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ORIGINAL PAPER

Genome-wide DArT and SNP scan for QTL associated with resistance to stripe rust (*Puccinia striiformis* f. sp. *tritici*) in elite ICARDA wheat (*Triticum aestivum* L.) germplasm

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Abstract
Key message **Identified DArT and SNP markers including a first reported QTL on 3AS, validated large effect APR on 3BS. The different genes can be used to incorporate stripe resistance in cultivated varieties.**
Abstract Stripe rust [yellow rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*)] is a serious disease in wheat (*Triticum aestivum*). This study employed genome-wide association mapping (GWAM) to identify markers linked to stripe rust resistance genes using Diversity Arrays Technology (DArT®) and single-nucleotide polymorphism (SNP) Infinium 9K assays in 200 ICARDA wheat genotypes, phenotyped for seedling and adult plant resistance in two sites over two growing seasons in Syria. Only 25.8 % of the genotypes showed resistance at seedling stage while about 33 and 44 % showed moderate resistance and resistance response, respectively. Mixed-linear model adjusted for false discovery rate at $p < 0.05$ identified 12 DArT and 29 SNP markers on chromosome arms 3AS, 3AL, 1AL, 2AL, 2BS, 2BL, 3BS, 3BL, 5BL, 6AL, and 7DS significantly linked to *Pst* resistance genes. Of these, the locus on 3AS has not been previously reported to confer resistance to stripe rust in wheat. The QTL on 3AS, 3AL, 1AL, 2AL, and 2BS were effective at seedling and adult plant growth stages while those on 3BS, 3BL, 5BL, 6AL and 7DS were effective at adult plant stage. The 3BS QTL was validated in Cham-6 × Cham-8 recombinant inbred line population; composite interval analysis identified a stripe resistance QTL flanked by the DArT marker, wPt-798970, contributed by Cham-6 parent which accounted for 31.2 % of the phenotypic variation. The DArT marker "wPt-798970" lies 1.6 cM away from the 3BS QTL detected within GWAM. Epistatic interactions were also investigated; only the QTL on 1AL, 3AS and 6AL exhibited interactions with other loci. These results suggest that GWAM can be an effective approach for identifying and improving resistance to stripe rust in wheat.

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Abbreviations

APR	Adult plant resistance
CIMMYT	International Maize and Wheat Improvement Center
cM	Centimorgan
DArT	Diversity array technology
GWAM	Genome-wide association mapping
ICARDA	International Center for Agricultural Research in the Dry Areas
LD	Linkage disequilibrium
MAF	Minor allele frequency
MLM	Mixed linear model
QTL	Quantitative trait loci
SSR	Simple sequence repeat
SNP	Single nucleotide polymorphism
Yr	Stripe rust

Introduction

Stripe rust caused by the *Puccinia striiformis* Westend. f. sp. *tritici* Erikss. (*Pst*) is a serious, widespread and damaging disease in wheat (*Triticum aestivum* L.). Losses in grain yield of up to 40 % have been reported in many countries, and in some cases the affected fields are completely destroyed (Mumtaz et al. 2009). Stripe rust epidemics have been reported in some major wheat producing regions such as China (Saari and Prescott 1985), Continental Europe, Australia, Ethiopia (Johnson 1992; Wellings et al. 2003), South Africa in 1996 (Boshoff et al. 2002), and USA (Chen 2007; Milus et al. 2006). Recently, many countries in the Central West Asia and North Africa (CWANA) and Sub-Saharan Africa regions reported significant losses in yield which ranged from 10 to 80 % due to the breakdown in major gene resistance in some widely grown cultivars as a result of the evolution of new pathotypes (Solh et al. 2012; Zegeye et al. 2014). Examples of major gene resistance breakdown are the acquisition of virulence towards *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*+ and *Yr27* genes deployed in many varieties (Singh et al. 2008). Various strategies have been adopted to control stripe rust disease but the development and deployment of host resistance remain the most effective, economical and environmentally friendly means to manage the disease.

Thus, the identification and mapping of stripe rust resistance gene(s) in wheat are very crucial for the attainment of this goal. To date, about 67 stripe rust resistance genes (*Yr1–Yr67*) plus 42 temporarily designated genes have been catalogued in cultivated wheat and its wild relatives (McIntosh et al. 2013; Macceferri et al. 2015; Cheng et al. 2014; Zhou et al. 2014). Considerable progress has been made to develop robust markers linked to genes that confer resistance to stripe rust that can be utilized in marker assisted selection (MAS). For example, diagnostic molecular markers that enabled the early detection of some of the stripe rust resistance genes in breeding programs, including *Lr34/Yr18* (Suenaga et al. 2003; Lagudah et al. 2006, 2009; Kolmer et al. 2008; Krattinger et al. 2009), *Yr5* resistance (Yang et al. 2003), *YrH52*, *Yr15* (Peng et al. 2000), *Yr36* (Distelfeld et al. 2004) and *Yr48* (Lowe et al. 2011), are major advancement in wheat breeding. However, the constant adaptation of the pathogen necessitates continued development of new stripe resistant wheat varieties (Pardey et al. 2013) to contain the threat.

The development of resistant varieties requires the availability of many sources of resistance to counter the continuing evolution of new virulence types within the pathogen population (Gill et al. 1985). The effective utilization of resistance genes requires the phenotypic and genotypic characterization of the mapping population under study. This has been widely exploited in many genetic studies—either through the use of classical bi-parental crosses and linkage mapping to determine the number and chromosomal location of stripe rust resistance genes (Yang et al. 2003) or the use of recent approaches such as genome-wide association mapping (GWAM) which involves a collection of adapted germplasm. The advantages of GWAM over bi-parental mapping population include higher mapping resolution, increase in allele number and time saving in establishing a marker-trait association and immediate application of its results in a breeding program (Flint-Garcia et al. 2003). Despite the advantages of GWAM, the major limitation is that it has higher probability of type I and II errors. The type I errors are attributed to confounding effects of population structure (Breseghello and Sorrells 2006), but studying the structure for traits that vary due to the environmental gradients which overlap with patterns of population structure, like flowering time, can also lead to type II errors (Brachi et al. 2011). Studies have shown that type I error can be controlled by taking into account the population structure and relatedness and, once these are correctly modelled; accurate marker-trait association due to linkage disequilibrium can be detected (Patterson et al. 2006; Price et al. 2006, 2010; Zhao et al. 2007; Rincent et al. 2014). In GWAM, the use of mixed linear models (MLM) (Yu et al. 2006; Kang et al. 2008; Stich and Melchinger 2009) that utilizes Q and K performs better than general linear model (GLM) in correcting for false-positive results. The MLM approach has been employed in many recent genetic studies to identify genes that conferred resistance to many biotic stresses as well as uncovering the genetic basis of agronomically useful traits (Maccaferri et al. 2011, 2015; Miedaner et al. 2011; Neumann et al. 2011; Yu et al. 2011, 2012; Wang et al. 2012; Kollers et al. 2013; Mulki et al. 2013; Joukhadar et al. 2013; Sela et al. 2014; Rasheed et al. 2014; Zegeye et al. 2014; Emebiri and Ogbonnaya 2015).

The objectives of this study were to (i) assess the diversity of stripe rust resistance in a collection of 200 elite spring wheat germplasm from ICARDA's breeding program in CWANA where resistance to stripe rust is a highly desirable trait to prevailing stripe rust races in Syria; (ii) identify genomic regions linked to seedling and adult stripe rust resistance in this set of germplasm using MLM approach; (iii) validate the results from GWAM using an F_2:F_8 derived recombinant inbred line (RIL) Cham-6 × Cham-8 population; and (iv) examine the epistatic interactions between the identified chromosomal regions to provide additional information on the most beneficial combinations of loci with potential synergistic effects.

Materials and methods

Plant materials

A total of 200 wheat genotypes from ICARDA spring wheat breeding program and few lines from CIMMYT and Australia were used in this study (Table S1). To validate the contribution of some of the stripe rust resistance QTL in the GWAM, we used 152 F_2:F_8 recombinant inbred population ((RIL) derived from the cross Cham-6 × Cham-8. Some of the genotypes used in the GWAM germplasm panel shared common ancestors with Cham-6 and Cham-8.

The studies were carried out at ICARDA research station, Tel-Hadya (36°16′N, 36°56′E), Syria, in 2010 and 2011 and in the Agricultural Research Centre, Al-Qamishly Malkiyeh (37°1′N, 42°08′E) in 2009 and 2010. Cham-8 (JUP/BJY//URES) is one of the CIMMYT mega-cultivars "Kauz" that carries the defeated stripe rust resistance genes *Yr9* and *Yr27* as well as the minor APR gene *Yr18* (research highlights of the CIMMYT Wheat Program 1999–2000). However, *Yr18* does not by itself confer enough protection under high disease pressure (Ma and Singh 1996). The response of Cham-8 to stripe rust ranged between 70 % to full susceptibility among locations/years. Cham-6 (W3918A/JUP) also carries *Yr27* but exhibits partial resistance derived from minor gene(s) (results of this study).

Disease phenotyping

The 200 wheat genotypes were screened against stripe rust under field conditions in adult plant growth stage at the GCSAR, Agricultural Research Centre, Al-Qamishly Malkiyeh, Yanboo station in 2009–2010 as well as at ICARDA, Tel-Hadya, in 2009–2010 and 2010–2011. At each location, seeds were sown in an alpha-lattice design with two replications. Each entry was planted in two rows 0.5 m length and 30 cm row space. Artificial inoculation was carried out using *Pst Yr27* avirulent isolate in 2010 and *Pst Yr27* virulent isolate in 2011. The inoculation was done three times during seedling (two leaf stage), tillering and booting stages using a local race of *Pst* virulent for *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *YrA*, *Yr25*, *Yr27* and *YrSd* genes. However, the experiment in Malkiyeh was exposed to natural epidemics during the growing season in both years.

The GWAM set in Malkiyeh 2009 and the Cham-6 × Cham-8 RIL validation population evaluated under field conditions were scored for adult-plant responses using a scale of 1–9 according to Bariana et al. (2004), where a host response score of 1 was considered very resistant, 2 resistant response, 3 resistant to moderately resistant, 4 moderately resistant, 5 moderately resistant to moderately susceptible, 6 moderately susceptible, 7 moderately susceptible to susceptible, 8 susceptible and 9 very susceptible. For Tel-Hadya and Malkiyeh 2010 adult-plant responses, the disease severity as percentage of the disease covered areas were multiplied by a value of 0.2, 0.4, 0.6, 0.8, or 1.0 for the host response of resistance (R), moderately resistanct (MR), intermediate (M), moderately susceptible (MS) or susceptible (S), respectively, to calculate the coefficient of infection (CI) following the procedures of Pathan and Park (2006). For association analysis, we used the mean adult plant resistance (APR) values, which was determined through the conversion of the CI estimates to a scale from 1 to 9 as (0 = 1; 1–9 = 2; 10–19 = 3; 20–29 = 4; 30–44 = 5; 45–59 = 6; 60–79 = 8; 80–100 = 9).

Evaluation of seedling resistance against commonly known *Pst Yr27* virulent race (with the virulence formula: *Yr2*, *Yr6*, *Yr7*, *Yr9*, *Yr25*, *Yr27*, *YrA*, *YrSd* and partially virulent on *Yr8* and *Yr17*) was carried out at ICARDA, Tel-Hadya, under control conditions in the glasshouse. Four replicates of each genotype were grown in a 9-cm diameter pot filled with standard potting mix. Plants were grown at 20 °C and supplementary light for 16 h. Urediniospores were removed from a deep freezer (−80 °C) and heat-shocked at 42 °C for 5 min. A light mineral oil (Soltrol 170; Chevron Phillips Chemical Company LP, the Woodlands, TX) was used in seedling inoculations of 9—to 10-day-old seedlings at two-leaf stage. Inoculated plants were incubated in a dew chamber at 10 °C, 100 % relative humidity under dark conditions for 24 h, and then moved to glasshouse with 18 °C with 16 h light/8 h dark cycle. Seedling infection types (ITs) were recorded 14 days after inoculation following the 0–4 scale as described by McIntosh et al. (1995). For the seedling trials, a set of stripe rust differential lines (Johnson et al. 1972) and Avocet near isogenic lines were included to reconfirm the race designation. The cultivar Morocco was used as susceptible check in all seedling trials. In order to get a better quantitative scale for the GWAM analysis, the 0–4 ITs scale was converted to 0–9 scale. The scores: 0, 1^-, 1, 1^+, 2^-, 2, 2^+, 3^-, 3, 3^+, and 4 were coded as 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 9, respectively.

The symbol for hypersensitive flecks (;) was converted to 0, while IT score of 4 was converted to 9. The special annotation codes C and N were ignored (Letta et al. 2014).

Genotyping

Genomic DNA was extracted from 2-week-old seedlings using pooled leaf samples from five plants per line, frozen in liquid nitrogen, and stored at −80 °C before DNA extraction. DNA extraction was carried out according to Ogbonnaya et al. (2001). The 200 GWAM panel and the bi-parental validation RIL populations were genotyped with high-density Diversity Arrays Technology (DArT®) markers from a PstI/BstNI representation ("wPt" markers) using 10 µl of a 100 ng μl^{-1} DNA of each sample sent to Triticarte Pty. Ltd. Australia (http://www.triticarte.com.au) as a commercial service provider for DArT markers. Of the 200 genotypes for GWAM, three genotypes were missing resulting in data for 197 genotypes. A subset of 2688 polymorphic marker loci out of 3051 DArT markers with a quality parameter and a call rate both greater than 80 % and minor allele frequency (MAF) >5 % were selected for genome-wide association analysis. Of the 2688 polymorphic DArT markers, 2007 markers were of known map locations. For the Cham-6 × Cham-8 RIL validation population, 1121 DArT markers were polymorphic between the parents. Additional set of 53 microsatellite (SSR) markers distributed across the wheat genome were also screened against the RIL population. The GWAM panel was also genotyped with the Illumina 9K iSelect SNP assay (Cavanagh et al. 2013). Both DArT and SNP data were filtered to contain <10 % missing values and the MAF >5 %; and the heterozygote genotypes in the SNP matrix were considered as missing values to avoid their bias since their frequency in the elite germplasm was very small, less than 2 %.

Statistical analysis

The stripe rust phenotypic data collected across test locations in two consecutive years were subjected to the analysis of variance (ANOVA). The genotype × environment interaction (GGE bi-plot) implemented in GenStat V.12 (http://www.GenStat.co.uk) was used to establish the level of variability among the wheat genotypes in response to the stripe rust races across environments. The phenotypic and genotypic variance components of the phenotypic data were estimated according to the standard methods described in Comstock and Robinson (1952) and Johanson et al. (1955). The coefficient of variance (CV), broad sense heritability (H_b) and the correlation coefficients of the phenotypes were also estimated among the genotypes across all environments.

Population structure and linkage disequilibrium (LD)

The population structure, (Q), was investigated using 50 and 72 unlinked DArT and SNP markers, respectively, distributed across the entire wheat genome. A clustering method based on a Bayesian model (Pritchard et al. 2000) and implemented in the STRUCTURE version 2.3.3 algorithm (available from http://www.pritch.bsd.ucicago.edu) was employed to infer the population structure. Both the length of burn-in period and the number of iterations were set at 100,000 with k value in the range of 1–15 with three replications. To reach the appropriate k value, we used two approaches. First, the estimated normal logarithm of the probability [Ln$P(D)$] values from the STRUCTURE output was plotted against k. This value reaches a plateau when the minimal number of groups that best describe the population substructure has been reached (Pritchard et al. 2000). Second, the Evanno method was also employed which calculates the Dk statistic based on the rate of change in the log probability of data between successive k values (Evanno et al. 2005). Principal component analysis (PCA) based on a simple matching coefficient was subsequently conducted using the polymorphic DArT and SNP markers with a PAST Program to ascertain the major groupings among the GWAM germplasm panel.

Pair-wise measures of LD (R^2) between markers were estimated for both the DArT and SNP markers as described by Hedrick (1987) and Weir (1996). The LD estimates were based on markers with minor allele frequency >5 % and pairwise comparison of $p < 0.001$. LD statistics were calculated per chromosome and across all chromosomes. The R^2 values were plotted as a function of genetic distance (cM) and the second LOESS decay curve fitted using the square root transformation of the equation described by Breseghello and Sorrells (2006) and Andreescu et al. (2007). The intra-chromosomal LD was calculated for each marker system separately because of limited consensus map that combines DArT and SNP markers together on the same linkage groups.

Association mapping

The association of the two marker sets (DArT and SNP markers) and stripe rust disease phenotype based on field evaluation was carried out using a unified mixed-model approach (MLM) as implemented in TASSEL 3 (Bradbury et al. 2007; http://www.maizegenetics.net). The MLM accounts for type I errors due to the inherent genetic relatedness or kinship (K) within the mapping population, with and without considering the effect of population structure (Q), MLM-Q model (Thornsberry et al. 2001; Yu et al. 2006). Only the markers that showed significant marker-trait associations in both models, MLM and MLM-Q, were reported and the statistics related to the associated markers

were obtained from the MLM-Q model. The Q matrix was obtained from both the inferred ancestry output from STRUCTURE that accounts for a coarse population structure and the first three components obtained from the PCA analysis. The K matrix is a measure of relative kinship and quantifies the probability that two homologous genes are identical by descent (Massman et al. 2011). The K matrix was generated within TASSEL utilizing the DArT and the SNP markers (Lynch and Ritland 1999).

Significant associations between DArT and SNP markers, with minor allele frequency MAF >5 %, and stripe rust resistance genes were determined based on the initial F test. The marker-trait association was declared to be significant for stripe rust resistance if the p value exceeds the qFDR ($p < 0.05$) threshold for accepting a significant association as implemented in Qvalue program (Storey and Tibshirani 2003). In order to avoid false-negative FDR results, only single markers were considered in the FDR analysis among the clusters of markers with tight LD ($R^2 > 0.95$) since FDR assumes independent variables in its algorithm while markers are dependent because they are linked to each other. The map locations of DArT markers which exhibited significant associations with stripe resistance QTL were determined from the released consensus genetic map of wheat (Detering et al. 2010) (http://www.DiversityArrays.com; Australia), while the SNP locations were obtained from the consensus map developed for 9K SNP (Cavanagh et al. 2013). The association analysis for Tel-Hadya in both years revealed similar results with minor differences in the p and R^2 values; thus, the mean values of both years were utilized in the association analysis.

Linkage mapping of Cham-6 × Cham-8 RIL validation population

A total of 1121 DArT and 53 SSR markers, polymorphic between the parents, were used to generate a dense linkage map for the F_2:F_8 Cham-6 × Cham-8 RIL population using JoinMap4 software (Van Ooijen 2006) under a maximum distance of 50 cM and the Haldane's mapping function. Segregating markers were placed into linkage groups under high stringency with a LOD score >10 (Stam 1993). The composite interval mapping procedure implemented in MapQTL6 (Van Ooijen 2009) was used for QTL analysis and loci with LOD score >3 were considered as putatively linked to stripe resistance. The proportion of the phenotypic variation (R^2) explained by the QTL and the additive effects of significant QTL were obtained from the MapQTL6 software output.

Gene–gene interaction

Only the DArT and SNP markers that showed significant associated with the stripe rust resistance in the field and seedling phenotypes were used for the pair-wise interaction analysis between markers. Detection of gene—gene interaction was done by fitting a linear model with $Q + K$ variables, additive effects of the markers and their interaction. Further, the p values for the gene–gene interaction and the associated contributions (R^2) in the residual sum of squares (obtained from fitting $Q + K$ variables and additive effects) were computed. Only the pairs of markers that showed $p \leq 10^{-5}$ were tabulated. The interaction graph was drawn using the software Circos 0.63-4 (Krzywinski et al. 2009).

Results

Phenotypic variations for stripe resistance in elite ICARDA germplasm

Disease development was good at the test locations: Malkiyeh (2009 and 2010) and Tel-Hadya (2010 and 2011). The disease incidence scores obtained in Tel-Hadya (2010 and 2011) were highly repetitive (Fig. 1a, b) accounting for the observed high $H_b \geq 0.97$ for both years. However, minor differences were observed at the Malkiyeh site across the 2 years with H_b value of 0.77. The response distribution of the GWAM germplasm panel revealed that considerable proportion of the genotypes had moderate to resistant response which ranged from 32.5 % in Malkiyeh 2009 to 43.5 % in Malkiyeh 2010 (Fig. 1c, d) against the prevailing stripe rust pathotype(s) in adult plant under field conditions across years/locations. At seedling stage under controlled glasshouse environment, 22.5 % of the genotypes exhibited resistance response while 77.5 % genotypes were susceptible. However, genotypes which were susceptible during the seedling stage were found to vary in their response at the adult plant stage. 23, 32, 25 and 25 % of the genotypes exhibited resistant to moderate resistance responses in Malkiyeh 2009, Malkiyeh 2010, Tel-Hadya 2010 and Tel-Hadya 2011, respectively (Fig. 1a–d).

The analysis of variance also revealed significant differences among genotypes in response to the stripe rust infection at both test locations (Table 1). The effect of environment, genotypes, and their interactions were highly significant ($p < 0.001$) while the replication effect was non-significant. The heritability (H_b) for disease severity ranged from 0.77 to 0.97 for Malkiyeh and Tel-Hadya environment, respectively and 0.88 between the different environments. The correlation between seasons was highly significant ($p < 0.001$) and the correlation coefficient values ranged from 0.63 for Malkiyeh 2009/Tel-Hadya 2011 to 0.95 for Tel-Hadya 2010/2011. The CV between the replications was down to 0.9 ± 0.03 % while the genotype/replication CV reached up to 23.9 ± 0.86 %. The principal component analysis showed that the first two principal components (PC1 and PC2) explained 96.3 % of the total GGE variation, with PC1

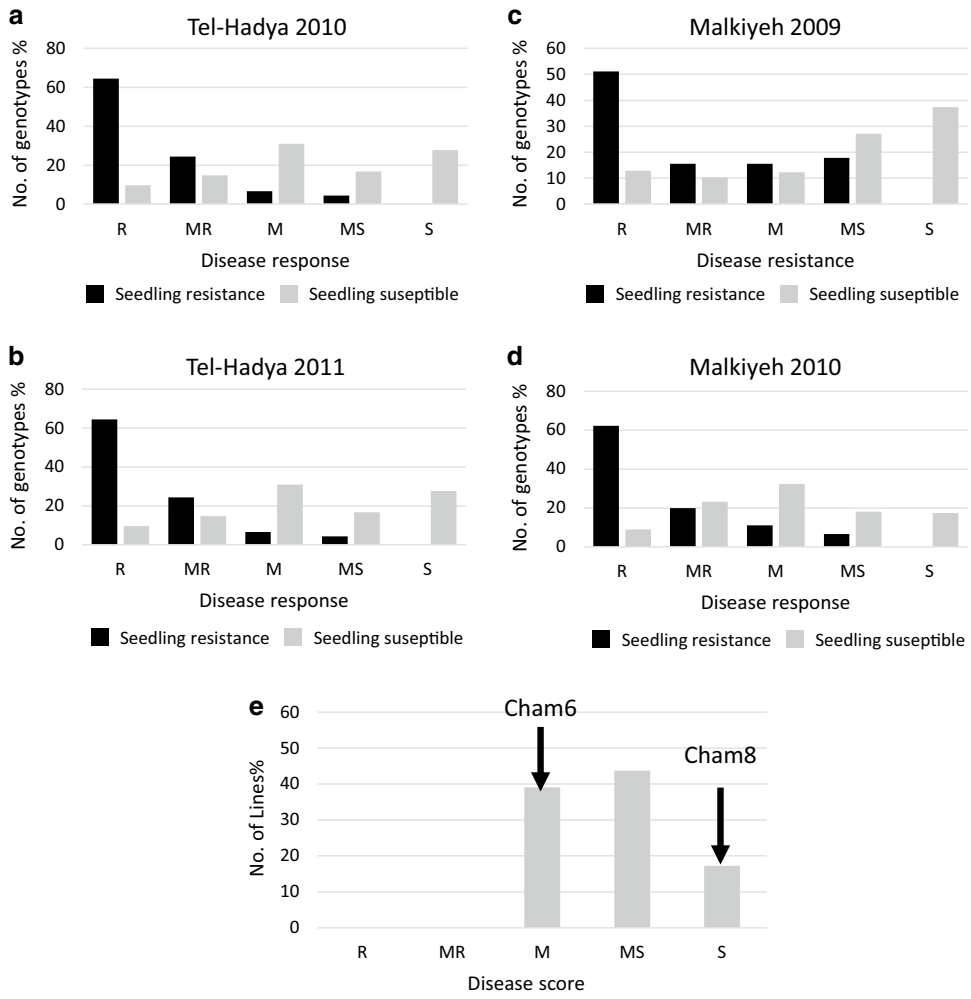

Fig. 1 The frequency distribution of the adult stage plant response to stripe rust infection of 200 elite ICARDA wheat germplasm as five classes, R, MR, M, MS and S (**a**–**d**). The *black bars* represent the genotypes that were resistance at the seedling stage (a total of 41 genotypes) while the *grey bars* represent the percentage of the genotypes that were susceptible at the seedling stage (159 genotypes) in all sites **a** Malkiyeh 2009, **b** Malkiyeh 2010, **c** Tel-Hadya 2010, **d** Tel-Hadya 2011. **e** Represents the F_2:F_8 Cham-6 × Cham-8 recombinant inbred population

Table 1 Analysis of variance of stripe rust severity of the 200 elite spring wheat genotypes at the adult plant growth stage among the studied environments

Source	DF	SS	MS	VR	*F* pr.
Replication	1	0.56	0.56	0.76	0.382
Environment[a]	2	226.4	113.2	153.61	<.001
Genotype	199	1904.65	9.57	12.99	<.001
Genotype × environment	398	469.93	1.18	1.6	<.001
Residual	599	441.44	0.7		
Total	1199	3042.99	2.54		

[a] We used only three environments which are Malkiyeh 2009, Malkiyeh 2010 and Tel-Hadya (2010, 2011 as two reps since those were highly repetitive with high heritability value of 0.96)

and PC2 explaining 80.7 and 15.6 % of the total variation, respectively. The genotype reactions were stable across the four environments with the majority distributed close to zero in both PC1 and PC2 (Fig. 2). However, some genotypes deviated largely away from the centre which resulted in the significant genotype/environment interaction (Table 1).

Stripe rust resistance amongst parents and Cham-6 × Cham-8 RILs

The two wheat cultivars Cham-6 and Cham-8 possess the stripe rust ineffective seedling resistance gene, *Yr27*, and were fully susceptible at the seedling stage. However, results from this study suggest that Cham-6 may contain additional uncharacterised minor gene(s) independent of *Yr27* gene. The response of the RILs ranged from M to S for stripe rust severity (Fig. 1e). None of the genotypes within the RIL scored higher than Cham-6 suggesting a null contribution from Cham-8 to stripe rust resistance variation in the population; however, the distribution of the RIL population skewed towards a MR response.

Marker coverage, population structure and linkage disequilibrium

A total of 2688 DArT and 4252 SNP markers were polymorphic in the GWAM panel out of the 3051 DArT and 8632 SNP markers obtained using the high-density DArT

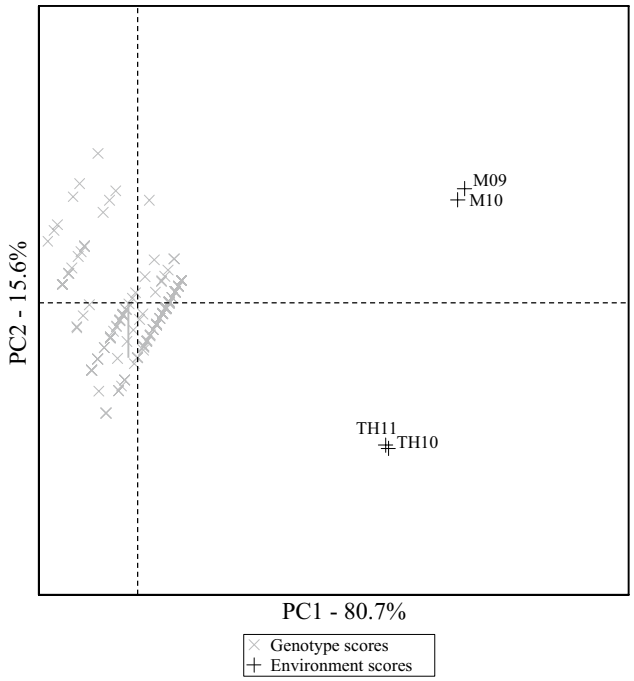

Fig. 2 GGE Bi-plot of the reaction of elite ICARDA GWAM panel to stripe rust adult resistance across four environments. The *grey dots* represent the genotypes while the *black dots* represent the environments. Both PC1 and PC2 explained about 96.3 % of the total variation

and Illumina 9K iSelect SNP arrays. This represents 88 and 49 % polymorphism for DArT and SNP markers, respectively. Of these, 2007 DArT and all polymorphic SNP markers were of known map position and thus were used to prepare two framework genetic maps comprising of 21 wheat chromosomes. Seven hundred and forty-one, 887 and 379 DArT loci were located on the A, B and D genomes, respectively, covering a genetic distance of 918.9, 933.8 and 861.3 cM, with an average density of 1.25, 1.06 and 2.32 cM for the A, B and D genomes, respectively. The SNP map had more markers in which a total of 2012 SNPs were located on the A genome and covered 906.4 cM with an average density of 0.68 cM while 1986 SNPs located on the B genome covered 1139 cM with average density of 0.62 cM. Two hundred and fifty-four SNPs were located on D genome and covered a genetic distance of 906.1 cM with average density of 2.65 cM. The DArT map spanned a total genetic distance of 2714 cM at an average density of 1.37 cM while the SNP map spanned 2951.5 cM with average density of 0.77 cM. Both the DArT and SNP markers were independently used to infer the population structure for the GWAM panel, as implemented in the STRUCTURE software. With the DArT markers, the highest [Ln$P(D)$] showed a plateau after $k = 11$ and thereafter tended to fluctuate, indicating 11 subgroups amongst the GWAM germplam panel used (Table S1). However, six subgroups were obtained with

Table 2 Inter- and intra-chromosomal linkage disequilibrium patterns between DArT and SNP marker

Pattern	LDs	Sig. LDs	Sig. (%)	Mean R^2
Intra. DArT	125,904	24,050	19.1	0.39
Intra. SNP	617,137	180,090	29.2	0.39
Intra. DArT/SNP	584,404	147,854	25.3	0.37
Intra. total	1,327,445	351,994	26.5	0.38
Inter. DArT	2,491,634	153,677	6.2	0.21
Inter. SNP	8,911,722	436,257	4.9	0.14
Inter. DArT/SNP	10,604,488	293,518	2.8	0.14
Inter. total	22,007,844	883,452	4.0	0.15
Total	23,335,289	1,235,446	5.3	0.24

SNP markers (Table S1). The pairwise genetic differentiation (*Fst*) among all populations was found to be relatively low: 0.23 and 0.31 for DArT and SNP maker, respectively. With the concordance of both marker types with the low *Fst* values, the sub-populations were dispersed equally on both components of the PCA analysis with a better differentiation by SNP markers (Figure S2). The population structure of the GWAM panel was also inferred using the Δk method (Evanno et al. 2005). With both the DArT and SNP marker datasets, a maximum Δk value at $k = 2$ was obtained using the six different sets of markers. Thirty-four crosses contributed to 128 genotypes with different selection histories in our germplasm. Noticeably, most crosses of similar pedigree were grouped in the same sub-populations using both the highest [Ln$P(D)$] and Evanno approaches (Table S1).

Table 2 shows the percentage of significant LDs for DArT, SNP and DArT/SNP patterns. For both DArT and SNP markers, the intra-chromosomal pairs of loci constituted about 5.7 % of all interactions while the remaining were inter-chromosomal LDs; 5.3 % were significant with mean R^2 value of 0.24. Despite the low percentage of the intra-chromosomal LDs, 26.1 % were significant, which resulted in about 0.38 of the total significant interactions with average R^2 value of 0.38. On the contrary, only 4 % of the total inter-chromosomal pairs were significant with mean R^2 value of 0.15. DArT markers showed higher significance level in the inter-chromosomal pairs (6.2 %) in contrast to 4.9 % for SNP. The mean R^2 value for DArT is 0.21 while SNP is 0.14. SNP pairs showed higher intra chromosomal pairs, 29.2 % compared to 19.1 % for DArT with similar average R^2 of 0.39 (Table 2). Figure S3a and S3b shows the plot of intra chromosomal LD at $p < 0.001$ against genetic distance. The LD started to decay below R^2 value of 0.2 after 30 cM for DArT markers (Figure S3a) and 45 cM for SNP markers (Figure S3b). However, combined pairwise LD for both DArT and SNP markers indicated that the LD decayed after 40 cM (Figure S3c). Most of the markers on chromosome arm 1BS were in very high LD (Figure S1).

Association analysis of QTL for stripe rust resistance based on DArT and SNP markers

Table 3 shows the results of marker-trait associations for stripe rust resistance with DArT and SNP markers using both the MLM and MLM-Q models at FDR value <0.05 for seedling and adult plant growth stages. No significant differences were identified between the results from both models except for the slight decrease in *p* values observed when the effect of population structure (*Q* matrix) obtained with either DArT or SNP markers was used as a covariate (MLM-Q model). Figure S4 shows the QQ plots of the expected vs observed *p* values. The plots for both models showed little or no inflation of the independent test at the base of the diagonals which is indicates that both models accurately accounted for the false-positive results in the GWAM.

GWAM analysis for stripe rust resistance

A total of 12 DArT markers were identified to be significantly associated with stripe rust resistance on chromosome arms 1AL, 2BS, 3AS, 3BS, 6AL and 7DS (Table 3) in the GWAM panel, out of which seven DArT markers which overlapped on the consensus map on 1AL were linked to stripe rust resistance QTL during the seedling stage with phenotypic variation explained (R^2) of 10.1 %. The 1AL markers linked to stripe rust resistance spanned a genetic interval from 134.2 to 135.6 cM (Table 3) while LD analysis of this region indicated that the DArT markers are in significant LD ($R^2 \geq 0.8$) with each other. Further, six DArT markers with linkage to APR to stripe rust were identified in the GWAM panel on chromosome arms 1AL, 2BS, 3AS, 3BS, 6AL and 7DS with R^2 values, which ranged from 3.5 % for *wPt-734285* on 1AL to 6.6 % for *wPt-668026* on 7DS (Table 3; Fig. 3).

Twenty-nine SNP markers were significantly linked to stripe rust resistance at seedling and adult plant stages on nine genomic regions (2AL, 2BS, 2BL, 3AS, 3AL, 3BS, 3BL, 5BL and 6AL) in the GWAM germplasm panel. Of these, six SNPs on 2AL, 2BS, 3AS and 3AL were associated with seedling resistance (Table 3). The phenotypic variation (R^2) explained by the SNPs ranged from 3.5 % for *wsnp_Ex_c21092_30220702* on 2BS to 6.3 % for *wsnp_CAP11_rep_c7339_3306558* on 3AS (Table 3). Twenty-four SNPs were linked to stripe rust resistance at adult plant growth stage with R^2 values, which ranged from 3.2 % for *wsnp_Ex_c14711_22788263* on 2BS to 11.3 % for *wsnp_Ex_c965_1845447* on 6AL. LD analysis identified a haplotype block of three SNPs on 6AL to be linked to adult stage stripe rust resistance within the genetic distance of 131.8–138.6 cM. Similarly, two SNPs (wsnp_Ex_c33431_41918732 and wsnp_Ex_c210_411604) in a haplotype block were linked to APR on 5BL and both are in significant LD with each other ($R^2 = 0.93$) at 86.1 cM.

Congruency of genomic regions linked to stripe rust resistance identified by DArT and SNP markers

Out of the 11 genomic regions associated with stripe rust resistance identified using DArT and SNP markers in this study, four genomic regions on 2BS, 3AS, 3BS and 6AL were detected by both DArT and SNP markers, with the SNPs exhibiting slightly lower *p* values (Table 3). The regions on 2AL, 2BL, 3AL, 3BL, and 5BL were detected with SNP markers only while the genomic regions on 1AL and 7DS, were identified with DArT markers alone in the GWAM panel. The DArT and SNP markers linked to stripe rust resistance in the GWAM germplasm panel on the same chromosome arm were in high LD ($R^2 > 0.36$).

Validation of GWAM using Cham-6 × Cham-8 recombinant inbred population

Figure 4 summarises the results of QTL analysis of the Cham-6 × Cham-8 RIL population. The DArT marker *wPt-798970* was significantly linked to stripe rust resistance on chromosome arm 3BS. The R^2 explained by *wPt-798970* was 31.2 % with a LOD score of 12.3. The allele for resistance on the 3BS QTL was contributed by Cham-6, which was also included in the GWAM panel used in this study. The marker *wPt-798970*, linked to stripe rust resistance in the RIL population, had a significant LD ($R^2 = 0.87$) with the DArT marker *wPt-800213*, associated with stripe rust resistance in the GWAM germplasm panel. The genetic distance between both markers is about 1.6 cM (Fig. 4). However, the marker *wPt-800213* was monomorphic in the Cham-6 × Cham-8 RIL population.

Gene–gene interaction

A total of 29 significant interactions between pairs of loci representing four different interactions on chromosome arms 3AL with 2AL; 6AL with 3AS; 5B and 6AL were identified to be involved in APR resistance to stripe rust. Out of the 20 markers linked with stripe rust resistance in the mean APR phenotype, only five markers located on chromosome arms 3AL and 6AL had interactions. The mean R^2 value for the stripe rust interactions was about 12.7 %. Table 4 summarized the representative interactions among the stripe rust markers in different chromosomes as was further illustrated in Fig. 5. Chromosome 6A exhibited intra-chromosomal APR interaction between a cluster of

Table 3 Stripe rust adult plant resistance QTL identified in Malkiyeh 2009, 2010 and Tel-Hadya plus seedling resistance QTL

Trait	Marker	Chr	Pos	*p*	FDR	R^2	Allele	Effect	Obs	Gene	Source	References
Seedling	*wPt-0164*	1A	134.2	6.3E−05	0.008	9.9	0	2.2	85	*QRYr1A.1*	Pastor	Rosewarne et al. (2012)
Seedling	*wPt-2976*	1A	134.2	8.4E−05	0.01	9.8	0	2.3	85	*QRYr1A.1*	Pastor	Rosewarne et al. (2012)
Seedling	*wPt-666087*	1A	134.2	3.8E−04	0.03	7.3	0	1.8	85	*QRYr1A.1*	Pastor	Rosewarne et al. (2012)
Seedling	*wPt-669294*	1A	134.2	3.4E−05	0.006	10.1	0	2.3	86	*QRYr1A.1*	Pastor	Rosewarne et al. (2012)
Seedling	*wPt-669800*	1A	134.2	2.8E−04	0.03	8.2	0	1.8	85	*QRYr1A.1*	Pastor	Rosewarne et al. (2012)
Seedling	***wPt-734285***	1A	134.2	5.6E−05	0.008	10.1	0	2.3	85	*QRYr1A.1*	Pastor	Rosewarne et al. (2012)
Tel-Hadya	***wPt-734285***	1A	134.2	0.011	NS	3.5	0	14.5	85	*QRYr1A.1*	Pastor	Rosewarne et al. (2012)
Seedling	*wPt-3712*	1A	135.6	1.1E−04	0.02	9.6	0	2.1	86	*QRYr1A.1*	Pastor	Rosewarne et al. (2012)
Mean_APR	***wsnp_BG274584B_Ta_2_3***	2A	158.9	0.007	NS	7.1	B	1.8	14	*Yr1*	NA	Bansal et al. (2009)
Seedling	***wsnp_BG274584B_Ta_2_3***	2A	158.9	9.6E−04	0.05	5.2	B	2.5	14	*Yr1*	NA	Bansal et al. (2009)
Malkiyeh 2009	***wsnp_BG274584B_Ta_2_3***	2A	158.9	0.002	0.05	8.0	B	2.2	14	*Yr1*	NA	Bansal et al. (2009)
Malkiyeh 2010	***wsnp_BG274584B_Ta_2_3***	2A	158.9	0.010	NS	6.5	B	9.8	14	*Yr1*	NA	Bansal et al. (2009)
Tel-Hadya	***wsnp_BG274584B_Ta_2_3***	2A	158.9	0.011	NS	5.7	B	9.1	14	*Yr1*	NA	Bansal et al. (2009)
Malkiyeh 2009	*wPt-6271*	2B	0.2	5.7E−04	0.04	6.6	1	−1.2	144	*Yr31*	Pastor; Opata-85	Boukhatem et al. (2002)
Mean_APR	***wsnp_Ex_c14711_22788263***	2B	44.0	0.001	0.05	4.6	B	−0.9	95	*Yr31*	Pastor; Opata-85	Boukhatem et al. (2002)
Mean_APR	***wsnp_Ex_c14711_22788586***	2B	44.0	0.001	0.05	4.4	B	−0.9	84	*Yr31*	Pastor; Opata-85	Boukhatem et al. (2002)
Malkiyeh 2009	***wsnp_Ex_c14711_22788263***	2B	44.0	0.003	NS	5.9	B	−0.7	95	*Yr31*	Pastor; Opata-85	Boukhatem et al. (2002)
Malkiyeh 2010	***wsnp_Ex_c14711_22788263***	2B	44.0	1.6E−05	0.005	3.2	B	−15.4	95	*Yr31*	Pastor; Opata-85	Boukhatem et al. (2002)
Malkiyeh 2010	***wsnp_Ex_c14711_22788586***	2B	44.0	1.8E−05	0.005	4.7	B	−15.3	84	*Yr31*	Pastor; Opata-85	Boukhatem et al. (2002)
Malkiyeh 2009	***wsnp_Ex_c14711_22788586***	2B	44.0	1.8E−05	0.005	4.6	B	−1.8	84	*Yr31*	Pastor; Opata-85	Boukhatem et al. (2002)
Malkiyeh 2010	*wsnp_Ex_c163_320267*	2B	44.3	2.0E−04	0.02	4.0	B	−14.1	75	*Yr31*	Pastor; Opata-85	Boukhatem et al. (2002)
Malkiyeh 2010	*wsnp_Ex_c163_320858*	2B	44.3	2.6E−04	0.03	5.0	B	−13.9	111	*Yr31*	Pastor; Opata-85	Boukhatem et al. (2002)
Malkiyeh 2010	*wsnp_Ex_rep_c70756_69644826*	2B	45.9	9.6E−04	0.05	6.1	A	−13.1	139	*Yr31*	Pastor; Opata-85	Boukhatem et al. (2002)
Malkiyeh 2010	*wsnp_JD_c5064_6183978*	2B	48.1	8.4E−04	0.05	5.9	A	13.3	36	*Yr31*	Pastor; Opata-85	Boukhatem et al. (2002)
Seedling	*wsnp_Ex_c21092_30220342*	2B	56.2	3.7E−05	0.007	4.8	A	3.3	154	*Yr31*	Pastor; Opata-85	Boukhatem et al. (2002)
Seedling	*wsnp_Ex_c21092_30220702*	2B	56.2	4.9E−05	0.007	3.5	A	3.1	159	*Yr31*	Pastor; Opata-85	Boukhatem et al. (2002)
Malkiyeh 2010	*wsnp_JD_c744_1111659*	2B	163.4	0.001	0.05	3.6	A	8.7	32	*QRYr2B.2*	Opata-85	Boukhatem et al. (2002)
Malkiyeh 2010	*wsnp_Ex_c1758_3326792*	2B	166.2	0.002	0.05	5.9	A	−8.5	144	*QRYr2B.2*	Opata-85	Boukhatem et al. (2002)
Malkiyeh 2010	*wsnp_Ex_rep_c67411_65994109*	2B	166.2	2.3E−04	0.03	5.4	B	18.5	42	*QRYr2B.2*	Opata-85	Boukhatem et al. (2002)
Malkiyeh 2010	*wsnp_Ex_rep_c68194_66973114*	2B	166.2	2.4E−04	0.03	4.2	B	−18.5	142	*QRYr2B.2*	Opata-85	Boukhatem et al. (2002)
Malkiyeh 2010	*wsnp_Ex_rep_c68194_66973531*	2B	166.2	1.5E−04	0.02	5.3	A	18.7	42	*QRYr2B.2*	Opata-85	Boukhatem et al. (2002)
Malkiyeh 2010	*wsnp_Ex_c54998_57670603*	2B	168.0	0.001	0.05	5.0	B	−8.6	142	*QRYr2B.2*	Opata-85	Boukhatem et al. (2002)
Malkiyeh 2010	***wsnp_Ex_rep_c67561_66189356***	2B	185.1	7.4E−04	0.04	7.9	B	16.8	97	*QRYr2B.2*	Opata-85	Boukhatem et al. (2002)
Tel-Hadya	***wsnp_Ex_rep_c67561_66189356***	2B	185.1	0.003	NS	4.9	B	8.1	97	*QRYr2B.2*	Opata-85	Boukhatem et al. (2002)
Malkiyeh 2010	*wsnp_Ex_c9729_16071358*	2B	185.7	0.001	0.05	6.1	B	9.2	55	*QRYr2B.2*	Opata-85	Boukhatem et al. (2002)
Malkiyeh 2010	*wsnp_JD_c52_87219*	2B	185.7	5.4E−04	0.04	6.6	B	17.2	55	*QRYr2B.2*	Opata-85	Boukhatem et al. (2002)

Table 3 continued

Trait	Marker	Chr	Pos	*p*	FDR	R^2	Allele	Effect	Obs	Gene	Source	References
Seedling	*wsnp_CAP12_c2692_1286812*	3A	26.2	5.4E−05	0.007	3.5	A	3.1	12	–	NA	–
Seedling	*wsnp_CAP11_rep_c7339_3306558*	3A	35.9	4.3E−04	0.04	6.3	A	2.3	35	–	NA	–
Mean_APR	***wPt-4868***	3A	52.2	0.003	NS	5.4	0	−0.9	117	–	NA	–
Malkiyeh 2009	***wPt-4868***	3A	52.2	0.002	0.05	5.5	0	−1.0	117	–	NA	–
Malkiyeh 2010	***wPt-4868***	3A	52.2	0.002	0.05	5.6	0	−8.4	117	–	NA	–
Tel-Hadya	***wPt-4868***	3A	52.2	0.002	0.05	5.5	0	−15.3	117	–	NA	–
Malkiyeh 2009	***wsnp_JD_c14691_14352459***	3A	100.8	0.001	0.05	4.5	B	1.4	85	–	NA	Sela et al. (2014)
Malkiyeh 2010	***wsnp_JD_c14691_14352459***	3A	100.8	0.001	0.05	5.7	B	8.0	85	–	NA	Sela et al. (2014)
Tel-Hadya	***wsnp_JD_c14691_14352459***	3A	100.8	0.003	NS	5.5	B	15.1	85	–	NA	Sela et al. (2014)
Seedling	*wsnp_Ex_c4094_7399975*	3A	110.2	3.6E−04	0.03	5.1	A	2.2	56	–	NA	Sela et al. (2014)
Malkiyeh 2009	***wPt-800213***	3B	26.7	0.001	0.05	6.2	0	1.0	42	*Yr30*	Pastor; Opata-85; Cham-6	Börner et al. (2000)
Malkiyeh 2010	***wPt-800213***	3B	26.7	0.001	0.05	3.6	0	7.0	42	*Yr30*	Pastor; Opata-85; Cham-6	Börner et al. (2000)
Tel-Hadya	***wPt-800213***	3B	26.7	0.002	NS	3.6	0	12.5	42	*Yr30*	Pastor; Opata-85; Cham-6	Börner et al. (2000)
Mean_APR	***wPt-800213***	3B	26.7	0.002	NS	3.4	0	0.9	42	*Yr30*	Pastor; Opata-85; Cham-6	Börner et al. (2000)
Malkiyeh 2009	*wsnp_Ex_c1558_2976128*	3B	33.2	0.001	0.05	4.0	B	0.9	150	*Yr30*	Pastor; Opata-85; Cham-6	Börner et al. (2000)
Malkiyeh 2009	***wsnp_Ku_c1391_2771050***	3B	88.5	0.001	0.05	3.7	B	1.1	154	*QRYr3B.2*	Pastor	Rosewarne et al. (2012)
Malkiyeh 2010	***wsnp_Ku_c1391_2771050***	3B	88.5	0.001	0.05	3.2	B	7.0	154	*QRYr3B.2*	Pastor	Rosewarne et al. (2012)
Mean_APR	***wsnp_Ex_c210_411604***	5B	86.1	0.002	0.05	5.3	B	1.5	162	*QRYr5B.1*	NA	Rosewarne et al. (2012)
Malkiyeh 2009	***wsnp_Ex_c210_411604***	5B	86.1	9.4E−04	0.05	10.9	B	2.4	162	*QRYr5B.1*	NA	Rosewarne et al. (2012)
Malkiyeh 2009	***wsnp_Ex_c33431_41918732***	5B	86.1	0.002	0.05	3.2	B	1.5	156	*QRYr5B.1*	NA	Rosewarne et al. (2012)
Malkiyeh 2010	***wsnp_Ex_c33431_41918732***	5B	86.1	0.004	NS	4.3	B	12.2	156	*QRYr5B.1*	NA	Rosewarne et al. (2012)
Tel-Hadya	***wsnp_Ex_c210_411604***	5B	86.1	0.003	NS	5.0	B	11.6	162	*QRYr5B.1*	NA	Rosewarne et al. (2012)
Malkiyeh 2009	*wsnp_Ex_rep_c105594_89968727*	6A	131.8	0.002	0.05	5.9	B	1.4	130	*QRYr6A.1*	NA	Rosewarne et al. (2012)
Malkiyeh 2009	*wsnp_Ex_c965_1845447*	6A	138.6	6.0E−04	0.04	11.3	A	1.8	150	*QRYr6A.1*	NA	Rosewarne et al. (2012)
Malkiyeh 2009	*wsnp_Ex_c34641_42914170*	6A	138.6	6.9E−04	0.04	5.0	B	1.7	107	*QRYr6A.1*	NA	Rosewarne et al. (2012)
Malkiyeh 2009	*wPt-731936*	6A	–	9.0E−04	0.05	5.9	0	1.5	161	*QRYr6A.1*	NA	Rosewarne et al. (2012)
Mean_APR	***wPt-668026***	7D	1.1	0.004	NS	5.1	1	1.0	157	*Yr18*	Opata-85; Kauz	Singh et al. (2000)
Malkiyeh 2009	***wPt-668026***	7D	1.1	0.002	0.05	6.6	1	1.6	157	*Yr18*	Opata-85; Kauz	Singh et al. (2000)
Malkiyeh 2010	***wPt-668026***	7D	1.1	0.005	NS	4.8	1	8.2	157	*Yr18*	Opata-85; Kauz	Singh et al. (2000)
Tel-Hadya	***wPt-668026***	7D	1.1	0.003	NS	6.1	1	10.7	157	*Yr18*	Opata-85; Kauz	Singh et al. (2000)

Significant markers using both MLM and MLM-Q analyses were included and *p* values and R^2 values were obtained from the MLM-Q model

The duplicated markers were bolded while the unknown source of resistance was indicated with NA "not applicable"

Chr chromosome, *Pos* position, *Obs* the number of the observation of the allele in the germplasm

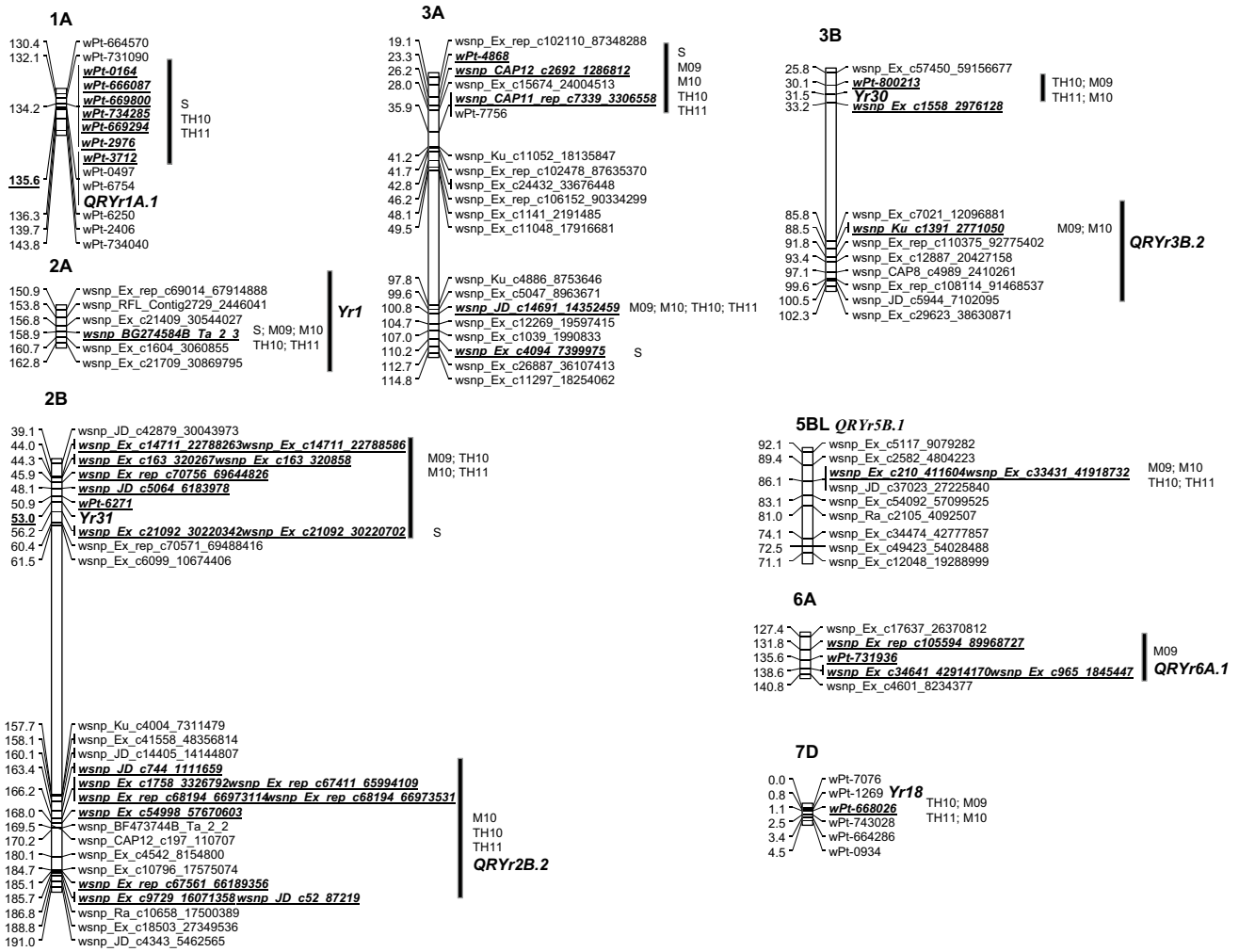

Fig. 3 Linkage map of wheat chromosomes showing DArT and SNP markers in QTL regions linked to stripe rust resistance in the GWAM panel based on wheat consensus map. Associated genes/QTL names are indicated in the groups. The locations and years are indicated next to the associated marker name

three markers and three different loci on the same chromosome (Fig. 5). The intra-chromosomal interaction between the DArT marker *wPt-731936* on 6AL and the SNP marker *wsnp_Ex_c2236_4189774* was the only interaction between two markers associated with APR to stripe rust.

For seedling resistance, three major interactions were detected by 46 different interacted pair of loci (Table 4) with a mean R^2 value of about 13.2 %. Out of the five detected seedling QTL, only the 1AL and 3AS QTL showed interactions. The 1AL QTL interaction has two overlapping markers on 7BL at 212.3 cM. The 3AS QTL interaction has three regions, 1AS, 7BS and 7DS. The seven interacting markers on 1AS for the 3AS/1AS interaction distributed between 84.9 and 85.1 cM. However, all the 7BS loci interacting with the 3AS Yr QTL at the seedling stage overlapped at 57.4 cM.

Discussion

The recent outbreak of stripe rust epidemic in many wheat producing countries in East and North Africa, Middle East and central west Asian countries poses a serious threat not only to wheat production and economic livelihoods in these countries but also has serious implications for global food security. The most viable option to abate the continuous risk of stripe rust disease is through the development and deployment of resistant wheat cultivars in stripe rust prone areas through gene pyramiding using marker assisted selection. The greenhouse and the field screening of the ICARDA GWAM germplasm panel confirmed that the genotypes possessed both seedling and adult resistance genes. Only 22.5 % of the germplasm showed resistance during the seedling stage while about 54 % exhibited resistance

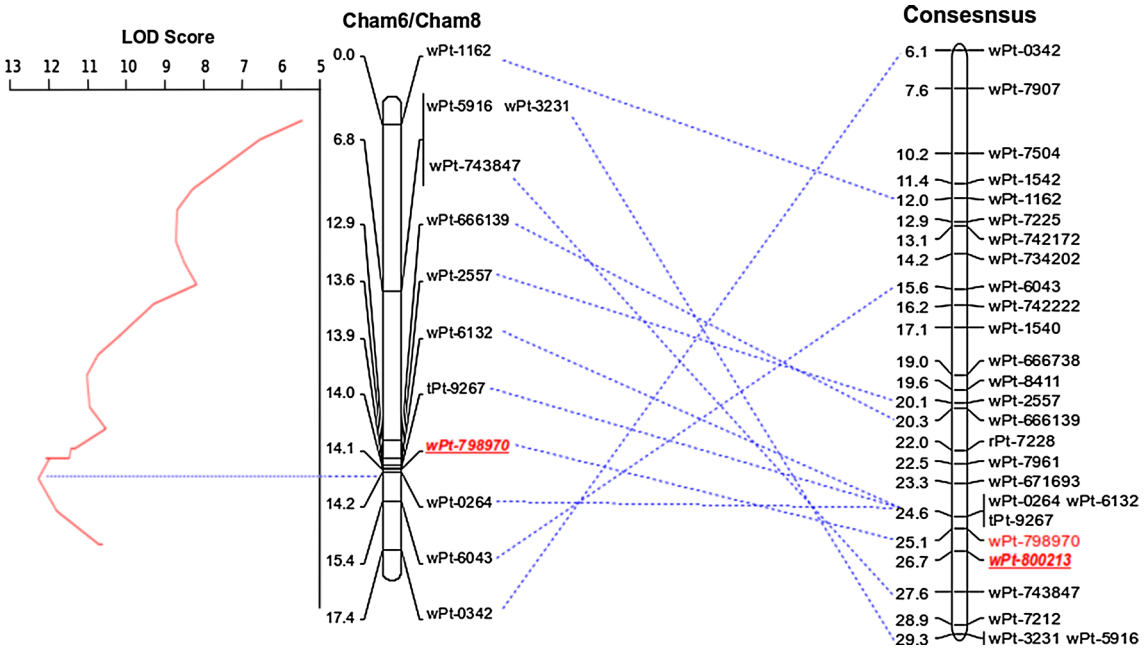

Fig. 4 The position of the 3BS QTL identified with both linkage mapping (on the *left*) and association mapping (on the *right*) approaches. Partial linkage map of chromosome 3B comparing position of the DArT marker *wPt-800213* linked to APR identified on 3BS derived from F_2:F_8 Cham-6 × Cham-8 RIL population to that identified in the GWAM panel based on the consensus DArT map (Detering et al. 2010)

Table 4 The representative QTL-whole genomic interactions for mean APR stripe rust and seedling resistances

Marker1	Chr1	Marker2	Chr2	Best R^2	Best *p*
APR Yr					
wsnp_JD_c14691_14352459	3AL	4 markers	2AL	11.43	8E−06
wPt-731936; *wsnp_Ex_c34641_42914170*	6AL	4 markers	3AS	13.63	1.6E−06
wPt-731936	6AL	11 markers	5B	14.34	7.5E−07
wsnp_Ex_c34641_42914170; wPt-731936; *wsnp_Ex_rep_c105594_89968727*	6AL	10 markers	6AL	15.55	4.3E−07
Seedling Yr					
7 markers	1AL	*wPt-8040*; *wPt-5646*	7BL	13.9	1.0E−06
wsnp_CAP11_rep_c7339_3306558; *wsnp_CAP12_c2692_1286812*	3AS	7 markers	1AS	16.4	1.4E−07
wsnp_CAP11_rep_c7339_3306558; *wsnp_CAP12_c2692_1286812*	3AS	9 markers	7BS	18.3	5.4E−08

to moderate resistance responses at the adult plant growth stage. Results obtained from the ANOVA indicated the availability of considerable variation among the GWAM germplasm panel while the correlation analysis showed significant concordance among the data generated over different environments/seasons and between replications. These results were confirmed by the high heritability estimates obtained across different environments ($H_b = 0.88$) indicating reliability of the dataset. Arguably, there are sufficient numbers of resistant genotypes in the ICARDA GWAM germplasm panel that could be used to increase the diversity of genes for stripe rust resistance in countries and regions threatened by the recent breakdown of *Yr27* resistance gene. Chen (2013) suggested combining both major and minor genes to achieve durable resistance. Most of the resistant genotypes have been sent as an international public good to the national agricultural research systems (NARs) from ICARDA and as such can be valuable for breeding stripe rust resistance wheat varieties to replace existing susceptible varieties.

To effectively utilize the stripe rust resistance present in the germplasm evaluated, genetic characterization is needed. An underlying step forward is to consider as to what would be the most effective approach to identify

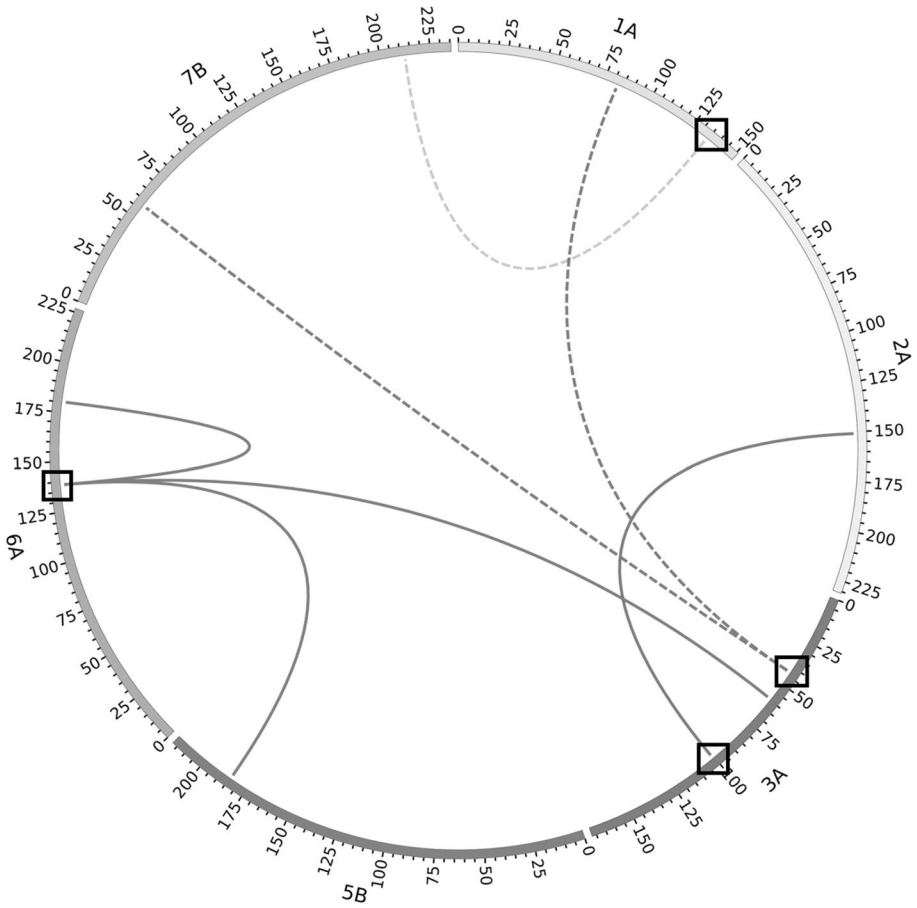

Fig. 5 The network of gene–gene interactions for the APR (*straight linkers*) and seedling (*dashed linkers*) stripe rust resistance QTL and other genomic regions. *Each curved bar* represents one chromosome and the numbers represent the chromosome positions in cM. The linkers connect the interacted QTL/genomic regions, and *squares* indicate the interacted QTL positions

stripe rust resistance in the germplasm that could facilitate effective utilisation in wheat breeding programs. Standard bi-parental crosses have been initiated using some of the potentially diverse germplasm based on varying disease reaction. However, despite the development of cost-effective, high-throughput marker systems such as DArT and SNP, QTL mapping efforts in individual bi-parental populations will not reveal, in the most efficient way, the diverse alleles present in large germplasm collections and their chromosomal locations (Roy et al. 2010). Several studies have employed GWAM to characterize many agronomic traits, disease and insect pest resistance in wheat consistent with the approach adopted in this study (Maccaferri et al. 2011, 2015; Miedaner et al. 2011; Neumann et al. 2011; Yu et al. 2011, 2012; Wang et al. 2012; Kollers et al. 2013; Mulki et al. 2013; Joukhadar et al. 2013; Sela et al. 2014; Zegeye et al. 2014). The GWAM relies mostly on historical pattern of recombination that has occurred within a collection of varieties, landraces or breeder's lines (Vinod 2011) such as the collections used for this study.

The results of GWAM can be greatly influenced by population structure (Kang et al. 2008). In the present study, most of the genotypes with similar pedigrees clustered in the same groups with some exemptions (Table S1). Despite the concordance between the population structure results and the pedigree data, the inclusion of population structure (obtained from PCA components and STRUCTURE software) as a covariate in the GWAM analysis resulted in slightly lower p values. It is apparent that the population structure did not inflate type 1 error rate, as can be seen in the QQ plots (Figure S4). Although 6 and 11 sub-populations were obtained using SNP and DArT markers, respectively, the mean Fst estimates of the subpopulations were very low: "0.23" for DArT and "0.31" for "SNP which explains the negligible effect of population structure on the GWAM germplasm panel in this study. Further, repeating the population structure analysis with Evanno method resulted in a maximum Δk value at $k = 2$. According to Evanno et al. (2005), inferring a k of $= 2$ in a GWAM panel is an indication that there is no population structure in the germplasm or the methodology failed to determine the real structure of the germplasm. With six replicates of the analysis, the resultant low Fst values from higher number of subpopulations, plus the results from PCA analysis, we believe that the population structure effect on the association mapping in this study is minimal". Our elite germplasm consists of 200 genotypes derived from 217 ancestors through 697 crosses. This artificial outcrossing and recombination

of the germplasm would create a highly diverse germplasm stock without major population subdivisions (Rostoks et al. 2006). Minimal population differentiation for $k = 2$ have also been reported in inbreeding crop such as barley (Tondelli et al. 2013).

The effectiveness of whole genome association studies for rust resistance and other traits depends on the decay of LD initially present within the mapping population at a rate determined by the genetic distance between loci and the number of generations since it arose (Mackay and Powell 2006). The results of pair wise intra- and inter-chromosomal LDs for the DArT and SNP markers are inconsistent. DArT markers showed a higher percentage of significant pairs in the inter-chromosomal LDs while SNP markers showed a higher percentage in the intra-chromosomal pairs. These discrepancies may be attributed to the multi-loci nature of some DArT clones, which resulted in significant LD between markers located on different chromosomes or between distant markers on the same chromosome. Markers on the 1BS chromosome arm showed high LD with each other (Figure S1) which could be attributed to the presence of the 1BS/1RL translocation in some of the genotypes within the GWAM germplasm panel. In this study, the second loess smoothing trend line showed that the R^2 declined to below 0.2 after 40 cM for both the DArT and SNP marker assays which also showed long-range LD, although SNP markers exhibited longer-range LD compared with DArT. These results are comparable to the earlierreport by Dreisigacker et al. (2008) who obtained an LD at 30 cM using DArT for a collection of synthetic hexaploid wheat. Similarly, Crossa et al. (2007) obtained an LD at 40 cM in historical bread wheat germplasm, but higher than that observed by Chao et al. (2007) and Joukhadar et al. (2013) at 20 cM; and Mulki et al. (2013) and Tadesse et al. (2014) at less than 10 cM.

Association study and identification of potentially novel genomic regions linked to stripe rust resistance gene

In this study, 41 markers, including 12 DArT and 29 SNP markers, were identified to be significantly linked to stripe rust resistance in the elite ICARDA wheat germplasm using the mixed linear model (MLM-Q). The MLM-Q model has the advantage of controlling both population structure and cryptic familial relatedness in addition to capturing different types of long range LD (Larsson et al. 2013). Both MLM and MLM-Q gave similar results with the later exhibiting slightly lower p values for MLM (Figure S4). The markers identified correspond to nine distinct regions on chromosome arms 1AL, 2AL, 2BS, 2BL, 3AL, 3BS, 3BL, 5BL, 6AL and 7DS, which were previously reported to harbour stripe rust resistance genes (Table 3). On the other hand, the locus on chromosome arm 3AS found to be associated with stripe rust resistance in this study the best of our knowledge, has not been previously reported. Only one QTL associated with APR was previously tagged on chromosome arm 3AS (Lillemo et al. 2008) which appears to be different from the QTL identified on 3AS in the present study which instead confer seedling resistance. It appears that the sources of resistance in the GWAM panel seem to come from Pastor, Cham-6, Seri and Ns732/Her (Table S2) This QTL on 3AS provides an opportunity to pyramid diverse seedling and adult stripe rust resistance genes into locally adapted elite germplasm to improve the stripe rust resistance in wheat.

Comparison to previously reported stripe rust resistant genes

Seedling resistance

The defeated stripe rust seedling resistance gene, *Yr27,* is located on 2BS; thus, it is most likely that the resistance gene identified on 2BS resistance in this study is a different gene. Rosewarne et al. (2013) reported that there are at least four QTL regions associated with rust resistance on 2BS including the seedling resistance *Yr31* gene (Rosewarne et al. 2012). The stripe rust QTL *QYr.Orr-2BS* linked to the marker *wPt-5738* (Vazquez et al. 2012) is about 1–2 cM away from the DArT markers, *wPt-6271,* identified in the present study. The 2BS QTL identified in this study could be traced to two wheat genotypes Opata-85 and Pastor (Table S2) which are ancestral parents of some of the genotypes in the present study and are known to carrier of *Yr31* gene (Boukhatem et al. 2002; Rosewarne et al. 2012). Haplotype analysis (Table S2) showed that Cham-6, Kauz, Seri and Croc_1/*Ae. Squarrosa* (224) may be potential sources for this QTL for stripe rust resistance. To the best of our knowledge, there is no marker reported for *Yr31* yet and Rosewarne et al. (2012) used its seedling reaction to define the presence of *Yr31* in Pastor/Avocet population. Our results suggested that the 2BS DArT marker *wPt-6271* is linked to *Yr31*. Similarly, in a recent study, Sela et al. (2014) identified two SNP markers *wsnp_Ex_c15100_23284023* and *wsnp_Ra_c27831_37346894* on chromosome arm 3AL linked to seedling resistance in a collection of wild emmer wheat. The latter SNP marker *wsnp_Ra_c27831_37346894* was located 5.4 cM away from the SNP, *wsnp_Ex_c4094_7399975* linked to seedling resistance on 3AL identified in the present study. The marker *wsnp_Ra_c27831_37346894* was in significant LD with *wsnp_Ex_c4094_7399975*, with R^2 value of 0.07 suggesting the existence of two possible stripe rust resistance genes within this region.

Two other seedling resistance QTL on 1AL and 2AL identified in the present study may be the same as the

previously reported QTL. A DArT marker *wPt-6005* was previously reported to be linked to a QTL for stripe rust resistance on chromosome arm 1AL and was attributed to one of the ancestral parents Pastor (Rosewarne et al. 2012); Pastor is also present in some of the GWAM panel used in this study. Other sources for this QTL are Croc_1/*Ae. Squarrosa* (224) and Opata-85 (Table S2). This marker is located at 135.6 cM on the DArT consensus map and is associated with stripe rust resistance in the GWAM germplasm panel in the present study at $p < 0.01$. We also identified a cluster of seven markers on 1AL that overlap within the DArT *wPt-6005* marker interval linked to stripe rust resistance. This QTL was effective in the adult stage in Tel-Hadya but not in Malkiyeh supporting the proposition that the *Pst* isolates in Malkiyeh were virulent on the seedling resistance gene in Tel-Hadya. Rosewarne et al. (2013) reported that chromosome 2A contains two regions associated with stripe rust resistance; the first lies on the short arm while the second region is on the long arm. The SNP marker *wsnp_BG274584B_Ta_2_3* linked to stripe rust resistance identified in the present study lies on the long arm of chromosome 2A, a region known to carry a seedling resistance gene *Yr1* which is located in 2AL and linked to the marker *stm673acag* (Bansal et al. 2009).

Adult resistance

Six APR QTL on 2BL, 3BS, 3BL, 5BL, 6AL and 7DS were previously reported to be linked to stripe rust resistance (reviewed in Rosewarne et al. 2013). In the present study, one DArT marker, *wPt-800213*, and one SNP *wsnp_Ex_c1558_2976128* on 3BS were found to be associated with stripe rust resistance. The DArT and SNP markers showed significant pair-wise LD ($R^2 = 0.88$) suggesting that they are associated with the same QTL. The same QTL was further validated in the Cham-6 × Cham-8 RIL population, with the resistance contributed by Cham-6 (Fig. 4). Previous studies have reported that at least two loci are involved in contributing to stripe rust resistance on 3BS (Rosewarne et al. 2013). The first cluster of QTL is located near the telomeric region of 3BS, while the second cluster is located more towards the 3BS centromere. The DArT marker *wPt-800213* in our study mapped very close, 1–2 cM, away from two SSR markers *Xgwm493* and *Xgwm533.1* which are linked to many reported QTL conferring resistance to stripe rust (Börner et al. 2000; William et al. 2006; Dedryver et al. 2009; Lowe et al. 2011). This region was reported to harbour the *Sr2*/*Lr27*/*Yr30* gene (Börner et al. 2000; Dedryver et al. 2009; Spielmeyer et al. 2005; William et al. 2006; Yang et al. 2013). *Yr30* is an interesting gene in many wheat breeding programs and is reported to work well in combination with other stripe rust resistance genes such as *Yr18* also present in some of the genotypes used in the present study (Yang et al. 2013). Some of the QTL for stripe rust resistance on the 3BS chromosome arm in this study was derived from the wheat cultivars Cham-6, Pastor and Opata-85, sources of the resistance gene *Yr30* in the present study. Haplotype analysis indicated that additional sources for this resistance within ICARDA germplasm include Croc_1/*Ae. Squarrosa* (224), Kauz, Ns732/Her and Seri (Table S2). The DArT marker *wPt-800213* should be further converted into a user-friendly co-dominant marker to be utilized in MAS for characterizing different germplasm for the presence of *Yr30* having been confirmed using two different mapping approaches. Another APR QTL that could be attributed to the ancestor Opata-85 is located on chromosome arm 2BL, *QRYr2B.2* (Boukhatem et al. 2002) while the 3BL APR QTL could be attributed to the parent Pastor (Rosewarne et al. 2012). The 3BL QTL was previously reported to be unstable across environments (Dedryver et al. 2009; Rosewarne et al. 2012) as with our study in which the QTL was detected in Malkiyeh but not in Tel-Hadya.

Four markers, including three SNPs (*wsnp_Ex_rep_c105594_89968727*, *wsnp_Ex_c965_1845447* and *wsnp_Ex_c34641_42914170*) and one DArT (*wPt-731936*) were located on the chromosome arm 6AL in our study. Results from LD analysis showed high LD between these markers (R^2 ranged between 0.27 and 0.56). The Chromosome 6A is known to harbour three stripe rust resistance QTL (Rosewarne et al. 2013) including *Yr42* on the long arm (Marais et al. 2009) and *Yr38,* which was not assigned to a specific arm (Marais et al. 2006). These QTL were located (i) at the telomere of 6AS (Lin and Chen 2009), (ii) near the centromere but on the 6AL (Lillemo et al. 2008; William et al. 2006; Rosewarne et al. 2012) and, (iii) near the telomere of 6AL (Vazquez et al. 2012). In the present study, this group of DArT and SNP markers linked to stripe rust resistance were located near the centromere on 6AL, which is in concordance with a region previously reported for stripe rust resistance QTL, *QRYr6A.2* (Lillemo et al. 2008; William et al. 2006; Rosewarne et al. 2012). Similarly, the DArT marker *wPt-731936* identified in the present study to be linked to stripe rust resistance on 6AL was earlier reportedly linked with Hessian fly resistance gene, *QHf.ugu-6AL* QTL on the 6AL chromosome arm (Hao et al. 2013), suggesting that 6AL region may likely be involved in conferring multiple-disease resistance in wheat, and the marker *wPt-731936* can be used to select simultaneously for stripe rust and Hessian fly resistances.

The DArT marker *wpt-668026* associated with stripe rust resistance was identified on chromosome arm 7DS. This region is known to carry the cloned durable and slow rusting gene which confers multiple disease resistance to various pathogens *Yr18*/*Lr34*/*Sr57*/*Pm38*/*Sb1*/*Bdv1* (Singh et al. 2000; Börner et al. 2002; Boukhatem et al.

2002; Krattinger et al. 2009; Suenaga et al. 2003; Schnurbusch et al. 2004a, b; Rosewarne et al. 2012). Similarly, *wpt-668026* is also flanked by the markers *wPt-2551* and *wPt-0366* reported to flank a stripe rust resistance QTL which various studies have linked with lowering infection type in the field (Singh et al. 2000; Ramburan et al. 2004; Lowe et al. 2011). Further, *wpt-668026* has a very high LD ($R^2 = 0.63$) with the marker *wPt-1269* which was previously reported to be associated with *Yr18* (Crossa et al. 2007). The APR locus on 7DS is from the ancestor Opata-85 and Kauz (Boukhatem et al. 2002), which are common in the pedigree of some of the germplasm used in this study. Nineteen out of the 24 genotypes carrying the resistance allele for *Yr18* have Opata-85 and Kauz in their pedigrees (Table S2).

Two significant SNPs *wsnp_Ex_c210_411604* and *wsnp_Ex_c33431_41918732* were identified on 5BL with high LD among each other in our study. The *YrExp2* gene (Lin and Chen 2008) was reported on 5BL in addition to other QTL such as *QRYr5B.1* flanked by the SSR markers *Xgwm335* and *Xgwm777* (Suenaga et al. 2003). The QTL *QRYr5B.2 was* associated with stripe rust resistance and is sandwiched within 2 cM interval between *wPt-2707* and *wPt-3030* (Bariana et al. 2010; Ren et al. 2012). Lu et al. (2009) also mapped a QTL *QRYr5B.3* (*QYr.caas-5BL.2*), with *Xgwm604* and *Xbarc142* and Crossa et al. (2007) also reported three significant DArT markers *wPt-3569*, *wPt-9467* and *wPt-9116* to be associated with stripe rust resistance on 5BL. The results from this study identified the presence of stripe resistance genes within the GWAM panel such as *Yr1*, *Yr18* and *Yr30* offering the opportunity to more effectively design targeted crosses and pyramid diverse stripe rust resistance sources into cultivated varieties.

Gene–gene interaction

Optimizing stripe rust resistance studies using MAS requires careful tracking of the epistatic interactions to avoid pyramiding unfavourable interacted alleles. Based on the epistasis analysis using the mean APR phenotype, most of the stripe rust resistance QTL seems to be neutral, and only two out of the six APR resistance QTL on chromosome arms 3AL and 6AL showed four interactions with other loci on chromosome arms 2AL, 3AS, 5B and 6AL. Additionally, two QTL for seedling stripe rust resistance also showed four different interactions (Fig. 5). For APR resistance, hotspot interactions were found on chromosome arm 6AL (Fig. 5). The stripe rust resistance QTL on chromosome arm 6AL has an intra-chromosomal interaction with other stripe rust resistance QTL. Similar intra-chromosomal interactions between QTL were previously detected for stem rust resistance on chromosome arms 3BS (*Sr2* with another QTL on the same arm) and 7DS (Yu et al. 2011). Regarding the seedling resistance, the 3AS QTL seems to be a hotspot. All the APR and seedling interactions identified in this study seem to be unique and have not been reported previously. Further studies may be needed to provide better understanding and role of these complex interactions and their contribution to stripe rust resistance gene networks.

Author contribution statement FCO designed the research; FCO, AJ and FM performed the research; AJ and BCO contributed new analytic and computational tools and analysed the data; AJ, FM, KN, OY, WT and OS contributed to the phenotyping of both seedling and adult growth stages and AJ, BCO, and FCO wrote the research paper.

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Conflict of interest The authors declare that they have no conflict of interest.

References

Andreescu C, Avendano S, Brown SR, Hassen A, Lamont SJ, Dekkers JCM (2007) Linkage disequilibrium in related breeding lines of chickens. Genetics 177:2161–2169

Bansal UK, Hayden MJ, Keller B, Wellings CR, Park RF, Bariana HS (2009) Relationship between wheat rust resistance genes *Yr1* and *Sr48* and a microsatellite marker. Plant Pathol 58:1039–1043

Bariana HS, Willey NJ, Venkata BP, Lehmensiek A, Standen GE, Lu M (2004) Breeding methodology to achieve durability for rust resistance in wheat. In: Black CK, Panozzo JF, Rebetzke GJ (eds) Proceedings of the 54th Australian cereal chemistry conference and 11th wheat breeders assembly, Canberra, pp 8–12

Bariana HS, Bansal UK, Schmidt A, Lehmensiek A, Kaur J, Miah H, Howes N, McIntyre CL (2010) Molecular mapping of adult plant stripe rust resistance in wheat and identification of pyramided QTL genotypes. Euphytica 176:251–260

Börner A, Röder MS, Unger O, Meinel A (2000) The detection and molecular mapping of a major resistance gene for non-specific adult-plant disease resistance against stripe rust (*Puccinia striiformis*) in wheat. Theor Apppl Genet 100:1095–1099

Börner A, Schumann E, Fürste A, Cöster H, Leithold B, Röder M, Weber W (2002) Mapping of quantitative trait loci determining agronomic important characters in hexaploid wheat (*Triticum aestivum* L.). Theor Appl Genet 105(6–7):921–936

Boshoff WHP, Pretorius ZA, Niekerk BDV (2002) Establishment, distribution, and pathologenicity of *Puccinia striiformis* f. sp. *tritici* in South Africa. Plant Dis 86:485–492

Boukhatem N, Baret PV, Mingeot D, Jacquemin JM (2002) Quantitative trait loci for resistance against yellow rust in two wheat derive recombinant inbred line populations. Theor Appl Genet 104:111–118

Brachi B, Morris GP, Borevitz JO (2011) Genome-wide association studies in plants: the missing heritability is in the field. Genome Biol 12:232

Bradbury JC, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES (2007) TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics 23:2633–2635

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

Cavanagh CR, Chao S, Wang S, Huang BE, Stephen S et al (2013) Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. PNAS 110(20):8057–8062

Chao S, ZhangW Dubcovsky J, Sorrells M (2007) Evaluation of genetic diversity and genome-wide linkage disequilibrium among US wheat (*Triticum aestivum* L.) germplasm representing different market classes. Crop Sci 47:1018–1030

Chen XM (2007) Challenges and solutions for stripe rust control in the United States. Aust J Agric Res 58:648–655

Chen XM (2013) Review article: high-temperature adult-plant resistance, key for sustainable control of stripe rust. Am J Plant Sci 4:608–627

Cheng P, Xu LS, Wang MN, See DR, Chen XM (2014) Molecular mapping of genes *Yr64* and *Yr65* for stripe rust resistance in hexaploid derivatives of durum wheat accessions PI 331260 and PI 480016. Theor Appl Genet. doi:10.1007/s00122-014-2378-8

Comstock RR, Robinson HF (1952) Genetic parameters, their estimation and significance. In: Proceedings of the 6th international Grassland congress, vol 1, pp 277–283

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

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

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

Distelfeld A, Uauy C, Olmos S, Schlatter AR, Dubcovsky J, Fahima T (2004) Microcolinearity between a 2-cM region encompassing the grain protein content locus *Gpc-6B1* on wheat chromosome 6B and a 350-kb region on rice chromosome 2. Funct Integr Genomics 4:59–66

Dreisigacker S, Arief V, DeLacy I, Davenport G, Manes Y, Reynolds M, Ravi S, Dieters M, Crossa J (2008) Patterns of linkage disequilibrium in multiple populations. In: Appels R, Eastwood R, Lagudah E, Langridge P, Mackay M, McIntyre L, Sharp P (eds) Proceedings of 11th international wheat genet symposium. Sydney University Press, Brisbane, pp 1–5

Emebiri LC, Ogbonnaya FC (2015) Exploring the synthetic hexaploid wheat for novel sources of tolerance to excess boron. Mol Breed 35:68 doi:10.1007/s11032-015-0273-x

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620

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

Gill BS, Sharma HC, Raupp WJ, Browder LE, Hatchett JH, Harvey TL, Moseman GJ, Waines JG (1985) Evaluation of *Aegilops* species for resistance to wheat powdery mildew, wheat leaf rust, Hessian fly and green bug. Plant Dis 69:314–316

Hao Y, Cambron SE, Chen Z, Wang Y, Bland DE, Buntin GD, Johnson JW (2013) Characterization of new loci for Hessian fly resistance in common wheat. Theor Appl Genet 126(4):1067–1076

Hedrick PW (1987) Gametic disequilibrium measures: proceed with caution. Genetics 198(117):331–374

Johanson HW, Robinson F, Comstock RE (1955) Estimate of genetic and environmental variability in soybeans. Agron J 47:314–318

Johnson R (1992) Past, present and future opportunities in breeding for disease resistance, with examples from wheat. Euphytica 63:3–22

Johnson R, Stubbs RW, Fuchs E, Chamberlain NH (1972) Nomenclature for physiologic races of *Puccinia striiformis* infecting wheat. Trans Br Mycol Soc 58:475–480

Joukhadar R, El-Bouhssini M, Jighly A, Ogbonnaya FC (2013) Genome-wide association mapping for five major pest resistances in wheat. Mol Breed. doi:10.1007/s11032-013-9924-y

Kang HM, Zaitlen NA, Wade CM, Kirby A, Heckerman D et al (2008) Efficient control of population structure in model organism association mapping. Genetics 178:1709–1723

Kollers S, Rodemann B, Ling J, Korzun V, Ebmeyer E et al (2013) Genetic architecture of resistance to *Septoria tritici* blotch (*Mycosphaerella graminicola*) in European winter wheat. Mol Breed 32(2):411–423

Kolmer JA, Singh RP, Garvin DF, Viccars L, William HM, Huerta-Espino J, Ogbonnaya FC, Raman H, Orford S, Bariana HS, Lagudah ES (2008) Analysis of the *Lr34/Yr18* rust resistance region in wheat germplasm. Crop Sci 48:1841–1852

Krattinger SG, Lagudah ES, Spielmeyer W, Singh RP, Huerta-Espino J, McFadden H, Bossolini E, Selter LL, Keller B (2009) A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. Science 323:1360–1363

Krzywinski M et al (2009) Circos: an information aesthetic for comparative genomics. Genome Res 19:1639–1645

Lagudah ES, McFadden H, Singh RP, Huerta-Espino J, Bariana HS, Spielmeyer W (2006) Molecular genetic characterization of the *Lr34/Yr18* slow rusting resistance gene region in wheat. Theor Appl Genet 114:21–30

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

Larsson H, Källman T, Gyllenstrand N, Lascoux M (2013) Distribution of long-range linkage disequilibrium and Tajima's *D* values in Scandinavian populations of Norway spruce (*Picea abies*). G3 3(5):795–806

Letta T, Olivera P, Maccaferri M, Jin Y, Ammar K, Badebo A, Salvi S, Noli E, Crossa J, Tuberosa R (2014) Association mapping reveals novel stem rust resistance loci in Durum wheat at the seedling stage. Plant Genome. doi:10.3835/plantgenome2013.08.0026

Lillemo M, Asalf B, Singh RP, Huerta-Espino J, Chen XM, He ZH, Bjornstad A (2008) The adult plant rust resistance loci *Lr34/Yr18* and *Lr46/Yr29* are important determinants of partial resistance to powdery mildew in bread wheat line Saar. Theor Appl Genet 116:1155–1166

Lin F, Chen XM (2008) Molecular mapping of genes for race-specific overall resistance to stripe rust in wheat cultivar Express. Theor Appl Genet 116:797–806

Lin F, Chen XM (2009) Quantitative trait loci for non-race-specific, high-temperature adult-plant resistance to stripe rust in wheat cultivar Express. Theor Appl Genet 118:631–642

Lowe I, Jankuloski L, Chao S et al (2011) Mapping and validation of QTL which confer partial resistance to broadly virulent

post-2000 North American races of stripe rust in hexaploid wheat. Theor Appl Genet 123:143–157

Lu YM, Lan CX, Linag SS, Zhou XC, Liu D, Zhou G, Lu QL, Jing JX, Wang MN, Xia XC, He ZH (2009) QTL mapping for adult-plant resistance to stripe rust in Italian common wheat cultivars Libellula and Strampelli. Theor Appl Genet 119:1349–1359

Lynch M, Ritland K (1999) Estimation of related with molecular markers. Genetics 152:1088–1753

Ma H, Singh RP (1996) Contribution of adult plant resistance gene *Yr18* in protecting wheat from yellow rust. Plant Dis 80:66–69

Maccaferri M, Sanguineti MC, Demontis A, El-Ahmed A, del Moral LG, Maalouf F, Nachit M, Nserallah N, Ouabbou H, Rhouma S, Royo C, Villegas D, Tuberosa R (2011) Association mapping in durum wheat grown across a broad range of water regimes. J Exp Bot 62:409–438

Maccaferri M, Zhang J, Bulli P, Abate Z, Shiaoman C, Cantu D, Bossolini E, Chen X, Pumphrey M, Dubcovsky J (2015) A genome-wide association study of resistance to stripe rust (*Puccinia striiformis* f. sp. *tritici*) in a worldwide collection of hexaploid spring wheat (*Triticum aestivvum* L.). Genes Genomes Genet (G3) 5:449–465

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

Marais GF, McCallum B, Marais AS (2006) Leaf rust and stripe rust resistance genes derived from *Aegilops sharonensis*. Euphytica 149(3):373–380

Marais GF, Marais AS, McCallum B, Pretorius Z (2009) Transfer of leaf rust and stripe rust resistance genes *Lr62* and *Yr42* from *Aegilops neglecta* Req. ex Bertol. to common wheat. Crop Sci 49:871–879

Massman J, Cooper B, Horsley R, Neate S, Dill-Macky Chao S, Dong Y, Schwarz P, Muehlbauer GJ, Smith KP (2011) Genome-wide association mapping of fusarium head blight resistance in contemporary barley breeding germplasm. Mol Breed 27:439–454

McIntosh RA, Wellings CR, Park RF (1995) Wheat rusts: an atlas of resistance genes. CSIRO Publications, Victoria

McIntosh RA, Yamazaki Y, Dubcovsky J, Rogers WJ, Morris CF, Appels R, Xia XC (2013) Catalogue of gene symbols for wheat. In: 12th international wheat genetic symposium, 8–13 September 2013, Yokohama

Miedaner T, Wurschum T, Maurer HP, Korzun V, Ebmeyer E, Reif JC (2011) Association mapping for Fusarium head blight resistance in European soft winter wheat. Mol Breed 28:647–655

Milus EA, Soyran E, McNew R (2006) Aggressive of *Puccinia striiformis* f. sp. *tritici* in eastern United States. Plant Dis 90:847–852

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

Mumtaz S, Khan IA, Ali S, Zeb B, Iqbal A, Shah Z, Swati ZA (2009) Development of RAPD based markers for wheat rust resistance gene cluster (*Lr37–Sr38–Yr17*) derived from *Triticum ventricosum* L. Afr J Biotechnol 8(7):1188–1192

Neumann K, Kobiljski B, Denčić S, Varshney RK, Börner A (2011) Genome-wide association mapping: a case study in bread wheat (*Triticum aestivum* L.). Mol Breed 1:37–58

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

Pardey PG, Beddow JM, Kriticos DJ, Hurley TM, Park RF, Duveiller E, Sutherst RW, Burdon JJ, Hodson D (2013) Right-sizing stem-rust research. Science 340(6129):147–148

Pathan AK, Park RF (2006) Evaluation of seedling and adult plant resistance to leaf rust in European wheat cultivars. Euphytica 149:327–342

Patterson N, Price A, Reich D (2006) Population structure and eigenanalysis. PLoS Genet 2:e190

Peng JH, Fahima T, Roeder MS, Huang QY, Dahan A, Li YC, Grama A, Nevo E (2000) High-density molecular map of chromosome region harboring stripe-rust resistance genes *YrH52* and *Yr15* derived from wild emmer wheat, *Triticum dicoccoides*. Genetica 109(3):199–210

Price A, Patternson N, Plenge R, Weinblatt M, Shadick N et al (2006) Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet 38:904–909

Price AL, Zaitlen NA, Reich D, Patterson N (2010) New approaches to population stratification in genome-wide association studies. Nat Rev Genet 11(7):459–463

Pritchard JK, Stephen M, Donnely P (2000) Inference on population structure using multi-locus genotype data. Genetics 155:945–959

Ramburan VP, Pretorius ZA, Louw JH, Boyd LA, Smith PH, Boshoff WHP, Prins R (2004) A genetic analysis of adult plant resistance to stripe rust in wheat cultivar Kariega. Theor Appl Genet 108:1426–1433

Rasheed A, Xia X, Ogbonnaya F, Mahmood T, Zhang Z, Mujeeb-Kazi A, He Z (2014) Genome-wide association for grain morphology in synthetic hexaploid wheats using digital imaging analysis. BMC Plant Biol 14:128

Ren Y, He ZH, Li J, Lillemo M, Wu L, Bai B, Lu QX, Zhu HZ, Zhou G, Du JY, Lu QL, Xia XC (2012) QTL mapping of adult-plant resistance to stripe rust in a population derived from common wheat cultivars Naxos and Shanghai 3/Catbird. Theor Appl Genet 125:1211–1221

Rincent R, Moreau L, Monod H, Kuhn E, Melchinger AE, Malvar RA, Moreno-Gonzalez J, Nicolas S, Madur D, Combes V, Dumas F, Altmann T, Brunel D, Ouzunova M, Flament P, Dubreuil P, Charcosset A, Mary-Huard T (2014) Recovering power in association mapping panels with variable levels of linkage disequilibrium. Genetics 197:375–387

Rosewarne GM, Singh RP, Huerta-Espino J, Herrera-Foessel SA, Forrest KL, Hayden MJ, Rebetzke GJ (2012) Analysis of leaf and stripe rust severities reveals pathotype changes and multiple minor QTL associated with resistance in an Avocet × Pastor wheat population. Theor Appl Genet 124:1283–1294

Rosewarne GM, HerreraFoessel SA, Singh RP, HuertaEspino J, Lan CX, He ZH (2013) Quantitative trait loci of stripe rust resistance in wheat. Theor Appl Genet 126(10):2427–2449

Rostoks N, Ramsay L, MacKenzie K, Cardle L, Bhat PR, Roose ML (2006) Recent history of artificial outcrossing facilitates whole-genome association mapping in elite inbred crop varieties. Proc Natl Acad Sci USA 103:18656–18661. doi:10.1073/pnas.0606133103

Roy JK, Smith KP, Muelbauer GJ, Chao S, Close TJ, Steffenson BJ (2010) Association mapping of spot blotch resistance in wild barley. Mol Breed 26:243–256

Saari EE, Prescott JM (1985) World distribution in relation to economic losses. In: Roelfs AP, Bushnell WR (eds) The cereal rusts. vol II. Diseases, distribution, epidemiology and control, pp.259–298. Academic Press, Orlanda

Schnurbusch T, Paillard S, Schori A, Messmer M, Schachermayr G, Winzeler M, Keller B (2004a) Dissection of quantitative and durable leaf rust resistance in Swiss winter wheat reveals a major resistance QTL in the *Lr34* chromosomal region. Theor Appl Genet 108:477–484

Schnurbusch T, Bossolini E, Messmer M, Keller B (2004b) Tagging and validation of a major quantitative trait locus for leaf rust resistance and leaf tip necrosis in winter wheat cultivar Forno. Phytopathology 94:1036–1041

Sela H, Ezrati S, Ben-Yehuda P, Manisterski J, Akhunov E, Dvorak J, Breiman A, Korol A (2014) Linkage disequilibrium and

association analysis of stripe rust resistance in wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*) population in Israel. Theor Appl Genet. doi:10.1007/s00122-014-2389-5

Singh RP, Nelson JC, Sorrells ME (2000) Mapping *Yr28* and other genes for resistance to stripe rust in wheat. Crop Sci 40:1148–1155

Singh D, Park RF, McIntosh RA, Bariana HS (2008) Characterisation of stem rust and stripe rust seedling resistance genes in selected wheat cultivars from the United Kingdom. J Plant Pathol 90(3):553–562

Solh M, Nazari K, Tadesse W, Wellings CR (2012) The growing threat of stripe rust worldwide Borlaug global rust initiative (BGRI) conference, Beijing

Spielmeyer W, McIntosh RA, Kolmer J, Lagudah ES (2005) Powdery mildew resistance and *Lr34*/*Yr18* genes for durable resistance to leaf and stripe rust cosegregate at a locus on the short arm of chromosome 7D of wheat. Theor Appl Genet 111:731–735

Stam P (1993) Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. Plant J 3:739–744

Stich B, Melchinger AE (2009) Comparison of mixed-model approaches for association mapping in rapeseed, potato, sugar beet, maize, and Arabidopsis. BMC Genomics 10:9

Storey JD, Tibshirani R (2003) Statistical significance for genome wide studies. Proc Natl Acad Sci USA 100:9440–9445

Suenaga K, Singh RP, Huerta-Espino J, William HM (2003) Microsatellite markers for genes *Lr34*/*Yr18* and other quantitative trait loci for leaf rust and stripe rust resistance in bread wheat. Phytopathology 93:881–890

Tadesse W, Ogbonnaya FC, Jighly A, Nazari K, Rajaram S, Baum M (2014) Association mapping of quantitative resistance to stripe rust (*Pucinia tritici*) in winter wheat cultivars and elite genotypes targeted to the CWANA region. Crop Sci 54(2):607–616

Thornsberry JM, Goodman MM, Doebley J, Kresovich S, Nielsen D, Buckler ES (2001) Dwarf8 polymorphisms associate with variation in flowering time. Nat Genet 28:286–289

Tondelli A, Xu X, Moragues M, Sharma R, Schnaithmann F, Ingvardsen C, Manninen O, Comadran J, Russell J, Waugh R, Schulman AH, Pillen K, Rasmussen SK, Kilian B, Cattivelli L, Thomas WTB, Flavell AJ (2013) Structural and temporal variation in genetic diversity of European spring two-row barley cultivars and association mapping of quantitative traits. Plant Genome 6:1–14. doi:10.3835/plantgenome2013.03.0007

Van Ooijen JW (2006) JoinMap 4, software for the calculation of genetic linkage maps in experimental populations. In: Kyazma BV (ed), Wageningen

Van Ooijen JW (2009) MapQTL 6, software for the mapping of quantitative trait loci in experimental populations of diploid species. In: Kyazma BV (ed), Wageningen

Vazquez MD, Peterson CJ, Riera-Lizarazu O, Chen X, Heesacker A, Ammar K, Crossa J, Mundt CC (2012) Genetic analysis of adult plant, quantitative resistance to stripe rust in wheat cultivar 'Stephens' in multi-environment trials. Theor Appl Genet 124:1–11

Vinod KK (2011) Association mapping in crop plants. In: Advanced faculty training on "impact of genomics in crop improvement: perceived and achieved", 20 January 2011–9 February 2011. Centre for Advanced Faculty Training in Genetics and Plant Breeding, Tamil Nadu Agricultural University, Coimbatore

Wang H, Smith KP, Combs E, Blake T, Horsley RD, Muehlbaue GJ (2012) Effect of population size and unbalanced data sets on QTL detection using genome-wide association mapping in barley breeding germplasm. Theor Appl Genet 124(1):111–124

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

Wellings CR, Wright DG, Keiper F, Loughman R (2003) First detection of wheat stripe rust in Western Australia: evidence for a foreign incursion. Australas Plant Pathol 32:321–322

William HM, Singh RP, Huerta-Espino J, Palacios G, Suenaga K (2006) Characterization of genetic loci conferring adult plant resistance to leaf rust and stripe rust in spring wheat. Genome 49:977–990

Yang WY, Yu Y, Zhang Y, Hu XR, Wang Y, Zhou YC, Lu BR (2003) Inheritance and expression of stripe rust resistance in common wheat (*Triticum aestivum*) transferred from *Aegilops tauschii* and its utilization. Hereditas 139:49–55

Yang EN, Rosewarne GM, Herrera-Foessel SA, Huerta-Espino J, Tang ZX, Sun CF, Ren ZL, Singh RP (2013) QTL analysis of the spring wheat "Chapio" identifies stable stripe rust resistance despite inter-continental genotype × environment interactions. Theor Appl Genet 126(7):1721–1732

Yu J, Pressoir G, Briggs WH, Vroh I, Yamasaki M, Doebley JF, McMullen MD, Gaut BS, Nielsen DM, Holland JB, Kresovich S, Buckler ES (2006) A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nat Genet 38:203–208

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

Yu LX, Morgounov A, Wanyera R, Keser M, Singh SK, Sorrells ME (2012) Identification of Ug99 stem rust resistance loci in winter wheat germplasm using genome-wide association analysis. Theor Appl Genet 125(4):749–758

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

Zhao K, Aranzana MJ, Kim S, Lister C, Shindo C et al (2007) An Arabidopsis example of association mapping in structured samples. PLoS Genet 3:e4

Zhou XL, Han DJ, Chen XM, Gou HL, Guo SJ, Rong L, Wang QL, Huang LL, Kang ZS (2014) Characterization and molecular mapping of stripe rust resistance gene *Yr61* in winter wheat cultivar Pindong 34. Theor Appl Genet. doi:10.1007/s00122-014-2381-0