same loci contributing to resistance conferred by *Lr53*, the gene derived from its progenitor, wild emmer wheat.

Besides the catalogued *Lr* genes, many QTL contributing to leaf rust resistance have been identified in bread and durum wheat using meta-QTL analysis (Amo and Soriano 2022). The moderate effect *S-QTr7A* of the emmer was present close to durum wheat QTL, *QTr.locus-7A.2* (~ 30 kb) (Table S7) (Wang et al. 2014; Aoun et al. 2016). These might represent the same QTL since the AB genomes of durum wheat are derived from emmer wheat. The remaining QTL (13/15) may represent novel loci in the emmer panel that potentially contribute to seedling resistance. A majority of these novel QTL exhibited moderate effects, among which, *S-QTr4A.1* and *S-QTr5A* were significantly detected by three statistical models, and *S-QTr3B.1* and *S-QTr6B.3* by two models (Table S5). These QTL are strong candidates for novel *Lr* genes for seedling resistance. In addition, *S-QTr4A.2* and *S-QTr2B.1* had major effects, and transfer of such QTL might enhance seedling resistance in modern wheat cultivars.

Adult plant resistance is effective at later stages of plant growth and often race non-specific and controlled by multiple minor effect resistance genes (Zhang et al. 2021). In the emmer panel, 11 unique QTL were associated with adult plant resistance to leaf rust. The moderate effect *A-QTr5B* was present within the marker regions flanking *Lr18* (Table 5, S6) (Carpenter et al. 2018; Fatima et al.

2020). Since *Lr18* was originally derived from G genome of *T. timopheevi* and translocated to chromosome arm 5BL of bread wheat (Friebe et al. 1996), it is unlikely for the cultivated emmer to acquire the same *Lr18* gene without translocation. Hence, the QTL found near the region of *Lr18* might be a novel locus.

However, the minor effect *A-QTr6B* mapped to the overlapping regions as the markers (*Xbarc5-Xgwm469.2*) of *QTr.fcu-6BL*, which was previously identified to contribute to adult plant resistance in synthetic hexaploid wheat (SHW) (Table S6) (Chu et al. 2009). Hence, these QTL may represent the same loci in hexaploid wheat and emmer wheat. The remaining QTL (10/11) are potentially novel loci that confer adult plant resistance to leaf rust in the emmer panel. These QTL mostly provide minor to moderate effects and exist mainly on chromosomes 2B, 3B and 6A. Among these, *A-QTr3B.2* and *A-QTr3B.3* that contributed highest to the phenotypic variance were conferred significant by three or more statistical models (Table S6). In addition, *A-QTr5B* and *A-QTr6A.1* were detected significant by two models. These QTL are strong candidates for adult plant resistance.

In this study, we identified 24 and nine cultivated emmer accessions having consistent seedling resistance to *Pt* race BBBQD and adult plant resistance to mixed races BBBQJ, CCMSS and MCDSS, respectively. These accessions should be a valuable resource for improving modern durum and bread wheat for resistance to leaf rust. A total of 15 and 11 QTL were identified to be associated with seedling and adult resistance, respectively. Interestingly, most of these QTL are in the genomic regions where no known *Lr* genes or QTL were previously reported. Furthermore, the cultivated emmer germplasm collections were not systematically explored for leaf rust resistance and none of the 79 catalogued *Lr* genes in wheat are derived from cultivated emmer. Therefore, some QTL might be potentially associated with new *Lr* genes. Many of the resistant emmer accessions (Tables 3 and 4) contain the resistant alleles of QTL identified herein especially *S-QTr2B.2* and *S-QTr4A.2* for seedling resistance (Table S9), and *A-QTr-2B.1*, *A-QTr-2B.2*, *A-QTr-3B.2*, *A-QTr-3B.3*, *A-QTr-4A* and *A-QTr-5B* for adult plant resistance (Table S10). These QTL likely represent novel *Lr* genes derived from the cultivated emmer.

However, the GWAS results presented in this study only provides preliminary information on the genomic regions potentially harboring *Lr* genes. The QTL identified need to be confirmed and validated through further linkage analysis, cloning, and functional genetics. The phenotypic and genotypic data and GWAS results presented in this study provide guidance to select certain emmer accessions as the parents for developing mapping populations that can be used to identify the causal genes underlying the QTL. Several emmer accessions, such as CItr 14916-1, CItr 14917-1,

PI 273981–1, PI 275996–1, PI 349046–1, CItr 14639, CItr 14751, PI 193878, PI 361833, PI 384318, PI 479957, PI 480307, and PI 480313 consistently exhibited a high level of seedling resistance to *Pt* race BBBQD. Because *Pt* race BBBQD poses a potential threat to durum crop in North America and sources of resistance is rare in durum germplasm, the emmer accessions with a high level of resistance can be used as resistance donors in developing adapted durum germplasm and varieties with resistance to this race. Furthermore, the cultivated emmer panel and its associated genotypic data sets presented in this study will be useful for identifying and mapping the genes for resistance to other fungal diseases.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s00122-023-04281-6>.

**Author contribution statement** SX and MA conceived and initiated the project. QZ prepared and increased seed stocks of the emmer panel. QS and MA performed the phenotype analysis and XL and JF (Fiedler) genotyped the emmer panel with the GBS method. QS conducted preliminary analysis for dissertation research advised by SX, XC, JF (Faris) and GX and assisted in manuscript preparation. DL performed all the data and bioinformatic analyses and wrote the manuscript assisted with SX and YG. UG and MA performed gene postulations. All authors reviewed and revised the manuscript.

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**Data availability** All data generated or analyzed during this study are included in this published article and its supplementary information files.

## Declarations

**Competing interest** The authors declare no competing interest.

**Code availability** Not applicable.

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