Theor Appl Genet (2018) 131:127–144
https://doi.org/10.1007/s00122-017-2990-5

ORIGINAL ARTICLE

Unlocking new alleles for leaf rust resistance in the Vavilov wheat collection

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Received: 4 July 2017 / Accepted: 21 September 2017 / Published online: 4 October 2017

Abstract

Key message **Thirteen potentially new leaf rust resistance loci were identified in a Vavilov wheat diversity panel. We demonstrated the potential of allele stacking to strengthen resistance against this important pathogen.**

Abstract Leaf rust (LR) caused by *Puccinia triticina* is an important disease of wheat (*Triticum aestivum* L.), and the deployment of genetically resistant cultivars is the most viable strategy to minimise yield losses. In this study, we evaluated a diversity panel of 295 bread wheat accessions from the N. I. Vavilov Institute of Plant Genetic Resources (St Petersburg, Russia) for LR resistance and performed genome-wide association studies (GWAS) using 10,748 polymorphic DArT-seq markers. The diversity panel was evaluated at seedling and adult plant growth stages using three *P. triticina* pathotypes prevalent in Australia. GWAS was applied to 11 phenotypic data sets which identified a total of 52 significant marker–trait associations representing 31 quantitative trait loci (QTL). Among them, 29 QTL were associated with adult plant resistance (APR). Of the 31 QTL, 13 were considered potentially new loci, whereas 4 co-located with previously catalogued *Lr* genes and 14 aligned to regions reported in other GWAS and genomic prediction studies. One seedling LR resistance QTL located on chromosome 3A showed pronounced levels of linkage disequilibrium among markers ($r^2 = 0.7$), suggested a high allelic fixation. Subsequent haplotype analysis for this region found seven haplotype variants, of which two were strongly associated with LR resistance at seedling stage. Similarly, analysis of an APR QTL on chromosome 7B revealed 22 variants, of which 4 were associated with resistance at the adult plant stage. Furthermore, most of the tested lines in the diversity panel carried 10 or more combined resistance-associated marker alleles, highlighting the potential of allele stacking for long-lasting resistance.

Communicated by Steven S. Xu.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-017-2990-5) contains supplementary material, which is available to authorized users.

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Introduction

Wheat (*Triticum aestivum* L.) is a major source of calories and protein in the human diet (Shewry and Hey 2015). However, the current global production is insufficient to meet the demand of a rapidly growing world population (Grassini et al. 2013). At the same time, wheat yields are consistently threatened by increasing climatic variations (Asseng et al. 2015) and rapidly evolving pests and pathogens (Chaves et al. 2013). Leaf rust (LR) caused by *Puccinia triticina* Eriks., is one of the most common and geographically widespread wheat diseases worldwide. LR causes more annual yield losses globally compared to losses attributed to stem and stripe rust (Bolton et al. 2008; Huerta-Espino et al. 2011). Among various disease management strategies, the cultivation of resistant wheat cultivars is the most effective and environment-friendly strategy (Kolmer et al. 2013).

Genetic resistance against LR is broadly categorised into seedling or all-stage resistance and adult plant resistance (APR). To date, 77 genes confering resistance to LR (*Lr*) have been successfully characterised, of which the majority confer seedling resistance (McIntosh et al. 2017). Typically, seedling resistance is controlled by a single gene with major effect that interacts with the pathogen in a 'gene-for-gene' relationship (Flor 1971). Usually, the seedling genes are pathogen race-specific and confer a hypersensitive response (HR)—a cell death phenomenon preventing the pathogen spread (Ellis et al. 2014; Mondal et al. 2016). This exerts intense selective pressure on the pathogen population, thus quickly rendering the deployed resistance gene ineffective (Burdon et al. 2014; Li et al. 2014; Niks et al. 2015). In contrast, APR is usually effective at the post-seedling growth stages, is either controlled by multiple genes each with minor effect or a single gene with major effect. Some APR genes provide partial resistance that is effective against all races of a given pathogen species (i.e. race-nonspecific) (Lagudah 2011; McCallum et al. 2012; Burdon et al. 2014). Mostly APR genes interact additively and enhance resistance to a level of immunity (Singh et al. 2014). Some APR genes confer pleiotropic resistance against multiple diseases, for instance *Lr34*, *Lr46*, and *Lr67* provide partial resistance to LR, stripe rust, stem rust, and powdery mildew diseases of wheat (Lagudah 2011; Risk et al. 2012; Ellis et al. 2014).

To date, six *Lr* genes (including seedling and APR) have been cloned: *Lr1* (Cloutier et al. 2007), *Lr10* (Feuillet et al. 2003), *Lr21* (Huang et al. 2003), *Lr22a* (Thind et al. 2017), *Lr34* (Krattinger et al. 2009), and *Lr67* (Moore et al. 2015). This has enabled the development of gene-specific molecular markers for rapid gene identification via marker-assisted selection (MAS). Markers further assist in pyramiding of 4–5 APR or seedling resistance genes or in combinations to generate durable rust resistant wheat cultivars (Ellis et al. 2014; Singh et al. 2014). To maintain and/or broaden the genetic diversity of durable rust resistance, the identification of novel genetic sources of resistance is required. One approach for the genetic enrichment of elite breeding pools is to exploit landraces by introducing genetic diversity from germplasm collections (Lopes et al. 2015; Sehgal et al. 2015; Kumar et al. 2016). More than 850,000 wheat accessions are stored in gene banks, representing a rich genetic resource to reinstate the variation of genetic bottlenecks (e.g. from domestication or selective breeding). Many of these accessions are already adapted to very specific target environments, possessing exclusive advantageous characteristics, such as resistances towards specific biotic and abiotic stresses (Mitrofanova 2012; Huang and Han 2014; Lopes et al. 2015), including resistance to rust diseases (Cavanagh et al. 2013; Lopes et al. 2015; Rinaldo et al. 2016; Vikram et al. 2016).

For instance, the *Lr* genes *Lr52* and *Lr67* (Hiebert et al. 2010; Bansal et al. 2013), and the stripe rust gene *Yr47* (Bansal et al. 2011) were identified in wheat landraces from the Watkins collection. Another historical yet relatively unexploited diverse wheat collection is the "N. I. Vavilov Institute of Plant Genetic Resources" (VIR) in St Petersburg, Russia, collected by the Russian botanist and geneticist N. I. Vavilov and his colleagues in the early 1900s. Recent studies have reported a large variety of novel alleles in the VIR wheat collection, revealing the promising basis for the genetic improvement of resistances to various biotic and abiotic stresses (Mitrofanova 2012; Sadovaya et al. 2015; Riaz et al. 2016a; Riaz et al. 2017). However, determining the genomic regions underpinning these resistances is challenging.

Traditionally, quantitative trait loci (QTL) mapping is used to identify underlying genetic variations that co-segregate with a trait of interest using a bi-parental mapping population (Koornneef et al. 2004; Zhu et al. 2008). Although traditional QTL mapping is successful, it is fundamentally limited to the comparative low allelic diversity of the two crossing parents and low recombination events which impair the mapping resolution (Zhu et al. 2008). Alternatively, genome-wide association studies (GWAS) represent a powerful tool to dissect the genetic architecture of complex traits in natural populations, such as germplasm collections (Zhu et al. 2008; Hall et al. 2010), by detecting genomic regions that are in linkage disequilibrium (LD) with genes affecting the trait of interest. Due to a greater number of historical chromosomal recombinations accumulated over a large number of generations in natural populations, GWAS can position QTL at a much higher resolution (Yu and Buckler 2006; Semagn et al. 2010).

Here, we present a large-scale association study for seedling and APR to LR under controlled and field conditions in a highly diverse panel of 295 bread wheat lines from the VIR. Using high-density Diversity Arrays Technology

(DArT-seq) markers and multi-year phenotypic data sets, we were able to map previously undescribed QTL for resistance against three major *P. triticina* pathotypes that are prevalent in Australia. We anticipate that this study provides breeders with a rich basis for the improvement of durable LR resistances in future wheat cultivars. Ongoing work based on these findings will help to functionally validate the significance of candidate genes in the identified novel genomic regions.

Materials and methods

Plant materials and genotyping

A diversity panel of 295 homozygous single seed descent (SSD) bread wheat lines from VIR, representing species-wide genetic diversity (Riaz et al. 2017), was selected for the assessment of LR response. DNA of each wheat line was extracted following the protocol recommended by Diversity Arrays Technology (DArT) (http://www.diversityarray.com) and the whole panel was genotyped with the DArT genotyping-by-sequencing (GBS) platform using the DArT-seq wheat *PstI* complexity reduction method, as described by Li et al. (2015), which returned a total of 56,306 raw DArT-seq markers. The DArT-seq markers are presence–absence dominant markers extracted in silico from sequences obtained from genomic representations. The raw marker data was filtered to retain only markers with ≤ 10% missing values, a minor allele frequency ≥ 3% and lines with ≤ 20% missing values, resulting in a selection of 10,748 high-quality, polymorphic DArT-seq markers for the subsequent genetic analyses. All used DArT-seq markers were ordered according to their genetic positions in a high-resolution DArT-seq consensus map (version 4.0), provided by Dr. Andrzej Kilian (Diversity Arrays Technology Pty Ltd, Canberra, Australia).

As described by Riaz et al. (2016a), the diversity panel was also screened for the polymerase chain reaction (PCR)-based markers *cssfr5* (Lagudah et al. 2009), *csLV46* (Lagudah, unpublished data) and SNP1-TM4 (Moore et al. 2015), which facilitated identification of the known LR APR genes *Lr34*, *Lr46*, and *Lr67*, respectively.

Evaluation of leaf rust resistance

For the resistance screening, we used the three *P. triticina* pathotypes (*pt*), namely *pt* 104-1,2,3,(6),(7),11,13, *pt* 76-1,3,5,7,9,10,12,13 + *Lr37* and *pt* 104-1,3,4,6,7,8,10,12 + *Lr37* (Table 1), which are prevalent in the eastern and western wheat growing regions of Australia (Park 2016). A summary of the experiments performed in this study at the seedling and adult plant stage for scoring LR response across years and pathotypes is presented in Table 2.

Integrated seedling and adult plant phenotyping

The 295 SSD lines in the diversity panel were evaluated using the integrated seedling and adult plant phenotyping method under controlled conditions, as described by Riaz et al. (2016a). For seedling infection, the *P. triticina* pathotype *pt* 104-1,2,3,(6),(7),11,13 was used (Table 2). Briefly, the diversity panel was sown in a standard glasshouse with diurnal temperatures (i.e. 22/17 °C day/night) and 12 h photoperiod. At the two-leaf stage, plants were inoculated using a *P. triticina* urediniospore suspension (Riaz et al. 2016a, b). Twelve days post-inoculation, seedlings were scored using the 0–4 Stakman scale (Stakman et al. 1962). Afterwards, plants were transferred to a temperature-controlled growth facility where the plants were subjected to "speed breeding" or "accelerated growth conditions" (AGC) by adopting a 12-h cycling temperature (22/17 °C) and 24 h photoperiod, which helps the plants to attain the adult plant stage rapidly (Riaz et al. 2016b; Watson et al. 2017). After 2 weeks, plants were re-inoculated using the same *P. triticina* pathotype 104–1,2,3,(6),(7),11,13 (Table 2). Twelve days post-inoculation, LR response was recorded for the flag-2 leaf using the 0–4 Stakman scale (Stakman et al. 1962). This provides representative measures of the adult plant response to LR, which are similar to field-based measures (Riaz et al. 2016b). The LR response in this experiment was converted

Table 1 Virulence and avirulence profiles of *P. triticina* pathotypes used in this study

Leaf rust pathotype[a]	Virulent on genes	Avirulent on genes
104–1,2,3,(6),(7),11,13	*Lr1, Lr3a, Lr14a, Lr16, Lr17a*[b], *Lr20, Lr24, Lr27 + 31*[b]	*Lr2a, Lr3ka, Lr13, Lr15, Lr17b, Lr23, Lr26, Lr28, Lr37*
76–1,3,5,7,9,10,12,13 + *Lr37*	*Lr3a, Lr3ka, Lr13, Lr14a, Lr16, Lr17a, Lr17b, Lr20, Lr24, Lr26, Lr27 + 31, Lr37*	*Lr1, Lr2a, Lr15, Lr23, Lr28*
104–1,3,4,6,7,8,10,12 + *Lr37*	*Lr1, Lr3a, Lr12, Lr13, Lr14a, Lr15, Lr17a, Lr17b, Lr20, Lr27 + 31, Lr28, Lr37*	*Lr2a, Lr3ka, Lr16, Lr23, Lr24, Lr26*

[a]The virulence/avirulence status of the leaf rust pathotype was reported by Park (2016)

[b]Pathotype is partially virulent on the gene

Table 2 Summary of experiments performed in this study at the seedling and adult stage for scoring leaf rust response across years and pathotypes used

Growth stage	Environment	Year tested	Phenotypic data sets	Number of lines assessed (*n*)	Leaf rust pathotypes
Seedling	Glasshouse	2014	Seedling	288	104–1,2,3,(6),(7),11,13
Adult	AGC[a]	2014	AGC	288	104–1,2,3,(6),(7),11,13
	Field	2014[b]	Field_2014_1 Field_2014_2 Field_2014_3 Field_2014_4	284	104–1,2,3,(6),(7),11,13
		2015[b]	Field_2015_1 Field_2015_2 Field_2015_3	288	104–1,2,3,(6),(7),11,13; 76–1,3,5,7,9,10,12,13 + *Lr37*
		2016[b]	Field_2016_1 Field_2016_2	261	104–1,2,3,(6),(7),11,13; 76–1,3,5,7,9,10,12,13 + *Lr37*; 104–1,3,4,6,7,8,10,12 + *Lr37*

[a]Accelerated growth conditions

[b]Multiple phenotypic data sets were recorded in each of the field environment

from the 0–4 to 0–9 scale to standardise data sets across all experiments (Ziems et al. 2014; Riaz et al. 2016b) and for subsequent GWAS analyses. Lines that depicted a LR response < 7 on the 0–9 scale were considered resistant.

Field trials

The SSD lines in the diversity panel were subjected to LR screening in the field over a 3-year period (2014, 2015, and 2016) at the Redlands Research Facility (27°31′40.8″S 153°15′05.7″E), Queensland, Australia, as detailed by Riaz et al. (2016b). Six seeds of each SSD line were sown as un-replicated hill plots, whereas four standards with known disease responses (i.e. Thatcher, Avocet, Avocet + *Lr34*, and Avocet + *Lr46*) were replicated five times throughout the test material to detect spatial variation in the nursery. About 5 weeks after sowing, the LR epidemic was initiated by transplanting rust-infected wheat (Morocco) seedlings into the field among the spreader rows. When the disease was sufficiently established on susceptible standards [i.e. Thatcher was scored 20 moderately susceptible to susceptible (MSS) in the field in 2014, 9 in the field in 2015, and 8 in the field in 2016], all SSD lines were assessed for LR response.

In 2014, the diversity panel was assessed for disease response in the LR nursery inoculated with *P. triticina* pathotype 104–1,2,3,(6),(7),11,13 (Table 2). The disease response for each line was assessed on a whole plot basis using the modified Cobb scale (Peterson et al. 1948). The disease severity data and IT were used to calculate the coefficient of infection (CI), as reported by Loegering (1959). Disease scoring was conducted at 70, 77, 86, and 96 days after sowing (DAS). Therefore, these multiple phenotypic data sets represent different time-points during the epidemic development in the nursery. The CI values of each disease score were then divided by 10 to convert to 0–9 scale. The converted scores were used to visualise the density distribution of LR response across phenotypic data sets.

In 2015, the LR nursery was inoculated with a mixture of two *P. triticina* pathotypes, namely *pt* 104-1,2,3,(6),(7),11,13 and *pt* 76-1,3,5,7,9,10,12,13 + *Lr37* (Table 2). Plants were assessed on a whole plot basis for disease response three times during the season (i.e. 78, 85, and 101 DAS) using the 1–9 scale where 1 = very resistant and 9 = very susceptible, as reported by Bariana et al. (2007). In 2016, the LR nursery was inoculated with a mixture of three *P. triticina* pathotypes, namely *pt* 104-1,2,3,(6),(7),11,13, *pt* 76-1,3,5,7,9,10,12,13 + *Lr37* and *pt* 104-1,3,4,6,7,8,10,12 + *Lr37* (Table 2). Therefore, the 2016 nursery comprised the most virulent composition of *P. triticina* pathotypes compared to nurseries conducted in 2014 and 2015. Plants were evaluated for disease response twice (i.e. 71 and 84 DAS) using the 1–9 scale, as detailed above.

In all the field trials, a threshold for 'resistance' to LR was determined as any line depicting a disease response ≤ 5 based on the 1–9 scale, where resistance was deemed as "moderately resistant to moderately susceptible" (MRMS) or better. Each disease reading within a field environment was regarded as a unique phenotypic data set and subsequently used for GWAS. The field phenotypic data sets were referred as Field_2014_1, Field_2014_2, Field_2014_3, Field_2014_4, Field_2015_1, Field_2015_2, Field_2015_3, Field_2016_1, and Field_2016_2.

Population structure, genetic diversity, and linkage disequilibrium

The population structure and genetic diversity for the diversity panel were previously described by Riaz et al. (2017). Briefly, population structure was estimated using the partitioning around medoids clustering algorithm and 'Jaccard distance' in R (Team 2014). The optimal number of clusters (i.e. $k = 2$) was determined using the 'fpc' package (Hennig 2014). Pairwise LD between markers were measured as r^2 (Hao et al. 2007). LD decay, the relationship between LD and genetic map distance between marker pairs in cM, was estimated as a locally estimated scatterplot smoothing (LOESS) curve and the LD cut-off threshold was set at $r^2 = 0.1$. The LD decay for the A, B, and D genomes was estimated for the whole population and the previously described clusters.

Genome-wide association analysis, allele stacking, and haplotype construction

Genome-wide marker-trait associations were calculated for data from a total of 11 phenotypic data sets (seedling, AGC and the field trials), using the R package GenABEL (Aulchenko et al. 2007). The applied mixed linear model was adjusted for population stratification by including identity-by-state estimates (kinship matrix) for genotype pairs and a principal component adjustment that uses the first four principal components as covariates. The significant cut-off value was arbitrarily set at $-\log_{10}(P) = 3.5$. Overlapping significant markers for different environments that were located at the same chromosomal position within a 5 cM window were considered the same QTL. Based on the predicted direction of the allele effect on the resistance score (e.g. negative effect $\triangleq$ resistance allele) (Table 3), we assigned resistance alleles for each significant marker.

To investigate an effect of accumulated alleles for LR resistance at the independent loci on the disease score, we assigned the lines to groups, based on the absolute number of resistance-associated alleles possessed and compared their relative disease indices that were calculated as

$$\mathrm{LRi} = \sum_{k}^{n} \frac{\text{individual Dis. score}[k]}{\text{mean Dis. score}[k]},$$

where the disease index LRi is the accumulated relative value of a line's disease score in experiment k in relation to the population mean in this experiment k over all n field experiments. Lines with high indices (above 0) are relatively more susceptible to LR infection than lines with indices below 0.

Two QTL were selected for haplotype analysis and subsequent network analysis: (1) a seedling QTL on chromosome 3A (*qNV.Lr-3A.3*) because it was deemed a new QTL with large effect, and (2) a QTL conferring APR (*qNV.Lr-7B.2*) on the long arm of chromosome 7B which was detected across many phenotypic data sets and reported by numerous previous studies. Haplotypes for seedling resistance and APR were constructed on the basis of LD around the respective identified QTL on chromosomes 3A (*qNV.Lr-3A.3*) and 7B (*qNV.Lr-7B.2*). All surrounding markers with pairwise r^2 values > 0.8 were included in the haplotype analysis, resulting in 7 and 22 haplotype variants, respectively. Haplotype networks, showing TCS genealogies between haplotype variants (Clement et al. 2000), were calculated using PopART (http://popart.otago.ac.nz.) (Leigh and Bryant 2015). The network nodes were coloured according to the average disease rating in the respective haplotype groups. A Tukey's test was performed to test for significant phenotypic differences between the haplotype groups. The origin information for lines within each haplo-group was used to visualise the geographic distribution of these haplotypes in the diversity panel.

Alignment of QTL identified in this study with previously reported *Lr* genes and QTL

For comparison, QTL identified in this study and already catalogued *Lr* genes (McIntosh et al. 2017) were projected onto the common integrated map developed by Maccaferri et al. (2015) using MapChart software version 2.3 (Voorrips 2002). A QTL was considered potentially new if the genetic distance was ≥ 5 cM of the reported *Lr* gene or QTL. Eight recent GWAS studies (Kertho et al. 2015; Jordan et al. 2015; Gao et al. 2016; Li et al. 2016; Aoun et al. 2016; Pasam et al. 2017; Turner et al. 2017; Kankwatsa et al. 2017) and two genomic prediction studies (Daetwyler et al. 2014; Juliana et al. 2017) using high-throughput marker platforms were considered for QTL comparison.

In silico annotation of significant markers

The genomic regions identified in this study were subjected to homology search for syntenic regions in *Brachypodium distachyon* and rice (*Oryza sativa* L.) genome. The marker sequences were annotated against the protein sequences to determine putative molecular functions, which could lead to the possible identification of candidates for disease resistance across species. The homology search was performed using EnsemblPlants; http://plants.ensembl.org/index.html (Kersey et al. 2016).

Table 3 Summary of the leaf rust resistance quantitative trait loci identified at both the seedling and adult plant stage in the diversity panel

QTL name	Chr.	Position (cM)[a]	Marker	Phenotypic data sets[b]	$-\log_{10}$ (p value)	Allele for resistance[c]	Effect on trait	Resistance growth stage	Co-located with *Lr* gene	Gene annotation
qNV.Lr-1A	1A	146.3	1133392	Field_2015_1	3.58	1	-0.73	Adult	[g]	–
qNV.Lr-1B.1	1B	51.3	1219818	Field_2015_2	3.66	0	-0.89	Adult	[g]	–
qNV.Lr-1B.2	1B	269.3	2276699	Field_2016_2	3.73	1	-0.61	Adult	[f]	–
qNV.Lr-2A.1	2A	67.8	1164339	Field_2015_1	3.56	1	-0.63	Adult	[f]	–
qNV.Lr-2A.2	2A	115.8	1242099	Field_2014_4	3.54	1	-7.97	Adult	[g]	–
qNV.Lr-2A.3	2A	123.6	1687763	Field_2015_3	6.28	1	-1.22	Adult	[g]	–
qNV.Lr-2B.1	2B	76.6	1232931	Field_2016_1	3.52	1	0.59	Adult	[f]	P-loop_NTPase
qNV.Lr-2B.2	2B	86.2	1140050	Field_2016_2	3.51	1	0.47	Adult	[g]	–
qNV.Lr-2B.3	2B	107.0	1092839	Seedling	4.02	1	-0.78	All stage	[f]	–
			1126885	Seedling	4.00	1	-0.76			NB-ARC, P-loop_NTPase
			2290750	Seedling	3.92	1	-0.80			Zinc finger, CCHC-type
			1109454	Seedling	3.81	1	-0.75			–
			1686496	AGC	4.05	1	-0.61			RNA-dependent DNA polymerase
			1095829	AGC	3.68	1	-1.10			–
			1112316	Field_2015_3	4.16	1	-0.81			–
qNV.Lr-3A.1	3A	47.7	1126760	Field_2015_3	3.62	1	-0.52	Adult	[f]	–
qNV.Lr-3A.2	3A	109.5	1116501	Field_2014_1	3.88	1	3.23	Adult	[g]	–
qNV.Lr-3A.3	3A	116.7	1254900	Seedling	4.10	1	-1.23	Seedling	[g]	–
		117.0	3941106	Seedling	6.26	1	-1.36			–
qNV.Lr-3B.1	3B	5.9	1203924	Field_2014_1	4.52	1	2.31	Adult	[g]	Domain of unknown function DUF1618
qNV.Lr-3B.2	3B	49.2	1101980	Field_2016_1	4.23	1	0.56	Adult	[f]	–
		53.6	1107825	Field_2014_2	3.98	1	-3.31			–
qNV.Lr-3B.3	3B	60.4	1696461	AGC	3.65	0	-1.07	Adult	[f]	LRR, protein kinase-like domain
qNV.Lr-3B.4	3B	94.1	1127641	Field_2015_3	4.51	1	-0.92	Adult	[f]	–
qNV.Lr-4A	4A	133.9	1266809	Field_2014_4	5.95	1	11.04	Adult	[f]	–
		135.1	1122735	Field_2014_4	4.15	1	9.40			–
qNV.Lr-5A	5A	112.9	1118643	AGC	3.55	1	-0.93	Adult	[f]	–
qNV.Lr-5B.1	5B	8.5	1139539	AGC	3.69	1	-1.16	Adult	[f]	–
qNV.Lr-5B.2	5B	37.9	1085450	Field_2015_1	3.56	1	-0.53	Adult	[g]	–
qNV.Lr-5B.3	5B	136.4	1106570	AGC	4.24	1	0.64	Adult	[g]	Glycosyl transferase, family 31, galectin, carbohydrate recognition domain
qNV.Lr-6A.1	6A	9.4	1138518	Field_2014_4	4.02	1	0.33	Adult	[f]	Cyclin-like F-box
				Field_2014_3	4.18	1	5.06			
qNV.Lr-6A.2	6A	27.5	1056198	Field_2015_3	4.05	0	-0.55	Adult	[f]	–
		27.8	1242792	Field_2015_3	4.15	1	-0.96			–
		28.0	1308654	Field_2015_3	5.09	1	-1.02			Glycosyl transferase family 29

Table 3 (continued)

QTL name	Chr.	Position (cM)[a]	Marker	Phenotypic data sets[b]	$-\log_{10}$ (p value)	Allele for resistance[c]	Effect on trait	Resistance growth stage	Co-located with *Lr* gene	Gene annotation
qNV.Lr-6A.3	6A	83.0	3950708	Field_2016_2	3.50	1	0.41	Adult	*Lr64*	–
qNV.Lr-6B.1	6B	18.9	1228013	Field_2014_2	3.54	1	2.59	Adult	[g]	–
qNV.Lr-6B.2	6B	79.7	2341955	AGC	3.55	1	-1.61	Adult	*Lr3*	LRR, NB-ARC, P-loop_NTPase, zinc finger
qNV.Lr-7A.1	7A	21.0	3021391	Field_2016_2	3.88	0	-0.43	Adult	[g]	P-loop_NTPase, Ran GTPase, small GTP-binding protein
qNV.Lr-7A.2	7A	40.3	3384641	AGC	4.18	0	0.64	Adult	[f]	–
qNV.Lr-7B.1	7B	67.3	1119801	AGC	4.02	1	-0.69	Adult	[g]	–
qNV.Lr-7B.2	7B	126.0	1140798	Field_2015_2	4.17	1	-0.88	Adult	*Lr14b, Lr68, LrBi16, LrFun*	–
		128.6	2303264	Field_2015_2	3.77	1	-0.82			–
			1073236	Field_2015_2	3.55	1	-0.82			–
		129.9	1207290	Field_2015_2	3.80	1	-0.83			–
		130.6	1048655	Field_2015_1	4.24	1	-0.85			–
			1200909	Field_2015_1	3.90	1	-0.87			–
				Field_2015_2	3.53	1	-0.81			
			1079125	Field_2015_2	4.63	1	-0.93			–
			1117456	Field_2015_3	4.91	1	-0.91			–
				Field_2015_2	4.90	1	-0.93			
			1214960	Field_2015_1	5.23	1	-0.96			–
				Field_2015_2	3.93	1	-0.83			
			1134022	Field_2015_2	4.07	1	-0.89			–
			2304335	Field_2015_2	3.95	1	-0.83			–
qNV.Lr-7D	7D	56.6	*cssfr5*[d]	Field_2015_3	6.19	1	-1.70	Adult	*Lr34*	ABC transporter
				Field_2015_1	4.74	1	-1.53			
				Field_2015_2	5.60	1	-1.64			
				Field_2015_3	3.92	1	-0.96			
				Field_2016_2	3.90	1	-1.03			

[a]The markers were positioned on the latest DArT-seq consensus map (version 4.0) provided by Dr. Andrzej Kilian (Diversity Arrays Technology, Pty, Ltd)

[b]*P. triticina* pathotypes used in seedling, AGC and in the field in 2014 *pt* 104-1,2,3,(6),(7),11,13; 2015—*pt* 104-1,2,3,(6),(7),11,13 and *pt* 76-1,3,5,7,9,10,12,13 + *Lr37*; 2016—104–1,2,3,(6),(7),11,13; 76–1,3,5,7,9,10,12,13 + *Lr37* and 104–1,3,4,6,7,8,10,12 + *Lr37*

[c]Allele for resistance, where 1 = present, 0 = absent

[d]*cssfr5* was projected using closest DArT marker *wPt-7171*

[e]Alignment with catalogued *Lr* genes

[f]Alignment with previously reported QTL from eight GWAS and two genomic prediction studies using high-throughput marker platforms

[g]QTL new to this study

Results

Disease response

Of the 288 lines in the diversity panel tested for LR response, 76.4% lines were seedling susceptible, and 23.6% lines were resistant (Fig. 1a, b). At the adult stage under AGC, 46.5% lines were resistant, and 53.5% showed a susceptible response (Fig. 1a; Supplementary material 1). In the 2014 field trial, 63.0, 75.4, and 63.0% of the tested 284 lines were resistant at the first three disease assessments (70, 77, and 86 DAS). However, at the fourth disease assessment (96 DAS) when the lines were at the flag leaf stage, and inoculum pressure in the nursery was highest, 71.2% lines displayed susceptibility, while only 28.8% lines displayed resistance (Fig. 1a). In 2015, 29.8% of the evaluated 288 lines showed resistance and 70.2% demonstrated susceptibility at the first disease assessment (78 DAS), while only 9% of the lines showed a resistant disease response at the third reading (101 DAS) (Fig. 1a). In 2016, of the 261 tested lines, 56.7 and 27.6% were resistant for the disease assessments performed at 71 and 85 DAS, respectively. The full description of disease responses observed for all lines in the diversity panel is provided in Supplementary material 1.

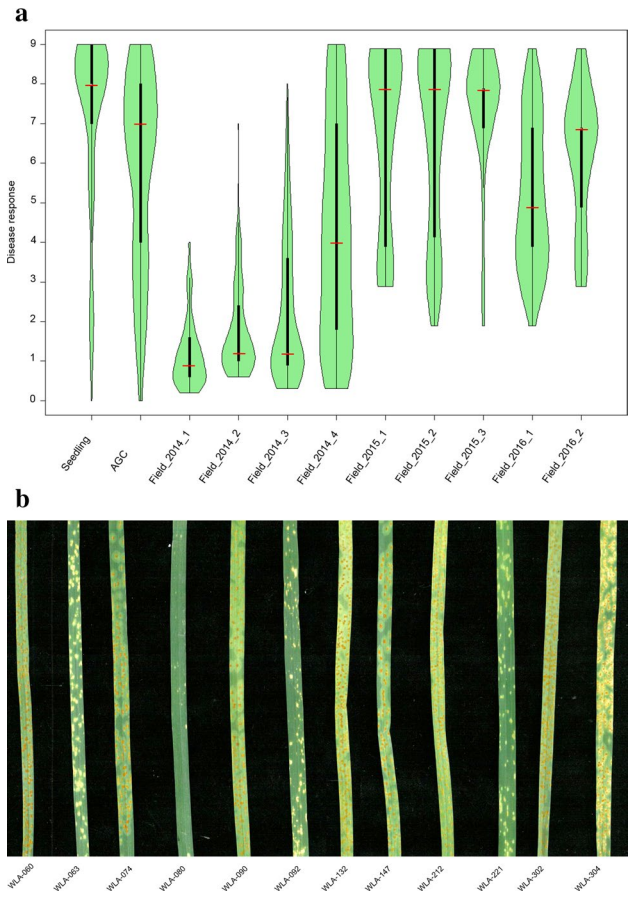


Fig. 1 **a** Violin plots illustrating the density distribution of leaf rust response for lines in the diversity panel based on 11 phenotypic data sets. The disease data for environments AGC and field (2014, 2015 and 2016) were converted to the 0–9 scale (9 = very susceptible) to allow comparison across all data sets. The red line displays the median, the top and bottom of the thick vertical bars represent first and third quartiles, respectively, and the green fill shows disease density estimates (n = 248). **b** A sample of the seedling leaf rust responses observed for the diversity panel

Marker properties, population structure, and linkage disequilibrium

After filtering, a total of 10,748 polymorphic mapped markers along with three PCR-based markers for known APR genes (*Lr34*, *Lr46*, and *Lr67*) were used for LD analysis and GWAS. Lower marker density and marker coverage were evident for the D genome compared to A and B genomes. Analysis of population structure in the diversity panel was previously described by Riaz et al. (2017), where distinct clustering was observed on the basis of cultivation status and geographical origin. The diversity panel was divided into two clusters (k = 2), containing 171 and 124 lines, respectively. Analysis of LD decay revealed strong differences between the three subgenomes. Overall, LD between marker pairs decayed quickly in the A and B genomes, especially in the latter, where the r^2 LOESS curve never exceeded the threshold line. In contrast, LD in the D genome was very pronounced, and LOESS curves did not drop below the threshold line until 19 cM for cluster 1 and 21 cM for cluster 2 (Fig. 2).

Marker–trait associations

A total of 52 significant markers ($p < 0.001$) were associated with LR resistance (Table 3; Supplementary material 2). Six markers were detected at the seedling stage and 46 markers at the adult stage (Table 3; Supplementary material 2). Most of the significant markers (n = 32) were detected in 2015 field environments. Manhattan plots depicting association between significant markers and LR response in different environments were displayed in Supplementary material 3. By considering chromosome position and LD between adjacent markers, a total of 31 QTL regions were assigned. These QTL were located on chromosomes 1A, 1B, 2A, 2B, 3A, 3B, 4A, 5A, 5B, 6A, 6B, 7A, 7B, and 7D (Table 3). Of the 31 QTL, 29 were associated with resistance at the adult stage and one QTL each was found to be associated with seedling (i.e. detected only at the seedling stage) and all-stage resistance (i.e. detected both at the seedling and adult stage) (Table 3). The QTL *qNV.Lr-2B.3* (all-stage resistance) and *qNV.Lr-7B.2* (adult plant stage) were detected in many of the environments. The gene-specific marker *cssfr5* for known APR gene *Lr34* on chromosome 7D (Lagudah

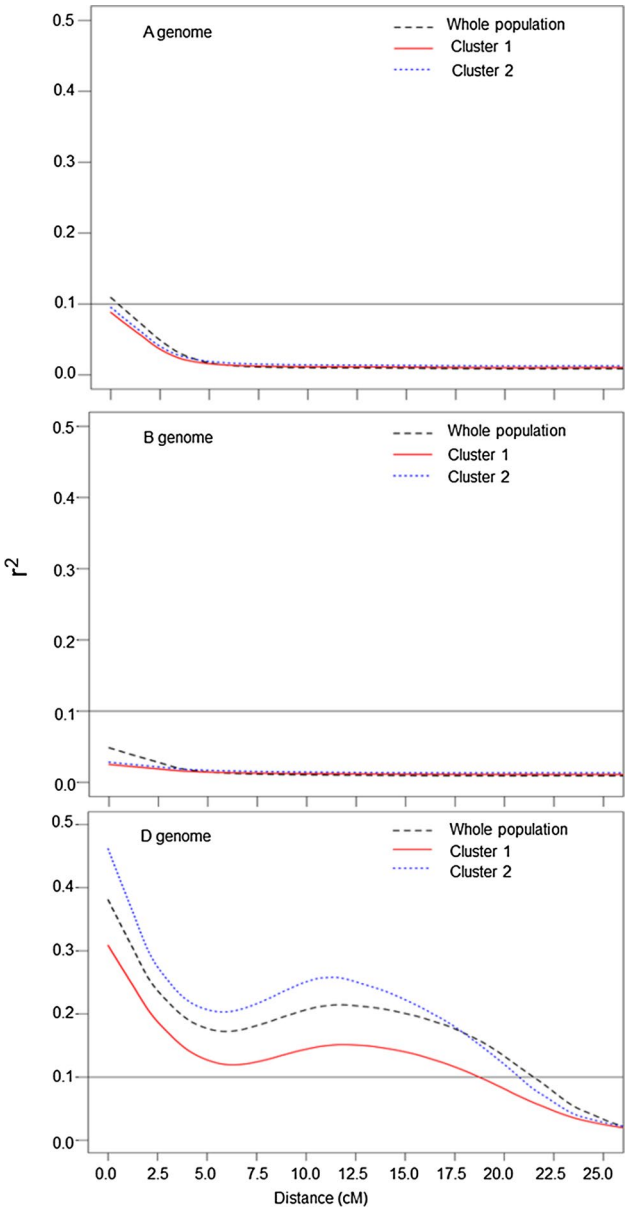


Fig. 2 Linkage disequilibrium (LD) decay as a function of genetic distance (cM) in A, B and D genomes for the diversity panel. LD was estimated for the whole population (black dotted line), and cluster 1 (red line) and cluster 2 (blue dotted line) as defined by Riaz et al. (2017). The LD decay was the point where the locally estimated scatterplot smoothing (LOESS) curves intersect the LD, whereas the threshold for LD decay was at $r^2 = 0.1$ (black line)

et al. 2009) was the only loci among the three PCR markers used that could be detected in GWAS with $-\log_{10}$ (p value) between 3.9 and 6.19 for the different field trials, and was designated QTL *qNV.Lr-7D* (Table 3).

Of the 31 QTL, 13 were identified as being new LR resistance loci (Table 3). Among the other 18 QTL, 4 were co-located with the catalogued *Lr* genes, namely *Lr3* on chromosome 6B, *Lr64* on 6A, *Lr14* (a and b alleles), *Lr68*, *LrBi16* and *LrFun* on 7B, and *Lr34* on 7D (McIntosh et al. 2017; Table 3 and Supplementary material 3). The remaining 14 QTL identified in our study were in alignment with the candidate regions reported in other GWAS studies (Table 3, Supplementary material 4). An in silico annotation of the identified significant markers showed that most sequences were uncharacterised regarding their molecular function (Table 3). However, 12 markers corresponded to the putative proteins carrying domains involved in disease resistance mechanisms, such as leucine rich repeat (LRR), NB-ARC, P-loop_NTPase, zinc finger, CCHC-type, RNA-dependent DNA polymerase, protein kinase-like domain, cyclin-like F-box, galectin, carbohydrate recognition domain, glycosyl transferase family 29, glycosyl transferase family 31, Ran GTPase, small GTP-binding protein, ABC transporter and domain of unknown function-DUF1618 (Table 3).

Haplotype analysis and allele stacking

A new QTL (*qNV.Lr-3A.3*) on the long arm of chromosome 3A (116.7–117.0 cM) represented by two highly significant markers for seedling LR resistance [$-\log_{10}(p$ value) = 6.26/4.1] which were in high LD ($r^2 = 0.7$), was selected for subsequent haplotype analysis (Table 3, Fig. 3a). This large effect QTL was considered a new genomic region conferring seedling resistance because it did not align with any previously reported *Lr* genes or QTL (Fig. 3b). Screening of allelic variation in our diversity panel resulted in seven different haplotype variants (*qNV.Lr-3A.3*—hap1–hap7), where hap1 was the most frequent variant in our diversity panel (frequency = 92.5%) (Fig. 3b). Hap2 was present in 4.7% of the lines while all other variants only occurred in 1% of the lines each. Inter-group comparisons of the disease responses for the first three haplotype groups showed that hap1 was associated with a significantly higher susceptibility to LR (8 on a 0–9 scale) than hap2 and hap3, where the median disease response ranged between 3.6 and 5.5, respectively (Fig. 3c). The lines carrying hap1 are geographically widespread and originate from 28 countries, including Russia ($n = 48$), India ($n = 37$), and Pakistan ($n = 30$). The lines carrying hap2 were from Armenia ($n = 3$), Azerbaijan ($n = 3$), Russia ($n = 2$), Pakistan ($n = 1$), Ethiopia ($n = 1$), and five were of unknown origin while hap3 was from Ukraine ($n = 1$) and two were of unknown origin (Fig. 3d). Interestingly, of the 14 lines carrying the resistant haplotype (hap2), only one line was deemed to also carry the known APR genes *Lr34* and *Lr46* (Supplementary material 5).

We also constructed a haplotype on the basis of the identified APR QTL *qNV.Lr-7B.2* on the long arm of chromosome 7B (126.0–130.6 cM) represented by 11 highly significant markers associated with LR resistance at the adult stage. Interestingly, several previously reported *Lr* genes and QTL have been reported in the region, including

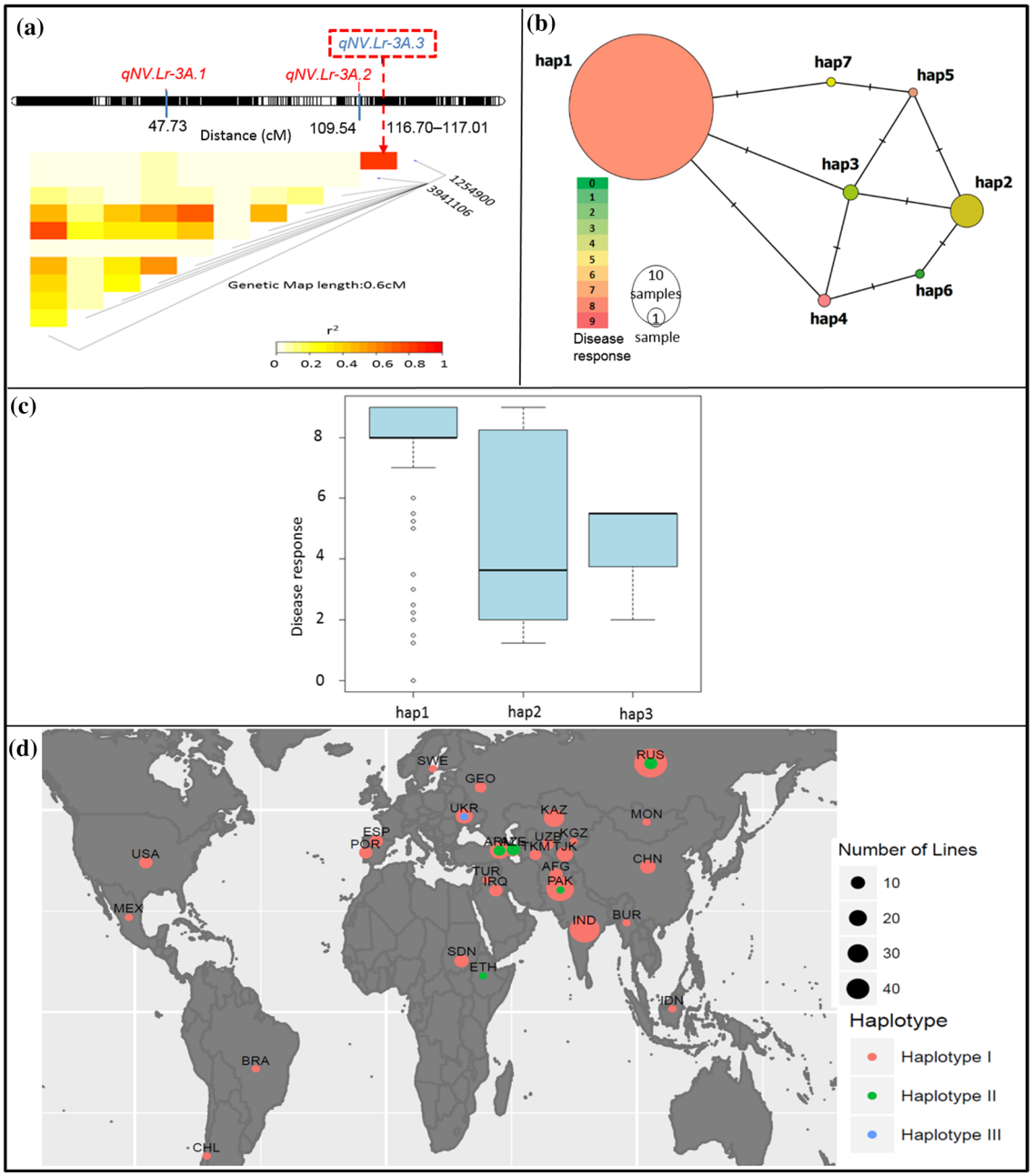


Fig. 3 Haplotype analysis of QTL *qNV.Lr-3A.3* on chromosome 3A associated with resistance to leaf rust at the seedling stage. **a** Chromosomal position of QTL *qNV.Lr-3A.*3 (116.7–117.0 cM based on the DArT-seq consensus map version 4.0 provided by Dr. Andrzej Kilian) and linkage disequilibrium for associated markers. **b** Haplotype network displaying seven haplotype variants, where the size of the node is proportional to the number of lines carrying that haplotype variant while colour indicates the mean disease response for those lines (0–9 scale, where 9 = very susceptible). **c** Box plots displaying the disease response for the lines carrying the three most common haplotypes. **d** The geographic distribution of the three most common haplotypes in the diversity panel

Lr14 (a and b alleles) (Dyck and Samborski 1970; Terracciano et al. 2013), *Lr68* (Herrera-Foessel et al. 2012), *LrBi16* (Zhang et al. 2011), and *LrFun* (Xing et al. 2014) (Fig. 4a). Around the identified QTL, the five DArT-seq markers (i.e. 1207290, 1117456, 1214960, 1134022, and 2304335) in very high LD ($r^2 > 0.75$) were used for the haplotype analysis (Table 3; Fig. 4a). In total, 22 haplotype variants were identified in our panel, of which hap1 and hap2 were the most frequent (78.3 and 7.8%, respectively). To construct the TCS haplotype network, only the variants which occurred at least twice in the panel (i.e. hap1-hap9) were used (Fig. 4b). Tukey's test and a comparison

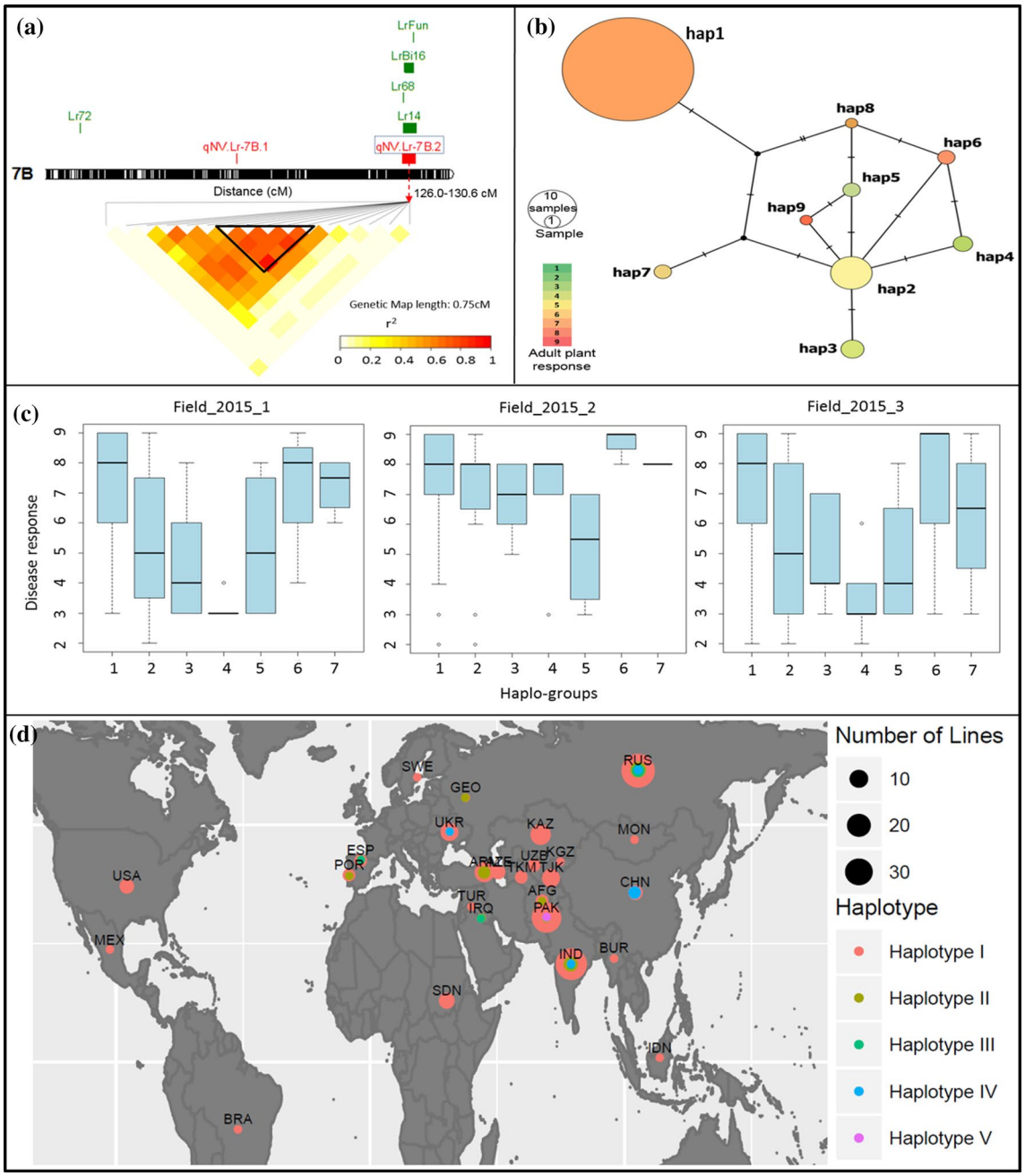


Fig. 4 Haplotype analysis of QTL *qNV.Lr-7B* on chromosome 7B associated with resistance to leaf rust at the adult plant stage. **a** Chromosomal position of the QTL *qNV.Lr-7B.3* (128.6–130.6 cM based on the DArT-seq consensus map version 4.0 provided by Dr. Andrzej Kilian) and comparison with catalogued *Lr* genes. The linkage disequilibrium block highlighted for the five associated markers. **b** Haplotype network displaying the nine most common haplotype variants, where the size of the node is proportional to the number of lines carrying that haplotype variant while colours indicate mean disease response for those lines (1–9 scale, where 9 = very susceptible). **c** Box plots displaying the disease response by lines carrying seven most common haplotypes in three phenotypic data sets in 2015. **d** The geographic distribution of the five most common haplotypes in the diversity panel

of median values for seven haplotypes showed that genotypes in hap1 were significantly more susceptible to LR (8 on a 1–9 scale) in all screenings of 2015 (Fig. 4c). Four haplotypes (hap2-hap5) displayed less susceptibility across three phenotypic data sets in 2015, where the median value of each haplotype across phenotypic data sets was variable i.e. hap2 (5–8 on a 1–9 scale), hap3 (4–7 on a 1–9 scale), hap4 (3–8 on a 1–9 scale) and hap5 (4–5.5 on a 1–9 scale) (Fig. 4c). The lines carrying hap2 originated from Russia ($n = 4$), India ($n = 2$), Armenia ($n = 1$), and 16 were of

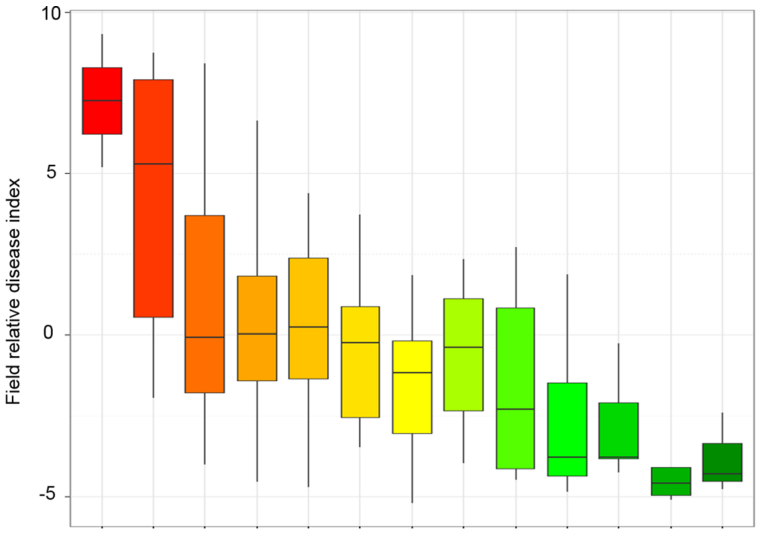
unknown origin. The lines carrying hap3 were from Russia ($n = 2$), unknown origin ($n = 2$) and one each from Iraq, Spain, and India. The hap4 originated from China ($n = 2$), and one each from Russia, India, and Ukraine. The hap5 was present in a line from Pakistan ($n = 1$) (Fig. 4d).

To test the effect of an accumulation of alleles for LR resistance at the independent loci, we assigned the lines from the diversity panel to groups, based on the absolute number of resistance-associated alleles possessed. This resulted in 13 different groups, ranging from two lines that carried ≤ 5 resistance-associated alleles, up to three lines that carried 29 or more (Fig. 5; Supplementary material 6). A comparison of their indices which represent the average LR response of a line in relation to the overall population evaluated in field trials from 2014 to 2016 revealed a very clear linear trend. While lines that combined relatively few of the identified resistance-associated alleles showed a comparatively high disease index, resistance to LR continuously increased with additional resistance-associated alleles. In total, 51 lines were detected that carry 19 or more resistance-associated alleles and showed index levels largely below zero (Fig. 5; Supplementary material 6).

Discussion

Novel sources of LR resistance

Deployment of resistant cultivars is the most economical and effective method to control rust diseases in the field (Ellis et al. 2014). However, deployed resistance genes can easily be overcome due to the rapid evolution of the pathogen and limited genetic diversity for resistance factors in modern wheat germplasm. Crop domestication and later selective breeding in modern breeding programs have led to a dramatic loss of genetic diversity in many important crop species, such as rice (*Oryza sativa* var. japonica) (Huang et al. 2012), maize (*Zea mays* subsp. mays L.) (Wright et al. 2005) and hexaploid bread wheat (Reif et al. 2005). The exploitation of genetic resources from the primary gene pool of wheat is considered a promising approach to identify new and durable resistance factors that can be utilised for the improvement of modern high-yielding varieties (Mujeeb-Kazi et al. 2013). The primary gene pool includes wild and early domesticated relatives of wheat, landraces, old cultivars and breeding lines. The use of landraces compared to wild relatives is advantageous as they carry homologous chromosomes that can easily recombine with hexaploid wheat (Wulff and Moscou 2014). As advances in genotyping technologies provide high-throughput genome information at an unprecedented resolution and low costs, vast germplasm collections stored in many gene banks worldwide represent a rich and now accessible genetic treasure chest for modern wheat improvement (Voss-Fels and Snowdon 2016). We have identified potentially new genomic regions that are highly associated with LR resistance at seedling and adult stage in the Vavilov wheat diversity panel. Analysis of LD for the three genomes revealed that LD decayed rapidly in the A and B genomes in both population clusters, reflecting the



Number of resistance alleles	≤5	≤7	≤9	≤11	≤13	≤15	≤17	≤19	≤21	≤23	≤25	≤27	29/31
Frequency (n)	2	15	45	86	52	25	19	9	12	14	5	8	3

Fig. 5 The effect of resistance-associated favourable alleles at quantitative trait loci for resistance to leaf rust response in the diversity panel. The field relative disease index is calculated using phenotypic data sets from field trials only. The frequency of lines carrying favourable alleles is also presented

high level of allelic diversity in the diversity panel. In our study, the highest LD was estimated for the D genome, which was also reported in numerous previous studies (Nielsen et al. 2014; Wang et al. 2014a; Zegeye et al. 2014; Voss-Fels et al. 2015). Across experiments, the lines identified as resistant include landraces, cultivars and breeding lines originating from different countries of the world (i.e. Russia, Kazakhstan, India, Pakistan, Ukraine, and China), thus providing diverse sources to achieve durable resistance in various eco-geographic contexts (Gurung et al. 2014; Maccaferri et al. 2015). These resources harbour promising novel resistances against a rapidly evolving pathogen. For instance, in Australia, a recent exotic introduction of *P. triticina* pathotype 104–1,3,4,6,7,8,10,12 + *Lr37* carried virulence on five *Lr* genes (*Lr12*, *Lr13*, *Lr20*, *Lr27* + *31*, and *Lr37*) which were widely deployed in cultivars (Cuddy et al. 2016; Park 2016). Thus, the identification of resistant lines in this study not only provides new sources of resistance, but likely different alleles for already known genes, which can help to enhance genetic diversity in modern wheat breeding programs.

Alignment of putative QTL to previously reported *Lr* genes and QTL

A large number of QTL were detected in our study ($n = 31$). Almost half ($n = 13$) of the identified QTL were considered novel, while the remainder ($n = 18$) aligned with previously reported QTL and/or catalogued *Lr* genes (discussed in Supplementary material 7). Interestingly, a locus corresponding to the seedling resistance gene *Lr3* on chromosome 6B was detected in our study, despite the use of *P. triticina* pathotypes that were virulent for *Lr3*. This suggests the probable presence of alternate alleles of the already 'extinct' resistance loci or a tightly linked gene with distinct resistance functions. The lack of availability of tightly linked or gene-specific markers for the known *Lr* genes hinders the ability to precisely position these genes on the respective chromosome. Further, allelism testing can also be performed to determine the association between the detected loci and previously reported genes and/or the QTL. A large number of QTL ($n = 30$) were identified in GWAS using more than one adult phenotype dataset. Of these, only six QTL were detected across different adult phenotypic data sets. This might be due to the fact the genomic regions underpinning APR often interact with the plant growth stage, inoculum pressure and the temperature conditions, thus affecting the resistance phenotype. An interesting region identified across both seedling, AGC and field data sets was QTL *qNV.Lr-2B.3* on chromosome 2B, which contained seven associated markers. Within this genomic region, we identified several candidate genes (i.e. NB-ARC, P-loop_NTPase, zinc finger, CCHC-type, and RNA-dependent DNA polymerase) that are known to encode proteins involved in pathogen recognition and subsequent activation of innate immune responses that lead to programmed cell death. It is well known that R genes tend to occur in clusters in plant genomes and give rise to many haplotypes via recombination (Friedman and Baker 2007; van Ooijen et al. 2008). Such 'hotspots' for resistance QTL could involve various combinations of classical R genes and other race-nonspecific genes (Burdon et al. 2014). A good example is the QTL region *qNV.Lr-7B.2* which contains seedling resistance gene *Lr14b* (Dyck and Sambroski 1970) and APR gene *Lr68* (Herrera-Foessel et al. 2012). It should be noted that QTL detected at the adult plant stage could also harbour genes regulating physiological characteristics, rather than classical R genes. For instance, in sorghum (*Sorghum bicolor* (L.) Moench), several QTL for resistance to rust (*Puccinia purpurea*) were found to co-locate with QTL for maturity and plant height (Wang et al. 2014b). These genetic factors could offer durable resistance to rust diseases.

Haplotype analysis

In GWAS, single marker scans are performed to understand the underlying genetic architecture of disease resistance in natural populations. In addition, a more powerful approach is to perform a haplotype analysis based on closely linked markers which are more likely to be inherited together as a block (Hayes et al. 2007). Haplotype analyses which typically depict marker-trait associations at a higher resolution due to an increased information content compared to bi-allelic molecular markers, such as SNPs or DArT markers, have been successfully applied in identifying genomic regions involved in effective Fusarium head blight resistance on wheat chromosome arm 3BS (Hao et al. 2012). In the present study, we performed haplotype analyses for two QTL, the seedling QTL *qNV.Lr-3A.3* and the APR QTL *qNV.Lr-7B.2*. Therefore, we followed previous studies and jointly defined markers in strong LD with the two identified QTL as a haplotype block (Hao et al. 2012; Diaz et al. 2011). Analysis of LD around the seedling QTL (*qNV.Lr-3A.3*) displayed a high level of LD between two associated markers, suggesting a high level of allelic fixation. One of the lines carrying the resistant haplotype (hap2) of QTL *qNV.Lr-3A.3* was also found positive to carry known APR genes *Lr34* and *Lr46*, thus providing a combination of R and APR genes/QTL. Such gene/QTL combinations are promising to achieve longer lasting resistance in elite cultivars. Similarly, of the markers located in the APR QTL *qNV.Lr-7B.2* on chromosome 7B, five markers with very high LD were considered a haplotype block. The results revealed a broad allelic variation for this chromosomal fragment and showed that four haplotype groups (hap2-hap5) were associated with

a reduction in susceptibility across three phenotypic data sets in 2015. This might be explained by the fact that this chromosomal region is known to carry *Lr* genes such as *Lr14* (*a* and *b* alleles), *Lr68*, *LrBi16* and *LrFun* (McIntosh et al. 2017).

Pyramiding of resistance-associated alleles for durable rust resistance

It has been well described that durable rust resistance in wheat can be achieved by pyramiding multiple QTL (Ellis et al. 2014; Mundt 2014). In our study, a comparatively high number of loci with variable LR resistance in the field were detected with a high fraction of lines that carried more than 20 resistance-associated alleles. Identification of lines with a large number of favourable alleles has been reported by previous studies (Kollers et al. 2014; Naruoka et al. 2015; Muleta et al. 2017). The landraces are the traditional varieties which were selected by the farmers in the field preferably for agronomic traits, but at the same time they were also indirectly selected for disease resistance (Zeven 2002). Since the rust pathogen has co-evolved with wheat landraces for thousands of years in the same environment, diverse resistance alleles and their combinations exist in the host population keeping epidemic development in check (Thrall and Burdon 2000; Ordonez and Kolmer 2007). Since the landraces in the seed bank were removed from their environmental context, it is hypothesised that they might hold new allelic variations against the modern *P. triticina* pathotypes. Detection of a large number of resistance alleles showed that these resistance-associated alleles have accumulated in landraces over time and occurred at variable frequencies (i.e. high, low, and rare) in the population. In particular, rare alleles are known to provide resistance to diseases and environmental stresses (Vikram et al. 2016). Therefore, the utilization of landraces for trait introgression may greatly increase the genetic diversity and frequency of rare alleles into modern wheat breeding programs. In our study, we were able to show that there is a close relationship between the level of LR resistance and number of resistance alleles from independent loci, highlighting the high potential of allele stacking for rust improvement in future cultivars. Combining seedling resistance and APR alleles is most effective and promising to provide sustainable resistance levels and also reduce the fitness cost associated with APR (Nelson 1978; Ellis et al. 2014; Consortium 2016). For example, durable resistance using combinations of resistances (seedling and APR) against stripe rust was achieved in Western Europe, while combining multiple minor genes provided durable resistance to stem rust and powdery mildew at the International Maize and Wheat Improvement Center (CIMMYT), Mexico (Singh et al. 2011; Basnet et al. 2014; Ellis et al. 2014; Singh et al. 2014; Brown 2015).

The detection of a large number of favourable alleles is promising. However, simultaneous consideration of all alleles in a breeding program is often challenging. To overcome this challenge, a small subset of alleles can be targeted by designing specific crosses in a breeding program, thus supporting recombination of favourable alleles at many loci. Furthermore, implementing trait introgression via MAS allows selection for various traits in early generations and can easily eliminate undesirable allele combinations. Recent advancements in genomic approaches such as marker-assisted backcrossing, whole-genome scans, genomic prediction and genomic selection enable rapid combination of multiple alleles in a single variety (Liu et al. 2014). Together with the latest advancements in plant phenotyping approaches and rapid generation advance systems, such as "Speed Breeding" (Watson et al. 2017), the development of rust resistant wheat cultivars can be accelerated (Hickey et al. 2012; Riaz et al. 2016b). Segregating populations can be screened and individuals selected by "phenotyping-on-the-go" during line development. The identified resistance can be rapidly introgressed using the rapid generation advance system in breeding programs. This could help fast-track the introgression of new LR resistance from the Vavilov wheat diversity panel into elite genetic backgrounds for future cultivars. The strategy is not limited to LR, as phenotyping methods adapted to speed breeding have been designed for other important traits in wheat, including stripe rust (Hickey et al. 2012), stem rust (Riaz and Hickey 2017), yellow spot (Dinglasan et al. 2016), seed dormancy (Hickey et al. 2009), and root architecture (Richard et al. 2015).

Author contribution statement AR, SP, EA, LH, and KVF conceived and designed the study; AR collected the data; AR, NK and SP performed PCR marker screening, EL contributed ideas and provided PCR markers for screening; AR and KVF analysed the data; AR wrote the manuscript; OA and OM provided geographic information about the Vavilov wheat collection; GP guided in designing the field nurseries and provided LR inoculum; RS contributed ideas for the haplotype analysis; SP, EA, RS, EL, LH and KVF edited and revised the manuscript.

Acknowledgements This research was supported by an Early Career Research Grant and a Ph.D. scholarship from the University of Queensland (UQ), Australia. We thank Dr. Laura Ziems and Dr. Dharmendra Singh from UQ for their valuable advice regarding the analyses performed in this study. We also greatly appreciate valuable advice from Prof. Robert McIntosh from the University of Sydney for positioning the catalogued *Lr* genes in the wheat genome.

Compliance with ethical standards

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

Ethical standards This article does not contain any studies with human participants or animals performed by any of the authors.

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