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Genome-Wide Association Study of Leaf Rust Resistance at Seedling and Adult Plant Stages in a Global Barley Panel

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Abstract: Barley leaf rust caused by *Puccinia hordei (Ph)* is one of the major limiting biotic stresses of barley production worldwide and causes yield losses of up to 60%. A diversity panel of 316 barley genotypes (AM2017) composed of released cultivars, advanced breeding lines and landraces was screened for *Ph* resistance at the seedling stage using two isolates (SRT-SAT and SRT-MRC), while the adult plant stage resistance screening was conducted at the disease hotspot location of Sidi Allal Tazi (SAT) for the cropping seasons of 2017 and 2019. The phenotypic responses were combined with 36,793 single nucleotide polymorphism (SNP) markers in a genome-wide association study (GWAS) using the general linear model (GLM), mixed linear model (MLM), settlement of MLM under progressively exclusive relationship (SUPER), multiple-locus MLM (MLMM), fixed and random model circulating probability unification (FarmCPU), and Bayesian-information and linkage-disequilibrium iteratively nested keyway (BLINK) in GAPIT3, and MLM (K+Q), MLM (K+PCA), and GLM (Q) models in TASSEL to identify genomic regions linked to *Ph* resistance. Fourteen barley genotypes were resistant (R) at the seedling stage to both *Ph* isolates, SRT-SAT and SRT-MRC, and twelve genotypes were either resistant (R) or moderately resistant (MR) at the adult plant stage, whereas only one genotype was resistant at the seedling stage, and moderately resistant at the adult plant stage. The genome scan revealed 58 significant marker trait associations (MTA) among which 34 were associated with seedling resistance (SR) and 24 with adult plant resistance (APR). Common genomic regions conferring resistance to *Ph* were identified at both stages on chromosome 2H (106.53 cM and at 107.37 cM), and on chromosome 7H (126.7 cM). Among the 58 MTA identified, 26 loci had been reported in previous studies, while the remaining 32 loci were regarded as novel. Furthermore, the functional annotation of candidate genes (CGs) adjacent to 36 SNP markers with proteins involved in disease resistance further confirms that some of the SNP markers from our study could be associated with *Ph* resistance in barley. The resistant barley genotypes and some of the SNP markers from this study with high R^2 and additive effects can be converted into high-throughput functional markers for accelerated selection and pyramiding of leaf rust resistance genes in North African barley germplasm.

Keywords: barley; *Hordeum vulgare*; leaf rust; *Puccinia hordei*; resistance; genome-wide association study; marker-trait-association

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1. Introduction

Barley (*Hordeum vulgare* L.) ranks fourth among the most important cultivated cereal crops worldwide for food, feed, and malting purposes [1]. It can tolerate harsh environmental conditions, such as low rainfall, fluctuating temperature, high altitude, and highly

saline soils, where other cereals grow poorly. In Morocco, barley has been grown on about 2 million hectares in the arid and semi-arid regions with 1.82 t/ha of average grain yield registered in 2018, which is significantly lower compared to the world's average grain yield (2.95 t/h) [1]. In addition, Morocco imported 240.5 thousand tons of barley from France, Spain, Ukraine, Greece, and Italy in 2018 [2]. Different abiotic and biotic stress factors contribute to lower barley yield in Morocco. In addition to low input conditions, many biotic stresses, such as leaf rust, powdery mildew, net form and spot form net blotches, scald, and spot blotch, limit barley grain and straw yields and quality.

Barley leaf rust (BLR) caused by the fungal pathogen *Puccinia hordei* is the most common and geographically widespread disease [3]. BLR causes serious yield losses in North Africa, Europe, New Zealand, Australia, and the Eastern and Midwestern United States [4–8]. Under epidemic conditions, up to 62% of yield losses have been reported in susceptible and late maturing barley cultivars [4]. The use of fungicides is effective to control BLR, but the deployment of genetically resistant barley cultivars is the most effective, economical, and environment-friendly control strategy. Genetic resistance is mostly categorized into qualitative and quantitative resistance. The qualitative resistance or major gene(s) resistance is often race-specific, governed by one or two resistance genes and follows the Flor's gene-for-gene concept [9]. Quantitative resistance, also known as 'partial or horizontal resistance' is usually controlled by several minor genes. Quantitative resistance is considered race non-specific in the field trials and is characterized by low infection frequency, prolonging latent period, and smaller pustule size to slow down pathogen infection and reproduction (slow rusting) [10,11]. This resistance is assumed to be durable because the chances that pathogens may mutate and develop virulence to all matching minor resistance genes are low.

The availability of sources of BLR resistance and understanding its genetics are crucial for efficient development of resistant barley cultivars [3]. Since the first genetic study on BLR resistance [12], 28 resistance genes to *P. hordei* (*Rph*) have been mapped on all seven barley chromosomes. Among them, three *Rph* genes, namely *Rph20*, *Rph23*, and *Rph24*, mediate adult plant resistance (APR) [13–15], whereas the remaining 25 *Rph* genes (*Rph1-Rph19*, *Rph21*, *Rph22*, and *Rph25-Rph28*) have been identified for seedling resistance [3,16–19]. However, the excessive use of dominant *Rph* genes exerts selection pressure on the pathogen population, which rendered BLR resistance genes ineffective within a few years [20–22]. This emphasizes the need for the identification of new sources of resistance to increase the genetic diversity of BLR resistance [14].

The majority of quantitative trait loci (QTL) conditioning resistance to BLR have been identified via traditional bi-parental mapping [7,23–26]. These bi-parental mapping studies were highly efficient in identifying QTL associated with resistance to *P. hordei* by map-based cloning [13,15,27], but they were limited by low allelic diversity and low recombination events, which affected the mapping resolution [28]. In addition, this approach is time-consuming as it involves phenotyping of successive generations. These limitations could be overcome with the use of GWAS, which is a powerful tool to dissect the genetic architecture of complex traits utilizing pre-existing germplasm [28]. This approach is based on detecting genomic regions that are in linkage disequilibrium (LD) with genes affecting the trait of interest. Owing to a large number of available SNP markers, GWAS is faster and more accurate as it can map QTL at a much higher resolution [29,30]. Furthermore, high efficiency of GWAS has been demonstrated in barley for genetic mapping of disease resistance to net form net blotch [31], spot form net blotch [32,33], spot blotch [22,34], leaf rust [35], stem rust [36], and stripe rust [22,37]. However, GWAS is prone to false-positive QTL identification due to the population structure [38], which has been circumvented by taking it as a covariate in the association mapping studies. However, bi-parental mapping is desirable to validate marker-trait associations (MTA) [39].

The objectives of the present study were to describe the phenotypic variation of the AM2017 panel for its response to two pure isolates of *P. hordei* from Morocco at the seedling stage (SRT) under controlled conditions, as well as in the field for adult plant stage resistance

(APR). In addition, these phenotypic responses were combined with 50k SNP marker data in GWAS analyses to identify genomic regions associated with *P. hordei* resistance.

2. Materials and Methods

2.1. Plant Material

A set of 316 genotypes of spring barley, designated here as the Association Mapping panel 2017 (AM2017), including landraces from the ICARDA (International Center for Agriculture Research in the Dry Areas) genebank, released cultivars from different countries, and advance breeding lines from the spring barley breeding program of ICARDA, was used in this study. The AM2017 panel has 173 two-row and 143 six-row type barley genotypes including feed and malting types from Europe, North America, South America, Africa, Australia, and Asia. The full list of barley genotypes with details is available in Supplementary Table S1.

2.2. Screening for Seedling Resistance to P. hordei

A seedling resistance test (SRT) was conducted with two pure isolates of *P. hordei* under controlled conditions in a growth chamber (Snijder Scientific, Tilburg, The Netherlands) at the International Center for Agricultural Research in the Dry Areas (ICARDA), Rabat, Morocco. The leaf rust pure isolates were collected from the experimental stations of Marchouch (LR-MRC) and Sidi Allal Tazi (LR-SAT) in 2017, and were multiplied on susceptible barley cultivars (Aglou, Baudin, and Bowman) followed by drying of urediniospores on silica gel and their storage at −80 °C until further use.

About 4–5 seeds of each test genotype were sown in peat moss supplemented with 14–14–14 NPK in 14 cm long cones of 3.8 cm diameter (Steuwe & Sons, Inc., Tangent, OR, USA), and the seedlings were raised under controlled conditions with a photoperiod of 16 h light/8 h dark at 20 ± 1 °C. Each tray contained 96-test genotypes along with resistant, 'Philadelphia' and susceptible, 'Lakhan' checks. About 10–12 days old seedlings with the first leaf fully extended were used for inoculation. For inoculation of each tray with 98-cones, 15 mg of dried urediniospores were resuspended in 10 mL of mineral oil (Novec 7100, Sigma Aldrich, St. Louis, Missouri, USA). The spore suspension was sprayed as a fine mist using an airbrush (Revell, Munchen, Germany) and the inoculated seedlings were left to dry for 30 min at room temperature to avoid phytotoxicity from mineral oil before placing them in a humid chamber for incubation in the dark for 24 h at 20 ± 1 °C. After 24 h of incubation, the seedlings were transferred to the growth chamber under the same conditions as described earlier. The experiment was conducted with three replications using a randomized complete block design. After 12–14 days post inoculation (dpi), the seedlings were classified into infection types according to the 0 to 4 scale developed by Stakman et al. [40]. The seedlings were classified either as immune (0), resistant (0; and 1), moderately resistant (2), moderately susceptible (3), or susceptible (4). The mean infection type of three replications was used for further analysis.

2.3. Screening for Adult Plant Resistance (APR) to P. hordei

The field phenotyping was performed at the National Institute for Agricultural Research (INRA) station of Sidi Allal Tazi (34°31′12.9″ N 6°14′30.1″ W) in the 2016–2017 (APR-SAT17) and 2018–2019 (APR-SAT19) cropping seasons, where the weather conditions (temperature and humidity) were favorable for leaf rust development. In 2016, planting was done during the last week of November, and in 2018 the planting was completed during the first week of December. The monthly average temperature, humidity, and precipitation during the cropping seasons of 2016–2017 and 2018–2019 in Sidi Allal Tazi have been described in Supplementary Table S2. The average temperature and the relative humidity during the cropping season of 2016–2017 (December–May) ranged from 13.48 to 26.72 °C and from 60.8 to 83%, whereas for the cropping season of 2018–2019, the average temperature and relative humidity ranged from 4.6 to 26.9 °C and from 56.3 to 71.7%, respectively.

In each trial, the test genotypes were sown as paired rows of one-meter length using an augmented block design with 20-test genotypes per block, and two susceptible checks (Rihane-03 and VMorales) were planted in each block. To allow the build-up and uniform distribution of *Ph* inoculum, each test block was surrounded by a spreader row composed of a mixture of susceptible cultivars (Rihane-03 and VMorales). The BLR trials were assessed under natural disease pressure as Sidi Allal Tazi is considered as an LR hot spot, and the spread of the disease was further favored by the periodic sprinkler irrigation.

In 2017, disease severity was assessed from 5 to 10 randomly chosen plants of each genotype at Zadok's growth stage (GS) 73–75 [41] using the modified Cobb's scale [42] followed by another observation after seven days. However, in 2019, only a single observation was recorded. Disease assessments combined the leaf rust severity (0 to 100%) and host response (Immune (I), Resistant (R), Moderately resistant (MR), Moderately susceptible (MS), and Susceptible (S)). The Coefficient of infection (CI) was calculated by multiplying the infection response value (R = 0.2, MR = 0.4, MS = 0.8, S = 1) with the percent disease severity (0–100%) [43] and the genotypes with CI of 0–8 were rated as R, 9–16 as MR, 17–24 as MS, and >25 as S. The area under the disease progression curve (AUDPC) was calculated using the following equation [44].

$$\text{AUDPC} = \sum_{a}^{1} i = 1^{n}[(LR_{i+1} + LR_i)/2][(t_{i+1} - t_i)]$$

where LR_i = leaf rust severity on ith days, t_i = time in days at ith observation, and n is the total number of observations.

2.4. Phenotypic Data Analysis

The phenotypic data of the LR response were subject to one-way analysis of variance (ANOVA) using GenStat 21st Edition (Version 21.1.0.25568) at a significance level of $p < 0.001$. Standard deviation (SD) and coefficient of variation (CV) of the LR response were calculated. In addition, GenStat was also used to analyze the differences among genotypes (G), between years (Y) and genotypes by year (G × Y) interaction.

2.5. Genotyping and Population Structure

The genomic DNA from 316 barley genotypes of AM2017 panel was extracted from lyophilized young leaf tissue from a single plant at the growth stage 12 [41] at the Cereal Crop Research Unit, USDA-ARS, Fargo, North Dakota, USA as described by Slotta et al. [45]. Genotyping was performed following the manufacturer's protocol using Illumina iSelect 50k SNP array [46], comprised of 49,267 mapped loci (https://ics.hutton.ac.uk/50k/ (accessed on 14 December 2017)). The genotyping and population structure of AM2017 has been reported by Verma et al. [47]. Briefly, the SNP markers with known genetic and physical map position were kept [46], but all monomorphic markers, markers with minor allele frequency (MAF) of less than 5%, and missing values of more than 10% were discarded. About 36,793 SNP markers encompassing seven barley chromosomes were used for further genetic analysis (Figure 3a).

Based on gene diversity (GD) and polymorphic information content (PIC), 2065 highly informative SNP markers (Table S3) with unique genetic map positions on seven barley chromosomes were selected for population structure analysis using the admixture model in STRUCTURE 2.3.4 [48]. The markers with PIC values between 0.10 and 0.25 were classified as moderately informative, followed by highly informative with PIC values between <0.25 and 0.375 [49]. The highest PIC value for bi-allelic markers was 0.375 [50]. For structure analysis, a burn-in period of 100,000 was run and the posterior probabilities were estimated with the Markov chain Monte Carlo (MCMC) method with 100,000 iterations. The STRUCTURE HARVESTER tool [51] was used to determine the number of sub-populations using ΔK [52]. The PCA analysis was computed using PLINK 1.9, and the PCA scatter plot was visualized using the ggplot2 package in R [53]. In addition, the kinship (K) among individuals was computed from the filtered set of SNP markers in the Genomic Association

and Prediction Integrated Tool (GAPIT) [54]. Based on the PCA, genotypes were assigned to subgroups or declared admixed using 80% membership criterion.

2.6. Genome-Wide Association Study (GWAS) of Leaf Rust Resistance

The GWAS was carried out by combining genotypic data and disease severity scores from the seedling and the adult plant stages. Marker-trait association(s) (MTA) were determined using a mixed linear model (MLM) in TASSEL version 5.2.53 [55]. SNP markers with minor allele frequency (MAF) of <5%, and SNP markers which were missing in >10% of barley genotypes were discarded from the analysis. The kinship matrix (K) was generated in TASSEL using filtered SNPs. General linear models GLM (Q) and GLM (PCA), and mixed linear models MLM (Q + K) and MLM (K + PCA) built in TASSEL version 5.2.53 were run to know the best fitting models. Both MLM models controlled effectively the false positives compared to the GLM models GLM (Q/PCA). Finally, the MLM (K + PCA) model was implemented in TASSEL for GWAS analysis. The statistical significance of multiple comparisons was tested based on pFDR ($q < 0.05$), as described by Storey [56]. In the output, R^2 explains the percent of phenotypic variation explained by the significant SNP marker, and a positive value of allele effect indicates susceptibility by increasing the disease score, whereas a negative value signifies resistance by reducing the disease score. Several GAPIT3 [54] models were further used to validate significant SNP markers/loci associated with LR resistance. In GAPIT3, GWAS was performed using the general linear model (GLM) [57], mixed linear model (MLM) [58], settlement of MLM under progressively exclusive relationship (SUPER) [59], multiple-locus MLM (MLMM) [60], fixed and random model circulating probability unification (FarmCPU) [61], and Bayesian-information and linkage-disequilibrium iteratively nested keyway (BLINK) [62]. The genetic linkage maps of significant SNP markers associated with the seedling and the adult plant stage resistance to *P. hordei* were drawn using MapChart software (v 2.32) [63].

2.7. QTL Alignment and Candidate Genes

To compare the marker-trait-associations (MTA) for BLR resistance from our study with the QTL reported in previous association mapping studies, marker sequences were retrieved from the GrainGenes database (https://wheat.pw.usda.gov/GG3/ (accessed on 5 April 2021)), and were aligned on the Morex genome using the Barleymap pipeline [64].

For candidate genes (CGs), the sequences of significant SNP markers were subject to the BLAST search tool of the IPK barley server [65] to know their homology to annotated barley gene(s) based on a threshold of BIT score (>200), sequence identity (90–100%), and an expected value (0–1^{-40}). The CG search considered the presence of functional domains implicated in plant disease resistance.

3. Results

3.1. Seedling Resistance to P. hordei

In the greenhouse, *P. hordei* infection's uniform and diverse infection responses (IR) were recorded for both isolates, SRT-SAT and SRT-MRC. The frequency distribution of IR of 316 genotypes of the AM2017 panel seemed to be positively skewed (Figure 1a). In addition, a detailed IR of the individual genotype has been presented in Supplementary Table S1. The mean IR of 'Philadelphia' (resistant) and 'Lakhan' (susceptible) was recorded as 1 and 4 for both *P. hordei* isolates tested, respectively. None of the tested genotypes were immune to both *Ph* isolates, while 66 genotypes (20.60%) were resistant to *Ph* isolate SRT-SAT, and 45 (14.10%) genotypes were resistant to *Ph* isolate SRT-MRC. Furthermore, 153 (47.81%) and 155 (48.4%) genotypes were moderately resistant to the *Ph* isolates LR-SAT and LR-MRC, respectively (Figure 1a and Table S4).

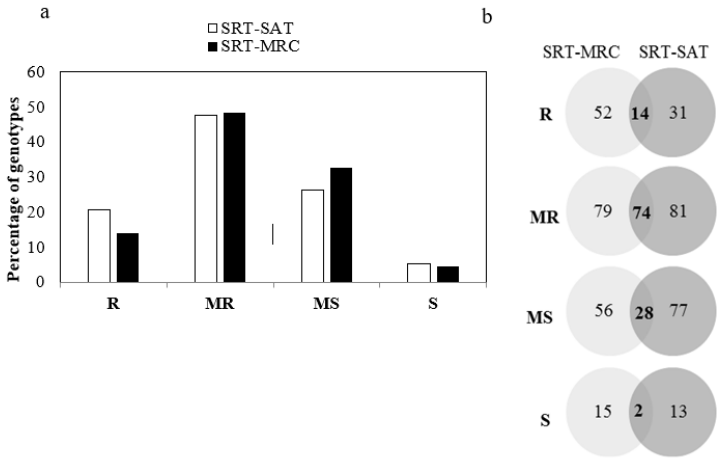

Figure 1. Frequency distribution of leaf rust resistance in 316 barley genotypes of AM2017 mapping panel at the seedling stage for *Ph* isolates, SRT-MRC and SRT-SAT. (**a**) Venn diagram of infection responses of 320 barley genotypes at the seedling stage to two *Ph* isolates under controlled conditions; (**b**) here, I, immune; R, resistant; MR, moderately resistant; MS, moderately susceptible; S, susceptible.

Among the tested genotypes, 14 genotypes were resistant to both *Ph* isolates, namely Philadelphia, Conchita, Orria, Frontier, Poet, SJ056089, SJ056576, UMBRELLA, Petunia1//Atahualpa/IraqiBlack, K 508, J02006004-09/2T0095, IG 144149, IG 17007, and Katara//SLB34-65/Arar. Whereas, 74 barley genotypes were moderately resistant to both *Ph* isolates (Figure 1b). The mean IR of *Ph* isolates SRT-MRC and SRT-SAT was recorded as 2.10 ± 0.69 and 2.00 ± 0.77, respectively. No difference was observed between the average IR of two-row genotypes to both *Ph* isolates, 2.00 ± 0.68 for SRT-MRC and 2.16 ± 0.75 for SRT-SAT. However, six-row genotypes were more susceptible (2.30 ± 0.68) to *Ph* isolate SRT-MRC than SRT-SAT isolate (1.94 ± 0.76).

3.2. Phenotyping for Adult Plant Stage Resistance

The frequency distribution of the adult plant stage reaction of 316 genotypes of the AM2017 panel to *Ph* at Sidi Allal Tazi in 2017 (APR-SAT17) and in 2019 (APR-SAT19) has been presented in Figure 2, where the disease severity seems to be negatively skewed towards resistance. Disease severity and the AUDPC of each genotype for 2017 and 2019 has been presented in Supplementary Table S1. The coefficient of infection of leaf rust at the adult plant stage ranged from 0 to 90 in 2017, and from 8 to 70 in 2019 (Table S4). Both susceptible checks, Rihane-03 and VMorales, scored a disease severity of 80S and 60S in APR-SAT17 and in APR-SAT19, respectively.

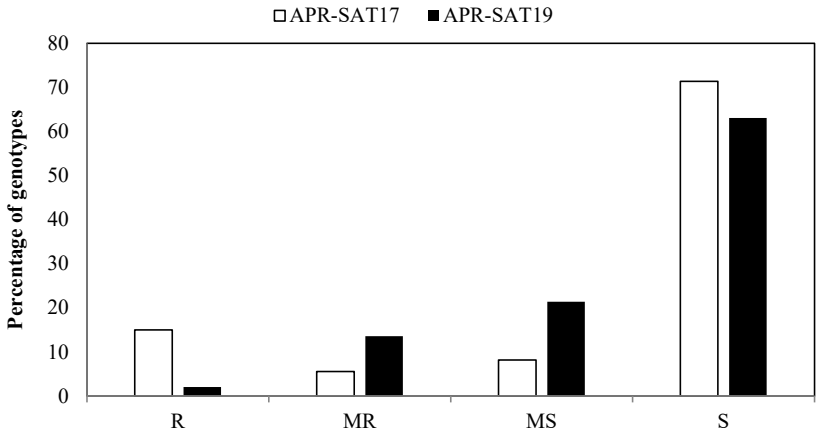

Figure 2. The frequency distribution of coefficient of infection of leaf rust in AM2017 mapping panel at the adult plant stage (APR) at Sidi Allal Tazi station in 2017 (APR-SAT17) and 2019 (APR-SAT19).

In 2017 (APR-SAT17) and 2019 (APR-SAT19), the overall values of AUDPC 262.20 ± 143.73, and of CI 36.87 ± 14.85 were observed. Among the genotypes tested, 46 (15%) genotypes were found resistant, and 17 (5.5%) genotypes were moderately resistant in APR-SAT17 (Figure 2 and Table S4), while only six genotypes (2%) were resistant and 40 (13.60%) genotypes were moderately resistant at APR-SAT19. Twelve barley genotypes were either resistant or moderately resistant during the two cropping seasons, namely Alanda 01, TR05671, SVANHALS-BAR/MSEL//AZAF/GOB24DH/3/NE167/CLE176, Cocktail, AZAF/SCARLETT, BRS195/ND19098-1, BREA/DL70//3*TOCTE/3/6B89.2027/CHAMICO, 15UCM9, 15UCM 92, IG 144107, RABAT 071, and Alanda//Lignee527/Arar/3/BF891M-617. Only one genotype AM164 (BREA/DL70//3*TOCTE/3/6B89.2027/CHAMICO) showed R-MR reaction at APR and SRT.

The ANOVA revealed significant differences ($p < 0.001$) in responses to *Ph* across genotypes at both growth stages, between genotypes (G), and in G × Y interactions, but the correlation among years (Y) was not significant ($p > 0.001$).

3.3. Population Structure

The population structure of AM2017 was analyzed with 2065 highly informative SNP markers (Table S3) using the STRUCTURE package [47]. Out of 2065 SNP markers, 86.8% (1794) were highly informative with PIC values ranging from 0.3 to 0.375, and 7.8% (161) were moderately informative with PIC values of <0.25 to 0.3. Furthermore, an average gene diversity of 0.44 was observed with 83% (1716) of the SNP markers with GD values between 0.4 and 0.5 (Table S3). Based on ΔK and K = 2 approaches [51,52], the AM2017 panel was categorized into two major clusters based on row types (Figure 3b,c). One cluster contained 40.18% of two-row genotypes, and the other cluster had 59.81% of six-row barley genotypes. The results were validated by principal component analysis (PCA), which clearly showed two distinct clusters using a filtered set of 36,793 SNP markers. Clusters 1 and 2 explained 21.10% and 9.29% of the total variation (30.4%) in the PCA scatter plot (Figure 3b).

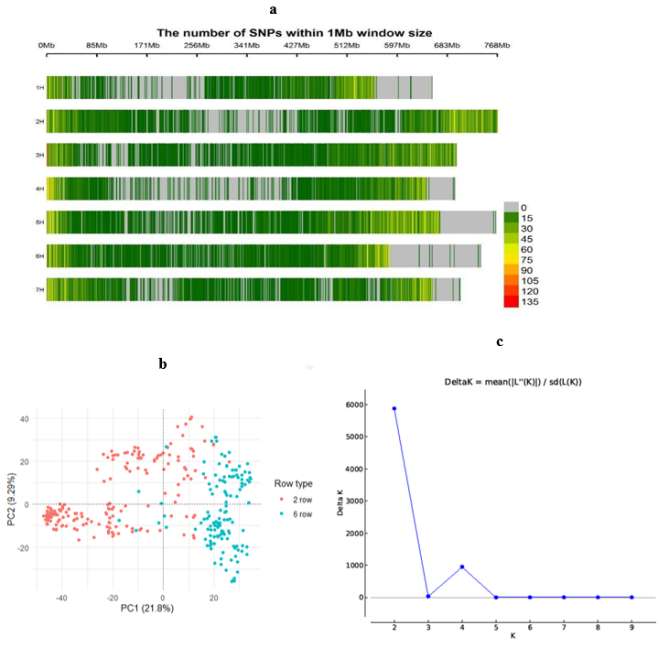

Figure 3. Marker distribution and population structure of barley panel AM2017. (**a**) Distribution of 36,793 SNP markers on the barley genome based on the physical map position. Color legend on the right shows the marker density; (**b**) principal component analysis (PCA) using 36,793 SNP markers with PC1 and PC2 explaining 21.10% and 9.29% of total variation in the AM2017 panel; (**c**) a line plot between delta-k and number of possible clusters [47].

3.4. GWAS of Seedling Stage Resistance to P. hordei

Performing GWAS for the assessment of resistance to two *Ph* isolates, SRT-MRC and SRT-SAT at SRT, the best fitting model in Tassel was the MLM procedure using PCA (K), accounting for population structure (Q) and relatedness (Figure 4). In addition, the GWAS analysis using the six models of GAPIT3 identified the common SNPs related to the resistance to LR especially from the three models MLMM, BLINK, and FarmCPU. The genome scan for the isolate SRT-MRC detected 10 MTA ($p < 0.001$) on chromosomes 1H, 2H, 3H, 5H, 6H, and 7H (Figures 4 and 5). The marker R^2 and additive effect for the isolate SRT-MRC ranged from 3.33% to 4.66%, and from −0.484 to 0.371, respectively. The total phenotypic variance explained by the 10 MTA was 37.86% (Table 1). For the isolate SRT-SAT, 24 MTA were detected on chromosomes 1H, 3H, 5H, 6H, and 7H (Figures 4 and 5) with marker R^2 and additive effect ranging from 3.3% to 13.67%, and from −1.14 to 1.14, respectively, and explained 48.41% of the total phenotypic variation (Table 1). The highest R^2 of 13.65% and the highest additive effect of −1.14 were caused by the two markers JHI-Hv50k-2016-111647 and JHI-Hv50k-2016-111819 on 2H (89.77 cM and 91.01 cM).

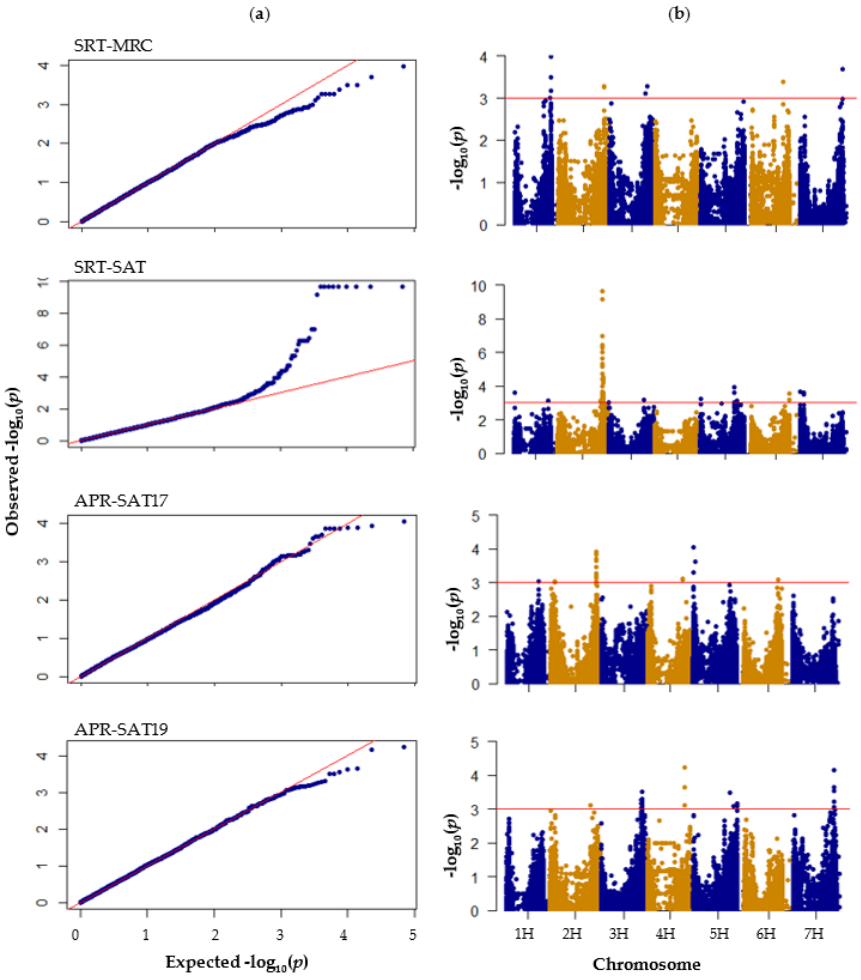

Figure 4. Genome-wide association mapping of barley leaf rust resistance at the seedling (SRT-MRC, SRT-SAT) and adult plant stages (APR-SAT17, APR-SAT19). (**a**) Quantile-Quantile (Q-Q) plots of marker-trait association at the seedling stage for P. hordei isolates SRT-MRC and SRT-SAT, and at the adult plant stage in Sidi Allal Tazi station in 2017 (APR-SAT17) and 2019 (APR-SAT19) using the MLM (PCA+K) model in Tassel; (**b**) the Manhattan plots shows –l0g10 of p-values from genome-wide association mapping against the positions of SNPs on all chromosomes of barley. The red horizontal line indicates the significance threshold ($p < 0.001$ [$-\log10(p) = 3$]).

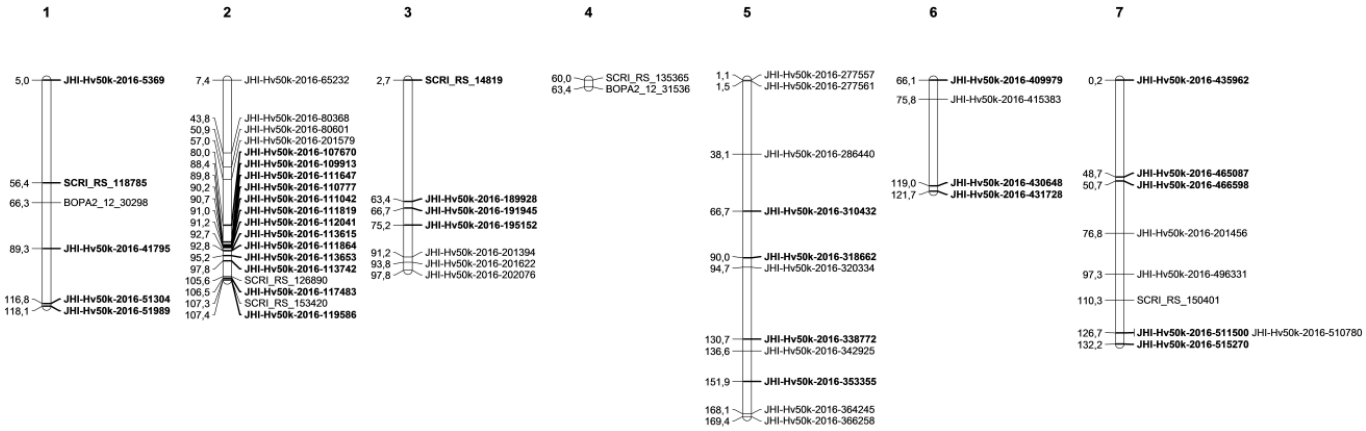

Figure 5. Genetic linkage map of significant SNP markers associated with the seedling and the adult plant stage resistance to *P. hordei* in barley association mapping panel AM2017. Markers are shown on the right and genetic distances (cM) are shown on the left. Markers in bold represent markers detected at seedling resistance.

3.5. GWAS of Adult Plant Stage Resistance to P. hordei

For GWAS of *Ph* resistance at the adult plant stage, the best fitting model in Tassel was the MLM procedure using PCA + K, accounting for population structure and relatedness (Q-Q plots are shown in Figure 4). For APR-SAT17, 10 MTA were detected on chromosomes 1H, 2H, 4H, 5H, and 6H explaining 50.52% of the total phenotypic variation (Figures 4 and 5). The marker R^2 and additive effect ranged from 3.62 to 5.25% and from -113.86 to 68, respectively. Among the MTA detected, peak marker JHI-Hv50k-2016-277557on 5H (1.11 cM) had the highest marker R^2 (5.25) and the lowest p-value. For APR-SAT19, a total of 16 MTA were detected on chromosomes 2H, 3H, 4H, 5H, and 7H, explaining 67.73% of the total phenotypic variation (Table 2 and Figures 4 and 5). The marker R^2 explained by these MTA ranged from 3.63 to 5.73%, and the additive effect from −14.27 to 13.85 with the peak marker BOPA2_12_31536 on 4H (63.39 cM) with marker R^2 of 5.73%.

Two SNP markers were associated with *Ph* resistance at both stages. On chromosome 2H, the seedling resistance SNP markers JHI-Hv50k-2016-119586 (107.37 cM), associated with SRT-SAT isolate, and JHI-Hv50k-2016-117483 (106.53 cM), associated with SRT-MRC isolate, overlapped with the APR SNP marker SCRI_RS_153420 located on the same chromosome at 107.26 cM for APR-SAT17. Similarly, SRT SNP marker JHI-Hv50k-2016-511500 on chromosome 7H at 126.7 cM for *Ph* isolate SRT-MRC was co-located with APR SNP marker JHI-Hv50k-2016-510780 on chromosome 7H at 126.7 cM for APR-SAT19.

3.6. QTL Alignment and Candidate Genes

Most of the MTA were located in genomic regions enriched with functional proteins involved in plant disease resistance and defense mechanism based on their annotation in the barley reference genome. Out of the 58 MTA detected in this study at the seedling and the adult plant stages, a BLAST search of query sequences of 36 SNP markers showed homologies with functional proteins/enzymes related to disease resistance, such as RPP13-like protein 4, receptor-like protein kinase, disease resistance RPP8-like protein 3, disease resistance protein (CC-NBS-LRR class), cyclic nucleotide-gated channel 14, ankyrin repeat family protein, B3 domain-containing protein, pentatricopeptide repeat-containing protein, alcohol dehydrogenase 1, glycine-rich domain-containing protein 2, leucine-rich repeat protein kinase family protein, ROP guanine nucleotide exchange factor 5, and UDP-Glycosyltransferase superfamily protein (Table 3). Furthermore, our sequence alignments showed that 25 of 58 SNP markers overlapped with the previously described QTL/gene involved in resistance to *Ph* (Table 3).

Table 1. Summary of SNP markers associated with *Puccinia hordei* resistance at the seedling stage of the barley AM2017 panel.

Marker	[a] Isolate	[b] Chr	[c] cM	*p*-Value	FDR	R^2(%)	Allele Frequency	[d] Allele Effect	GAPIT and TASSEL Models
SCRI_RS_118785	SRT-MRC	1	56.44	1.30×10^{-03}	1.36×10^{-03}	3.34	53	A (−0.400)	MLM (K + Q), MLM (K + PCA), MLMM, BLINK, FarmCPU
JHI-Hv50k-2016-51304	SRT-MRC	1	116.78	3.30×10^{-04}	8.58×10^{-04}	4.19	234	C (−0.382)	MLM (K + Q), MLM (K + PCA), MLMM, BLINK, FarmCPU
JHI-Hv50k-2016-51989	SRT-MRC	1	118.13	1.31×10^{-03}	1.36×10^{-03}	3.33	211	C (−0.309)	MLM (K + Q), MLM (K + PCA), MLMM, BLINK, FarmCPU
** JHI-Hv50k-2016-117483	SRT-MRC	2	106.53	5.50×10^{-04}	1.14×10^{-03}	3.86	286	A (−0.484)	MLM (K + Q), MLM (K + PCA), MLMM, BLINK, FarmCPU
JHI-Hv50k-2016-191945	SRT-MRC	3	66.69	7.90×10^{-04}	1.14×10^{-03}	3.67	127	A (−0.309)	MLM (K + Q), MLM (K + PCA)
JHI-Hv50k-2016-195152	SRT-MRC	3	75.21	5.30×10^{-04}	1.14×10^{-03}	3.91	135	C (0.355)	MLM (K + Q), MLM (K + PCA), MLMM, BLINK, FarmCPU
JHI-Hv50k-2016-353355	SRT-MRC	5	151.88	$1..21 \times 10^{-03}$	$1..33 \times 10^{-03}$	3.52	81	C (−0.374)	MLM (K + Q), MLM (K + PCA), BLINK, FarmCPU
JHI-Hv50k-2016-409979	SRT-MRC	6	66.08	4.20×10^{-04}	1.05×10^{-03}	4.03	263	A (−0.477)	MLM (K + Q), MLM (K + PCA)
** JHI-Hv50k-2016-511500	SRT-MRC	7	126.7	1.32×10^{-03}	1.36×10^{-03}	3.35	181	C (0.312)	MLM (K + Q), MLM (K + PCA)
JHI-Hv50k-2016-515270	SRT-MRC	7	132.22	2.00×10^{-04}	7.74×10^{-04}	4.66	229	C (0.371)	MLM (K + Q, MLM (K + PCA), MLMM, BLINK, FarmCPU
JHI-Hv50k-2016-5369	SRT-SAT	1	4.96	2.50×10^{-04}	7.74×10^{-04}	4.39	231	A (0.409)	MLM (K + Q), MLM (K + PCA), FarmCPU
JHI-Hv50k-2016-41795	SRT-SAT	1	89.31	7.10×10^{-04}	1.14×10^{-03}	3.7	297	A (0.691)	MLM (K + Q), MLM (K + PCA)
JHI-Hv50k-2016-107670	SRT-SAT	2	80.03	7.30×10^{-04}	1.14×10^{-03}	3.71	79	C (0.407)	MLM (K + Q), MLM (K + PCA), BLINK
JHI-Hv50k-2016-109913	SRT-SAT	2	88.39	4.50×10^{-04}	1.08×10^{-03}	3.99	223	A (−0.386)	MLM (K + Q), MLM (K + PCA), MLMM, BLINK
JHI-Hv50k-2016-111647	SRT-SAT	2	89.77	2.23×10^{-10}	3.74×10^{-09}	13.65	30	A (−1.14)	MLM (K + Q), MLM (K + PCA), BLINK, FarmCPU
JHI-Hv50k-2016-110777	SRT-SAT	2	90.16	2.23×10^{-10}	3.74×10^{-09}	13.65	286	C (1.14)	MLM (K + Q), MLM (K + PCA), MLMM

Table 1. *Cont.*

Marker	[a] Isolate	[b] Chr	[c] cM	*p*-Value	FDR	R^2(%)	Allele Frequency	[d] Allele Effect	GAPIT and TASSEL Models
JHI-Hv50k-2016-111042	SRT-SAT	2	90.72	9.51×10^{-07}	8.83×10^{-06}	7.94	55	A (−0.693)	MLM (K +Q), MLM (K + PCA), BLINK, FarmCPU
JHI-Hv50k-2016-111819	SRT-SAT	2	91.01	2.23×10^{-10}	3.74×10^{-09}	13.65	30	C (−1.14)	MLM (K +Q), MLM (K + PCA)
JHI-Hv50k-2016-112041	SRT-SAT	2	91.15	6.97×10^{-10}	9.06×10^{-09}	12.85	29	C (−1.13)	MLM (K + Q), MLM (K + PCA), MLMM
JHI-Hv50k-2016-113615	SRT-SAT	2	92.71	7.70×10^{-04}	1.14×10^{-03}	3,66	276	C (0.53029)	MLM (K + Q), MLM (K + PCA)
JHI-Hv50k-2016-111864	SRT-SAT	2	92.78	2.30×10^{-10}	3.74×10^{-09}	13.67	283	C (1.14)	MLM (K + Q), MLM (K + PCA)
JHI-Hv50k-2016-113653	SRT-SAT	2	95.16	3.00×10^{-05}	2.44×10^{-04}	5.69	261	C (0.584)	MLM (K + Q), MLM (K + PCA)
JHI-Hv50k-2016-113742	SRT-SAT	2	97.8	6.00×10^{-05}	3.90×10^{-04}	5.24	292	C (0.802)	MLM (K + Q), MLM (K + PCA), BLINK
** JHI-Hv50k-2016-119586	SRT-SAT	2	107.37	1.21×10^{-03}	1.33×10^{-03}	3.38	154	A (−0.336)	MLM (K + Q), MLM (K + PCA)
SCRI_RS_14819	SRT-SAT	3	2.69	9.10×10^{-04}	1.15×10^{-03}	3.6	21	A (−0.634)	MLM (K + Q), MLM (K + PCA), MLMM, BLINK
JHI-Hv50k-2016-189928	SRT-SAT	3	63.39	6.20×10^{-04}	1.14×10^{-03}	3.79	74	C (−0.469)	MLM (K + Q), MLM (K + PCA)
JHI-Hv50k-2016-338772	SRT-SAT	5	130.69	1.25×10^{-03}	1.35×10^{-03}	3.44	282	C (−0.534)	MLM (K + Q), MLM (K + PCA)
JHI-Hv50k-2016-310432	SRT-SAT	5	66.69	1.10×10^{-04}	5.50×10^{-04}	4.85	156	A (−0.373)	MLM (K + Q), MLM (K + PCA), MLMM
JHI-Hv50k-2016-318662	SRT-SAT	5	90.03	7.90×10^{-04}	1.14×10^{-03}	3.64	95	A (−0.422)	MLM (K + Q), MLM (K + PCA)
JHI-Hv50k-2016-430648	SRT-SAT	6	118.98	2.70×10^{-04}	7.98×10^{-04}	4.3	292	A (−0.677)	MLM (K + Q), MLM (K + PCA)
JHI-Hv50k-2016-431728	SRT-SAT	6	121.68	6.50×10^{-04}	1.14×10^{-03}	3.77	32	A (0.541)	MLM (K + Q), MLM (K + PCA), MLMM, BLINK
JHI-Hv50k-2016-435962	SRT-SAT	7	0.21	1.39×10^{-03}	1.39×10^{-03}	3.3	235	A (−0.354)	MLM (K + Q), MLM (K + PCA), MLMM, FarmCPU
JHI-Hv50k-2016-465087	SRT-SAT	7	48.73	2.50×10^{-04}	7.74×10^{-04}	4.36	299	G (−0.767)	MLM (K + Q), MLM (K + PCA), MLMM, BLINK
JHI-Hv50k-2016-466598	SRT-SAT	7	50.71	3.20×10^{-04}	8.58×10^{-04}	4.19	235	C (0.391)	MLM (K + Q), MLM (K + PCA)

** Common genomic regions between SRT and APR. [a] *P. hordei* isolates, SRT-SAT and SRT-MRC, used seedling stage screening [b] Chromosome [c] Genetic position of SNPs anchored using POPSEQ2017 [d] Allele effect contributed by the respective marker on a 0–4 scale at the seedling stage. The negative allele effect decreases the diseases severity (resistance) and the positive allele effect increases the diseases severity (susceptibility).

Table 2. Summary of SNP markers associated with *P. hordei* resistance at the adult plant stage of barley AM2017 panel.

Marker	[a] Env	[b] Chr	[c] cM	*p*-Value	FDR	R^2(%)	Allele Frequency	[d] Allele Effect	Best-Fit TASSEL and GAPIT Models
BOPA2_12_30298	APR-SAT17	1	66.29	9.23×10^{-04}	1.15×10^{-03}	3.7	78	A (−63.43)	GLM (Q), MLM (K + Q), MLM (K + /PCA)
JHI-Hv50k-2016-80368	APR-SAT17	2	43.84	9.57×10^{-04}	1.17×10^{-03}	3.65	19	A (−113.86)	GLM (Q), MLM (K + Q). MLM (K + PCA), MLMM, BLINK, FarmCPU
JHI-Hv50k-2016-80601	APR-SAT17	2	50.92	1.01×10^{-03}	1.22×10^{-03}	3.62	62	C (−68.16)	GLM (Q), MLM (K + Q), MLM (K + PCA), FarmCPU
SCRI_RS_126890	APR-SAT17	2	105.56	1.22×10^{-04}	5.66×10^{-04}	5.03	204	A (−69.22)	GLM (Q), MLM (K + Q), MLM (K + PCA), MLMM, BLINK, FarmCPU
** SCRI_RS_153420	APR-SAT17	2	107.26	6.90×10^{-04}	1.14×10^{-03}	3.89	194	C (−59.36)	GLM (Q), MLM (K + Q), MLM (K + PCA), MLMM, BLINK, FarmCPU
SCRI_RS_135365	APR-SAT17	4	59.99	8.39×10^{-04}	1.14×10^{-03}	3.85	263	C (−84.34)	GLM (Q), MLM (K + Q), MLM (K + PCA), BLINK, FarmCPU
JHI-Hv50k-2016-277557	APR-SAT17	5	1.11	9.20×10^{-05}	4.98×10^{-04}	5.25	162	A (−66.16)	GLM (Q), MLM (K + Q), MLM (K + PCA), MLMM, BLINK, FarmCPU
JHI-Hv50k-2016-277561	APR-SAT17	5	1.45	4.96×10^{-04}	1.14×10^{-03}	4.22	143	C (59.30)	GLM (Q), MLM (K + Q), MLM (K + PCA), MLMM, BLINK, FarmCPU
JHI-Hv50k-2016-286440	APR-SAT17	5	38.12	2.46×10^{-04}	7.74×10^{-04}	4.71	78	A (−70.43)	GLM (Q), MLM (K + Q), MLM (K + PCA), MLMM, BLINK, FarmCPU
JHI-Hv50k-2016-415383	APR-SAT17	6	75.78	8.41×10^{-04}	1.14×10^{-03}	3.76	43	A (−79.99)	GLM (Q), MLM (K + Q), MLM (K + PCA), BLINK
JHI-Hv50k-2016-65232	APR-SAT19	2	7.44	1.14×10^{-03}	1.32×10^{-03}	3.72	273	C (12.87)	MLM (K + Q), MLM (K + PCA), MLMM
JHI-Hv50k-2016-201579	APR-SAT19	2	57.01	7.72×10^{-04}	1.14×10^{-03}	3.98	104	A (7.31)	MLM (K + Q), MLM (K + PCA), MLMM, BLINK, FarmCPU

Table 2. *Cont.*

Marker	[a] Env	[b] Chr	[c] cM	*p*-Value	FDR	R^2(%)	Allele Frequency	[d] Allele Effect	Best-Fit TASSEL and GAPIT Models
JHI-Hv50k-2016-201394	APR-SAT19	3	91.22	8.84×10^{-04}	1.15×10^{-03}	3.89	169	C (−7.33)	MLM (K + Q), MLM (K + PCA), MLMM
JHI-Hv50k-2016-201622	APR-SAT19	3	93.81	7.17×10^{-04}	1.14×10^{-03}	4.03	194	A (−7.64)	MLM (K + Q), MLM (K + PCA)
JHI-Hv50k-2016-202076	APR-SAT19	3	97.8	1.19×10^{-03}	1.33×10^{-03}	3.69	107	A (−7.21)	MLM (K + Q), MLM (K + PCA)
BOPA2_12_31536	APR-SAT19	4	63.39	5.88×10^{-05}	3.90×10^{-04}	5.73	142	C (−8.31)	MLM (K + Q), MLM (K + PCA), MLMM,, BLINK, FarmCPU
JHI-Hv50k-2016-320334	APR-SAT19	5	94.72	3.20×10^{-04}	8.58×10^{-04}	4.6	188	A (−7.27)	MLM (K + Q), MLM (K + PCA), MLMM, BLINK, FarmCPU
JHI-Hv50k-2016-342925	APR-SAT19	5	136.6	8.23×10^{-04}	1.14×10^{-03}	3.94	21	C (−12.89)	MLM (K + Q), MLM (K + PCA)
JHI-Hv50k-2016-364245	APR-SAT19	5	168.12	8.04×10^{-04}	1.14×10^{-03}	4.12	15	C (13.85)	MLM (K + Q), MLM (K + PCA), MLMM
JHI-Hv50k-2016-366258	APR-SAT19	5	169.38	6.92×10^{-04}	1.14×10^{-03}	4.05	17	C (−14.27)	MLM (K + Q), MLM (K + PCA)
JHI-Hv50k-2016-201456	APR-SAT19	7	76.84	8.84×10^{-04}	1.15×10^{-03}	3.89	122	C (7.33)	MLM (K + Q), MLM (K + PCA), MLMM
JHI-Hv50k-2016-496331	APR-SAT19	7	97.31	1.36×10^{-03}	1.38×10^{-03}	3.63	262	C (−10.70)	MLM, (K + Q), MLM (K + PCA) MLMM, BLINK, FarmCPU
SCRI_RS_150401	APR-SAT19	7	110.27	7.06×10^{-05}	4.17×10^{-04}	5.64	38	C (11.35)	MLM (K + Q), MLM (K + PCA), MLMM, BLINK, FarmCPU
** JHI-Hv50k-2016-510780	APR-SAT19	7	126.7	1.06×10^{-03}	1.25×10^{-03}	3.77	261	C (−11.12)	MLM (K + Q), MLM (K + PCA), MLMM, BLINK, FarmCPU

** Common genomic regions between SRT and APR. [a] Environment and cropping season. [b] Chromosome [c] Genetic position of SNPs anchored using POPSEQ2017 [d] Allel effect contributed by the respective marker in terms of disease severity (0–100%) at the adult plant stage. The negative allele effect decreases the diseases severity (resistance) and the positive allele effect increases the disease severity (susceptibility).

Table 3. QTL alignment and candidate genes identified for the seedling and the adult plant stage resistance to *Puccinia hordei* in barley AM2017 panel.

Associated Marker	[a] Chr	[b] cM	Gene Identifier	Homology	[c] Previously Mapped LR QTL/Genes
Seedling resistance (SRT)					
SRT-MRC					
SCRI_RS_118785	1	56.44	HORVU1Hr1G059300	Receptor-like protein kinase	_
JHI-Hv50k-2016-51304	1	116.78	HORVU1Hr1G086590	ROP guanine nucleotide exchange factor 5	*RphQ3* [35]
JHI-Hv50k-2016-51989	1	118.13	HORVU1Hr1G087340	Guanylate-binding family protein	*RphQ18* (*RphQ3*) [35,66]
** JHI-Hv50k-2016-117483	2	106.53	HORVU2Hr1G103180	Terpene synthase 04	_
JHI-Hv50k-2016-191945	3	66.69	HORVU3Hr1G074330	Nucleic acid binding	*Rph10.o* [67]
JHI-Hv50k-2016-195152	3	75.21	HORVU3Hr1G079230	Leucine-rich repeat protein kinase family protein	_
JHI-Hv50k-2016-353355	5	151.88	HORVU5Hr1G114220	Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family	*Rph9.z* [67]
JHI-Hv50k-2016-409979	6	66.08	HORVU6Hr1G070350	Undescribed protein	_
** JHI-Hv50k-2016-511500	7	126.7	HORVU7Hr1G115100		*RphQ14* (*Rph3/19*); *Rph3.c*; RphQ28 [35,66,67]
JHI-Hv50k-2016-515270	7	132.22	HORVU7Hr1G118430	Disease resistance RPP13-like protein 4	_
SRT-SAT					
JHI-Hv50k-2016-5369	1	4.96	HORVU1Hr1G002310	50S ribosomal protein L2	*Rphq14* [68]
JHI-Hv50k-2016-41795	1	89.31	HORVU1Hr1G075680	Receptor-like protein kinase 5	_
JHI-Hv50k-2016-107670	2	80.03	HORVU2Hr1G092270	Lactoylglutathione lyase/glyoxalase I family protein	_
JHI-Hv50k-2016-109913	2	88.39	HORVU2Hr1G096190	Disease resistance protein RPP13	_
JHI-Hv50k-2016-111647	2	89.77	HORVU2Hr1G097860	Integrase-type DNA-binding superfamily protein	*Rphq11* [68]
JHI-Hv50k-2016-110777	2	90.16	HORVU2Hr1G096910	UDP-Glycosyltransferase superfamily protein	*Rphq11* [68]
JHI-Hv50k-2016-111042	2	90.72	HORVU2Hr1G097140	Zinc finger CCHC domain-containing protein 9	_
JHI-Hv50k-2016-111819	2	91.01	HORVU2Hr1G097940	Homeobox-leucine zipper protein 4	_
JHI-Hv50k-2016-112041	2	91.15	HORVU2Hr1G098100	Disease resistance protein	_
JHI-Hv50k-2016-113615	2	92.71	HORVU2Hr1G099350	Alcohol dehydrogenase 1	_
JHI-Hv50k-2016-111864	2	92.78	HORVU2Hr1G097980	Serine/arginine repetitive matrix protein 1 isoform X1	_
JHI-Hv50k-2016-113653	2	95.16	HORVU2Hr1G099440	Terpene synthase 04	_
JHI-Hv50k-2016-113742	2	97.8	HORVU2Hr1G099470	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein	_
** JHI-Hv50k-2016-119586	2	107.37	HORVU2Hr1G105090	Protein of unknown function (DUF581)	_
SCRI_RS_14819	3	2.69	HORVU3Hr1G002010	Pentatricopeptide repeat-containing protein	*QRph-3H.6*; *Rph5*; *Rph7.g* [67,69,70]

Table 3. *Cont.*

Associated Marker	[a] Chr	[b] cM	Gene Identifier	Homology	[c] Previously Mapped LR QTL/Genes
JHI-Hv50k-2016-189928	3	63.39	_	_	Rph10.o [67]
JHI-Hv50k-2016-310432	5	66.69	ADQ48070.1	bZIP transcription factor 27	_
JHI-Hv50k-2016-318662	5	90.03	HORVU5Hr1G078310	Protein FLOWERING LOCUST	11_11473 [22]
JHI-Hv50k-2016-338772	5	130.69		EKC/KEOPS complex subunit bud32	*RphQ25*; *QLr.S42-5H*.a (Rph9; Rph12) [66,71,72]
JHI-Hv50k-2016-430648	6	118.98	HORVU6Hr1G092660	Disease resistance RPP8-like protein 3	*QPh.6H-3* (QTL_Backes) [73,74]
JHI-Hv50k-2016-431728	6	121.68	HORVU6Hr1G093310	dTDP-4-dehydrorhamnose reductase	_
JHI-Hv50k-2016-435962	7	0.21	HORVU7Hr1G000040	Disease resistance protein (CC-NBS-LRR class) family	*RphQ12* [35]
JHI-Hv50k-2016-465087	7	48.73	HORVU7Hr1G030310	Formin Homology 14	_
JHI-Hv50k-2016-466598	7	50.71	HORVU7Hr1G031610	Protein tesmin/TSO1-like CXC 5	_
Adult Plant Resistance (APR)					
APR-SAT17					
BOPA2_12_30298	1	66.29	HORVU1Hr1G066050	Cyclic nucleotide-gated channel 14	_
JHI-Hv50k-2016-80368	2	43.84	HORVU2Hr1G022560	ABC transporter C family member 10	*Rph16*. *RphQ20* [66,75]
JHI-Hv50k-2016-80601	2	50.92	HORVU2Hr1G022900	Ankyrin repeat family protein	*RphQ7* [35]
SCRI_RS_126890	2	105.56	HORVU2Hr1G102880	Protein of unknown function (DUF760)	_
** SCRI_RS_153420	2	107.26	HORVU2Hr1G102930	High mobility group B protein 6	_
SCRI_RS_135365	4	59.99	HORVU4Hr1G064860	LisH and RanBPM domains containing protein	*MQTL9* (*Rphq19*) [26,76,77]
JHI-Hv50k-2016-277557	5	1.11	AAV49984.1	Nodulin-like/Major Facilitator Superfamily protein	_
JHI-Hv50k-2016-277561	5	1.45	AAV49985.1	Hordoindoline-B1	_
JHI-Hv50k-2016-286440	5	38.12	HORVU5Hr1G010930	Unknown function/putative serine protease do-like htrA	*Rph2.t*; *Rph2* (*Rph2.b*) [67,78]
JHI-Hv50k-2016-415383	6	75.78	HORVU6Hr1G077750	Phosphate-responsive 1 family protein	_
APR-SAT19					
JHI-Hv50k-2016-65232	2	7.44	HORVU2Hr1G005650	Cullin-associated NEDD8-dissociated protein 1	*Rph1.a*; *RphQ19* (*RphQ*5/6) [66,67]
JHI-Hv50k-2016-201579	2	57.01	_	_	_
JHI-Hv50k-2016-201394	3	91.22	HORVU3Hr1G086030	Eukaryotic aspartyl protease family protein	_
JHI-Hv50k-2016-201622	3	93.81	HORVU3Hr1G086290	Pentatricopeptide repeat-containing protein	_
JHI-Hv50k-2016-202076	3	97.8	ACB56486.1	Respiratory burst oxidase protein F	*MQTL7C* (*Rphq20*) [26,77]
BOPA2_12_31536	4	63.39	HORVU4Hr1G068990	Nodulin-related protein 1, putative	*MQTL9*; *Rphq19* [26,76,77]

Table 3. *Cont.*

Associated Marker	[a] Chr	[b] cM	Gene Identifier	Homology	[c] Previously Mapped LR QTL/Genes
JHI-Hv50k-2016-320334	5	94.72	HORVU5Hr1G080280	ATPase ASNA1 homolog	_
JHI-Hv50k-2016-342925	5	136.6	HORVU5Hr1G103380	Mitochondrial transcription termination factor family protein	*Rph9.i* [67]
JHI-Hv50k-2016-364245	5	168.12	HORVU5Hr1G123050	Chromatin accessibility complex protein 1	*QLr.HEB-5-5H.b5H MQTL13* (*Rphq7*), *MQTL14 (Rphq16)* [26,76,77]
JHI-Hv50k-2016-366258	5	169.38	HORVU5Hr1G124630	Glutathione S-transferase T1	_
JHI-Hv50k-2016-201456	7	76.84	HORVU3Hr1G086080	S-acyltransferase	_
JHI-Hv50k-2016-496331	7	97.31	HORVU7Hr1G100300	Disease resistance protein	*RphQ13*, *Rphq9* [23,35]
SCRI_RS_150401	7	110.27	HORVU7Hr1G106860	Glycine-rich domain-containing protein 2	*QPh.7H-3* (*Rphq9*), QTL_Castro, "Ris44-Bmac156" [26,73,79]
** JHI-Hv50k-2016-510780	7	126.7	HORVU7Hr1G114330	Homogentisate phytyltransferase 1	*RphQ14* (*Rph3/19*) Rph3.c; RphQ28 [35,66,67]

** Common genomic regions between SRT and APR. [a] Chromosome. [b] Genetic position of SNPs anchored using POPSEQ2017 [c] Previously QTL mapped in the same position for barley leaf rust.

4. Discussion

Recently, an increased incidence and prevalence of barley leaf rust has been observed in most barley growing regions, and the deployment of resistant cultivars is considered an important component of its integrated management. In this study, we have reported sources of resistance to *P. hordei* at the seedling and at the adult plant stages in a panel constructed by barley breeders to provide sources of valuable traits needed. Furthermore, GWAS analyses identified 58 marker-trait associations on all barley chromosomes, and common genomic regions were identified on chromosomes 2H and 7H for both growth stages. Our genome-wide scan had verified 26 already reported genomic loci in addition to the identification of 32 novel loci associated with *P. hordei* resistance, thus verifying our approach. To our knowledge, this is the first study on GWAS of leaf rust (LR) resistance in spring barley from North Africa.

The slow progress on LR resistance research in Morocco has been hampered partly by the lack of information on the Moroccan *P. hordei* pathotypes. Determination of pathogen population diversity and screening the germplasm with representative pathotypes is a key for the success of any resistance breeding program. Our results showed a large phenotypic diversity within the genotypes of AM2017 at the seedling stage to the Moroccan *Ph* isolates (SRT-SAT, SRT-MRC). More resistant genotypes (66) were noted when tested with the *Ph* isolate SRT-SAT compared to the *Ph* isolate SRT-MRC (45), which could be due to the differences in their virulence spectrum. This notion was supported by the race analysis of the two tested *Ph* isolates using 19-Bowman near isogenic lines (NILs). Among the 19 differentials tested, 11 (58%) showed differential interaction between both isolates (Amouzoune et al. unpublished data), and hence their differential response to AM2017 can be attributed to their diverse virulence spectrum (Figure 1b). The *Ph* isolate SRT-MRC was virulent on NILs carrying *Rph2.b, Rph3.c, Rph4.d, Rph5.e, Rph6.f Rph5, Rph7.g, Rph8.h, Rph9.i, Rph10.o, Rph11.p, Rph9.z Rph12, Rph2.j, Rph2.y,* and *Rph2.t*, whereas the *Ph* isolate SRT-SAT was virulent on NILs carrying *Rph1.a, Rph3.c, Rph4.d, Rph8.h,* and *Rph9*. Interestingly two alleles, *Rph15.ad* and *Rph13.x*, were found effective against both *Ph* isolates. Therefore, fourteen barley genotypes resistant to both *Ph* isolates might possess these two known *Rph* genes or have novel, but yet uncharacterized, *Rph* genes individually or in combinations. Park et al. [3] described seedling resistance (qualitative resistance) being effective at all growth stages of plants, and governed by a major or dominant resistance gene which recognizes directly or indirectly a dominant avirulence gene (effector) of the pathogen in a gene-for-gene interaction. A single loss or gain of functional mutation in a pathogen avirulence gene can render the deployed resistance gene ineffective within a short period of time due to selection pressure imposed by it on the pathogen population. The Australian barley breeding program deployed *Rph12* in cultivars such as Franklin (1989) in Tasmania, Tallon (1991) and Lindwall (1997) in the Eastern growing regions, and Fitzgerald and Gairdner (1997) in Western Australia, but a virulent pathotype (4610P+) on *Rph12* was observed in Tasmania in 1991, followed by two virulent pathotypes (5610P+ and 5453P−) in the Western Australia in 1997 and 2001, respectively. This demonstrates a rapid selection of virulent pathotypes of *Ph* once a single dominant resistance gene has been deployed in different varieties being grown on a large acreage [3]. Similarly, another dominant gene (*Rph3*) was deployed in Western Australia in the cultivar Bass (2012) and a virulent pathotype (5457P+) was detected in September 2013. These dominant resistance genes, however, are ineffective in providing adequate levels of protection at the post seedling stages. The variability in *Ph* could occur via simple mutation, exotic genotype introduction, asexual recombination, and via sexual hybridization [80].

Contrary to seedling resistance, APR or race-nonspecific resistance is considered a more durable and effective strategy for disease control. In this study, the phenotypic response to *Ph* at the adult plant stage was performed at Sidi Allal Tazi, which is considered the hot spot for LR screening of both wheat and barley in Morocco. During two cropping seasons (2016–2017 and 2018–2019), good natural infections with high disease pressure were recorded; however, late and light infection during the 2017–2018 cropping season

did not allow for good screening (data not presented). Out of the 316 genotypes tested for APR, 46 (15%) and 17 (5.5%) genotypes were resistant and moderately resistant in APR-SAT17, respectively. While only 6 (2%) and 40 (13.60%) genotypes were resistant and moderately resistant in APR-SAT19, respectively. In addition, ANOVA analysis revealed that correlation between years (Y) was not significant ($p > 0.001$). This can be due to the environmental conditions and changes in virulence of the pathogen population over the years. Based on the reaction of 19 Bowman differentials to LR at the adult plant stage in 2017, only one differential line carrying *Rph2* was moderately resistant. So far only three barley APR genes, *Rph20* on chromosome 5H, *Rph23* on 7H, and *Rph24* on 6H, against *Ph* have been characterized [13–15].

Barley is planted in Morocco in November–December under low input conditions, and LR is the last disease which affects the grain filling period in March–April by colonizing the flag leaf. Therefore, in the context of Morocco, APR to LR should be incorporated in the newly developed barley cultivars, and in our study, we have identified barley genotypes with good level of APR, which could be used in hybridization schemes to combat LR. However, their efficient utilization through MAS is hampered by the absence of tightly linked reliable diagnostic markers.

The AM2017 panel encompasses global genetic diversity, consisting of 134 advanced breeding lines from ICARDA's barley breeding program, 161 registered cultivars from Asia, Africa, Europe, the America, and 21 landraces. A total of 36,793 high quality SNPs were used to identify MTA associated with LR resistance at the seedling and at the adult plant stages in Morocco. An average SNP density of 37.09 SNPs/cM was observed in AM2017, whereas previous GWAS studies were successful with 5 SNP/cM [31], 1 DArT marker per 1.5 cM [81], and 1 SNP marker per 0.72 cM [82–84]. Therefore, the use of high SNP density per cM of AM2017 in GWAS was effective and resulted in higher resolution mapping of SNPs associated with *Ph* resistance. Furthermore, population structure was accounted for using PCA and kinship as covariates in MLM to avoid spurious MTA in this study.

Our genome scan identified 58 MTA associated with LR resistance at the seedling and adult plant stages (Tables 1 and 2). About 34 MTA were associated with SR (10 for isolate SRT-MRC, and 24 for isolate SRT-SAT), and 24 MTA were associated with APR (10 for APR-SAT17, and 14 MTA for APR-SAT19). Furthermore, at the seedling stage, 21 out of 34 MTA had negative allele effect, allowing a decrease in disease severity. For example, the SNP marker JHI-Hv50k-2016-111819 (91.01 cM) on 2H can reduce the disease severity by 28% (−1.14 units). Likewise, 18 out of 24 MTA associated with the adult plant stage resistance had negative allele effect and could reduce disease severity.

Interestingly, our study could verify 26 loci involved in LR resistance, which were mapped in previous mapping studies using different sets of genotypes (Table 3). However, 32 MTA were novel and had not been implicated previously in resistance against *Ph*. Historically, LR resistance loci have been mapped on all barley chromosomes [3,85], but we did not detect any SNP marker associated with LR resistance on chromosome 4H at SRT. The detection of various novel genomic regions in this study hints towards a complex interaction between *Ph* and barley.

Among the 34 MTA associated with the seedling resistance, 14 aligned with reported BLR loci and 20 were novel (Table 3). Among the five MTA on the chromosome 1H, two SNP markers, JHI-Hv50k-2016-51304 (116.78 cM) and JHI-Hv50k-2016-51989 (118.13 cM) associated with resistance to *Ph* isolate LR-MRC, were also reported from the Australian barley germplasm [35,66]. In addition, the SNP marker JHI-Hv50k-2016-5369 (4.96 cM) overlapped with the QTL *Rphq14* for LR resistance [86]. Similarly, two SR SNP markers associated with *Ph* isolate LR-SAT on 2H, JHI-Hv50k-2016-111647 (89.77 cM) and JHI-Hv50k-2016-110777 (90.16 cM), were reported in the double haploid mapping population Steptoe x Morex [26,68]. Interestingly, the novel SNP markers JHI-Hv50k-2016-111819 (91.01 cM), JHI-Hv50k-2016-112041 (91.15 cM), and JHI-Hv50k-2016-111864 (92.78 cM) explained 12.85–13.67% of total phenotypic variation to *Ph* isolate SRT-SAT and can reduce the disease severity by −1.14 (28%) on a disease rating scale of 0–4. Owing to their close

proximity, identical marker R^2, and allele effect, they may be linked to the same QTL (Table 1).

On chromosome 3H, two SNP markers, JHI-Hv50k-2016-191945 (66.69 cM; LR-MRC) and JHI-Hv50k-2016-189928 (63.39 cM; LR-SAT), were co-located with the genomic region of catalogued seedling resistance gene *Rph10* [67]. The genomic region in the proximity of SNP marker SCRI_RS_14819 (2.69 cM) on 3HS has been reported in several mapping studies, for example Berger et al. [69] mapped *QRph-3H.6* in the vicinity of this marker and it coincided with two mapped resistance genes *Rph5* [70] and *Rph7.g* [67].

On chromosome 5H, the SNP marker JHI-Hv50k-2016-318662 (90.03 cM), associated with *Ph* isolate SRT-SAT, overlapped with the SNP marker 11_11473 (89 cM) detected at APR to LR in the Latin American germplasm [22]. Another SNP marker, JHI-Hv50k-2016-338772 (130.69 cM), was co-located with *Rph12* [66,71,72] and it also coincides with SNP markers i_SCRI_RS_175848 and 11_11532 implicated in YR resistance [22,73]. Among three MTA on chromosome 6H, the SNP marker JHI-Hv50k-2016-430648 (118.98 cM) against *Ph* isolate LR-SAT was associated with a *QPh.6H-3*, which was reported by Bakes et al. [74] and confirmed later by Vatter et al. [73]. Likewise on chromosome 7H, the SNP marker JHI-Hv50k-2016-511500 (126.7 cM) against *Ph* isolate LR-MRC overlapped with *Rph3* and with *RphQ14* [67]. Interestingly, this locus was also aligned with a DarT marker 11_20847 linked to YR resistance [37]. In addition, the SNP marker on 7H (0.21 cM) against *Ph* isolate LR-SAT, JHI-Hv50k-2016-435962, overlapped with an APR QTL (*RphQ12*) effective against the two Australian *Ph* pathotypes, 5453 P+ and 5457 P+ [35].

Among the 24 MTA associated with APR, 12 MTA were aligned with the reported BLR loci and 12 were novel (Table 3). On chromosome 2H, four and two MTA were associated with *Ph* resistance at APR-SAT17 and SAT18-19, respectively. The SNP marker JHI-Hv50k-2016-80368 (43.84 cM) co-located with the resistance gene *Rph16* and a QTL *RphQ20* [66,75]. Interestingly, it reduced the disease severity by −113.86 AUDPC units and seems to be associated with a major non-host resistance gene. In addition, the SNP marker JHI-Hv50k-2016-80601 (APR-SAT17; 50.92 cM) was co-located with *RphQ7* reported from an association mapping study of the Australian barley breeding germplasm at both growth stages [35]. Likewise, the SNP marker JHI-Hv50k-2016-6523 2 (APR-SAT19; 7.44 cM) was co-located with *Ph* loci in two different studies [66,67].

On chromosome 3H, three MTA were detected for APR-SAT19. One SNP marker JHI-Hv50k-2016-202076 (97.8 cM) overlapped with *Rphq20* [26] and to the meta-QTL *MQTL7C* [77], whereas the other two MTA were novel. Similarly on chromosome 4H, two SNP markers, SCRI_RS_135365 (APR-SAT17) at 59.99 cM, and BOPA2_12_31536 (SAT18-19) at 63.39 cM, were associated with known *Ph* resistance locus [26,76,77].

On chromosome 5H, seven MTA were associated with APR. For APR-SAT17, the SNP marker JHI-Hv50k-2016-286440 (38.12 cM) was co-located with *Rph2* [67,78], while the other two markers were novel. For APR-SAT19, the SNP marker JHI-Hv50k-2016-342925 (136.6 cM) overlapped with *Rph9.i* [67], and the other SNP marker JHI-Hv50k-2016-364245 (168.12 cM) was found proximal to the QTL *QLr.HEB-5-5H.b5H* [76]. Likewise, four MTA were detected on chromosome 7H for SAT18-19, explaining a total phenotypic variation of 16.93%. The two SNP markers JHI-Hv50k-2016-496331 (97.31 cM) and SCRI_RS_150401 (110.27 cM) coincided with the reported *Ph* resistance loci [23,26,35,73,79].

Common genomic regions were detected for SRT and at APR. Two common loci associated with SRT were detected on the chromosome 2H, the SNP markers JHI-Hv50k-2016-117483 (SRT-MRC; 106.53 cM), JHI-Hv50k-2016-119586 (SRT-SAT; 107.37 cM), and SCRI_RS_153420 (APR-SAT17; 107.26 cM) may represent the same loci. Similar analogies can be drawn for the SNP markers JHI-Hv50k-2016-511500 (SRT-MRC) and JHI-Hv50k-2016-510780 (APR-SAT19) due to their common shared chromosomal location (7H; 126.7 cM).

To establish a link between the significant SNP markers and leaf rust resistance, the functional annotation of candidate genes (CGs) adjacent to SNP markers was obtained either using the BLAST search tool of the barley genome [65], or the Barleymap [64,87]. The identification of resistance genes and understanding their molecular mechanism will shed

more light on disease resistance signaling in barley to diverse biotrophic and necrotrophic pathogens to accelerate candidate gene-based resistance breeding via allele mining and genome editing. In the case of SRT, 21 putative CGs, and 15 in the case of APR, were involved in disease resistance (Table 3). The presence of CGs with putative functions in disease resistance further confirms that some of the SNP markers in our study could be associated with *Ph* resistance in barley.

A plant immune system is multilayered and is triggered upon pathogen recognition. PTI (pattern triggered immunity) constitutes the first layer of defense and is triggered by plasma membrane anchored extra-cellular pattern recognition receptors (PTT) upon recognition of conserved pathogen-associated molecular patterns (PAMPs). The second layer of defense constitutes the intracellular receptors (NLR), which directly or indirectly recognize pathogen secreted avirulence gene products (effectors) and trigger effector triggered immunity (ETI), which often culminates into hypersensitive response to halt a pathogen's invasion. The vast majority of the known NLR have a modular structure composed of a central nucleotide-binding (NBS) and C-terminal leucine-rich repeat (LRR) domain, where the LRR domain directly/indirectly interacts with the pathogen's effectors to initiate a plant defense response [88,89]. The sequence of eight SRT SNP markers showed homology with RLK and NLR (Table 3). On chromosome 1, two SRT SNP markers, SCRI_RS_118785 (56.44 cM) and JHI-Hv50k-2016-41795 (89.31 cM), encode the receptor-like protein kinase (RLK). RLKs contain an ectodomain, a transmembrane spanning region, and an intracellular cytoplasmic kinase domain to relay the pathogen recognition signal [88]. In barley, *Rph22* encodes a lectin receptor-like kinase and may possibly be involved in basal defense if an analogy is to be drawn with other extracellular pattern recognition receptors. Marcel et al. [26] reported the defense gene homologue, LR10 resistance-like protein (RLK), as a candidate gene associated with *Rphq9* on chromosome 7H. Similarly, in rice, RLK *Xa21* confers resistance to bacterial blast caused by *Xanthomonas oryzae* pv. *oryzae* [88,90].

Likewise, the sequences of five SRT SNP markers encoded disease resistance proteins belonging to the CC-NB-LRR family (Table 3). On chromosome 3H the SNP marker JHI-Hv50k-2016-195152 (75.21 cM) encoded leucine-rich repeat protein kinase family protein (HORVU3Hr1G079230) [73], JHI-Hv50k-2016-515270 (7H, 132.22 cM) shared homology with a disease resistance RPP13-like protein 4 (HORVU7Hr1G118430), JHI-Hv50k-2016-109913 (2H, 88.39 cM) encoded disease resistance protein RPP13 (HORVU2Hr1G096190), JHI-Hv50k-2016-112041 (2H, 91.15 cM) encoded a disease resistance protein (HORVU2Hr1G098 100), JHI-Hv50k-2016-430648 (118.98 cM) shared homology with disease resistance RPP8-like protein 3 (HORVU6Hr1G092660), and JHI-Hv50k-2016-435962 (7H, 0.21 cM) shared homology with a disease resistance protein (CC-NBS-LRR class; HORVU7Hr1G000040). Interestingly, a disease resistance protein (HORVU2Hr1G098100) had the lowest p-value (6.97×10^{-10}), and a higher additive value (-1.13), which alone can reduce the disease severity by 23% at the seedling stage. In *Arabidopsis*, RPP13 has been implicated exclusively in resistance to *Pseudomonas syringae* pathovars [91–94]. Similarly, RPP8 was induced in response to an oomycete fungus *Hyaloperonospora arabidopsidis* in *Arabidopsidis thaliana* [95]. It is important to note that four wheat leaf rust resistance genes, namely *Lr1* [96], *Lr10* [97], *Lr21* [98], and *Lr22a* [99], and one barley leaf rust resistance gene, *Rph1* [100], encode the CC-NBS-LRR class of disease resistance proteins. Recently, [101] mapped a new leaf rust resistance gene *Lr82* on chromosome 2H, and two of the putative candidate genes (TraesCS2B01G608500 and TraesCS2B01G608800) encoded NLR disease resistance proteins. In this study, we also found that the sequences of five SNPs on the chromosome 1, 2, 6, and 7H encode NLR proteins (Table 3), and the analysis of bi-parental mapping populations will help in cloning and validation of these putative resistance genes.

Another SNP marker, JHI-Hv50k-2016-110777 (2H, 90.16 cM), sequence was homologous to the UDP-Glycosyltransferase (UGT) superfamily protein (HORVU2Hr1G096910). Vatter et al. [73] also reported this candidate gene on chromosome 2H at 3.66 Mb. Glycosylation is the last step in the triterpenoid pathway to produce many plant defense-related compounds: phenolics, glucosinolates, salicylates, and anthocyanins. The glyco-

syltransferase gene family is ubiquitous in the plant genome, and *Arabidopsis thaliana* has 107 putative UGTs [102]. The UGT74F1 mutant *A. thaliana* was deficient in salicylic acid (SA) production with lower levels of resistance to *P. syringae*. Whereas, the UGT74F2 mutant produced higher levels of SA and was associated with resistance to *P. syringae*; however, overexpression of UGT74F2 rendered *Arabidopsis* highly susceptible to *P. syringae* infection [103,104]. Interestingly, the additive value of the UGT family member in this study is positive (1.14), and it can promote susceptibility to *Ph*.

Adult plant resistance and partial resistance have been used interchangeably in the literature, but Parlevliet and van Ommeren [105] differentiated partial resistance from SRT and APR. In partial resistance, the host plant remains susceptible to LR at all growth stages, but differences in infection frequency, latency period, and rate of spore production can be observed in barley genotypes. In addition, partial resistance is controlled by many minor genes that are additive in nature [106]. During field screening, barley genotypes are exposed to field populations of *Ph*, which may be constituted of different races. Therefore, adult plant stage resistance needs to be race-nonspecific in nature unlike SRT, which is conditioned by a dominant resistance gene. One APR SNP marker, JHI-Hv50k-2016-80368 (APR-SAT17) on chromosome 2H (43.84 cM), encoded an ATP-binding cassette transporter C (ABC) family member10 (HORVU2Hr1G022560). The ABC transporter proteins are involved in disease resistance by secreting the anti-fungal products into the apoplast, such as *PEN3* in *Arabidopsis* against *B. cinerea* [107]. Interestingly, the wheat leaf rust resistance gene *Lr34* on chromosome 7H encodes an ABC transporter [108]. Owing to its non-specific nature, it is believed that *Lr34* improves the durability of other major genes when used in combination. It provided resistance against stem rust, stipe rust, and powdery mildew of wheat [109], in addition to powdery mildew and rust resistance in barley [110], and against rice blast [111]. Interestingly, the marker allele of the SNP marker JHI-Hv50k-2016-80368 reduced the disease severity by −113.86 AUDPC units, and based on an analogy with *Lr34*, this ABC transporter could be a strong marker target for non-race specific resistance against *Ph*.

Two SNP markers, JHI-Hv50k-2016-277557 (5H, 1.11 cM) and BOPA2_12_31536 (4H, 63.39 cM), encoded nodulin-like proteins (AAV49984.1 and HORVU4Hr1G068990, respectively). The nodulin-like proteins have an important role in the transport of various solutes throughout plant development, and they are also involved in the interaction of plants with pathogenic microbes, highlighting the implication of solute transport in plant innate immunity [112]. In barley, the SNP marker 11_20162 was associated with a spot blotch resistance QTL (*Rcs-qtl-7H-32.81*) and encoded an early nodulin-93 (HORVU7Hr1G020770)-like protein [113]. Likewise, the wheat leaf rust resistance gene *Lr67* also encodes a hexose transporter, and a single amino acid substitution (Arg144Gly) in this hexose transporter, which conferred resistance to wheat LR. Furthermore, transgenic expression of *Lr67* in barley conferred seedling and adult plant resistance to *Ph* [114,115]. Moreover, the newly cloned BLR resistance gene *Rph3* encodes a transmembrane protein, and it showed similarity with various transport proteins [116]. Orthologues of existing known LR resistance genes could be sought as potential gene editing targets for broad spectrum resistance to LR.

Many of the SNPs identified in this study encode functional proteins which have been involved in plant defense against diverse fungal pathogens. Our study provides detailed information for GWAS of *Ph* resistance in the barley AM2017 panel that could be efficiently employed by barley breeders for fine mapping, gene cloning, and for MAS targeted at improving resistance to *Ph*.

5. Conclusions

We have reported effective sources of resistance to *P. hordei* at the seedling and adult plant stages in a diverse panel of barley genotypes (AM2017) supporting the potential of this breeder-constructed panel to supply sources of valuable traits sought by breeders. In addition, high density genotyping with 50k SNPs was combined with phenotypic data to detect genomic regions associated with the seedling as well as the adult plant stage

resistance in barley. This is the first comprehensive study published on the interaction of *P. hordei* with a unique collection of barley genotypes, and subsequent GWAS analyses. We have reported many of the previously identified QTL in addition to novel genomic loci against *P. hordei*, which will be of particular importance in improving leaf rust resistance in North African barley germplasm. Furthermore, SNP markers with high R^2 and additive effects can be converted into high-throughput functional markers for accelerated selection and pyramiding of LR resistance for their deployment at both growth stages using speed breeding and MAS.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12111829/s1. Supplementary figures; Quantile-quantile and the Manhattan plots of best-fit models for SRT-MRC (Figure S1), for SRT-SAT (Figure S2), for APR-SAT17 (Figure S3), and for APR-SAT19 (Figure S4). Supplementary tables; Table S1: Barley genotypes with row type, pedigree, and response to *P. hordei* at the seedling and at the adult plant stages; Table S2: Average monthly weather in Sidi Allal Tazi, Morocco during 2016/2017 and 2018/2019; Table S3: The list of 2065 highly informative SNP markers based on gene diversity (GD) and polymorphic information content (PIC), 2065 highly informative SNP markers; Table S4: Response of barley genotypes to *P. hordei* for each reaction class evaluated at the seedling and at the adult plant stages; Table S5: Principle components PC1 and PC2; Table S6: Q matrix from STRUCTURE analysis.

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