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ORIGINAL PAPER

Lr70, a new gene for leaf rust resistance mapped in common wheat accession KU3198

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Abstract
Key message **KU3198 is a common wheat accession that carries one novel leaf rust resistance (Lr) gene, *Lr70*, and another Lr gene which is either novel, *Lr52* or an allele of *Lr52*.**
Abstract Leaf rust, caused by *Puccinia triticina* Eriks. (*Pt*), is a broadly distributed and economically important disease of wheat. Deploying cultivars carrying effective leaf rust resistance (Lr) genes is a desirable method of disease control. KU3198 is a common wheat (*Triticum aestivum* L.) accession from the Kyoto collection that was highly resistant to *Pt* in Canada. An F_2 population from the cross HY644/KU3198 showed segregation for two dominant Lr genes when tested with *Pt* race MBDS which was virulent on HY644. Multiple bulk segregant analysis (MBSA) was employed to find putative chromosome locations of these Lr genes using SSR markers that provided coverage of the genome. MBSA predicted that the Lr genes were located on chromosomes 5B and 5D. A doubled haploid population was generated from the cross of JBT05-714 (HY644*3/KU3198), a line carrying one of the Lr genes from KU3198, to Thatcher. This population segregated for a single Lr gene conferring resistance to *Pt* race MBDS, which was mapped to the terminal region of the short arm of chromosome 5B with SSR markers and given the temporary designation *LrK1*. One F_3 family derived from the HY644/KU3198 F_2 population that segregated only for the second Lr gene from KU3198 was identified. This family was treated as an F_2-equivalent population and used for mapping the Lr gene, which was located to the terminal region of chromosome 5DS. As no other Lr gene has been mapped to 5DS, this gene is novel and has been designated as *Lr70*.

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Introduction

Leaf rust, caused by *Puccinia triticina* Eriks. (*Pt*), is a broadly distributed and economically important disease of wheat that is found in most of the World's wheat production areas (Saari and Prescott 1985). The disease can cause reductions in grain yield and end-use quality (Samborski 1985). In Canada, annual losses of approximately $80 M can be attributed to leaf rust (McCallum et al. 2007). While leaf rust can be controlled using leaf rust resistance (Lr) genes, the deployment of cultivars with simply inherited resistance often leads to a loss of resistance through the pathogen evolving virulence (Kolmer 1999). Combinations (stacks or pyramids) of effective and diverse Lr genes can mutually protect the genes in the stack and prolong effective resistance (Samborski and Dyck 1982). Thus, new Lr genes are desirable as they provide a means to increase the complexity and diversity of resistance and give breeders an array of options for leaf rust control through breeding (Dyck and Kerber 1985; Kolmer and Liu 2002).

Identifying or developing DNA markers linked to Lr genes facilitates marker-assisted breeding, particularly for selection of gene stacks, and parent selection within breeding programs. The vast majority of formally named Lr genes have been mapped with DNA markers (McCallum et al. 2012). However, the markers vary by their degree of linkage to the Lr genes and therefore applicability across germplasm. Developing tightly linked and informative

markers is a worthwhile goal for germplasm development and breeding programs.

Lr genes have been identified from a diversity of sources and transferred into common wheat stocks or elite germplasm. These sources includes the primary wheat gene pool such as common wheat (e.g. *Lr10*; Dyck and Kerber 1971) and progenitor species (e.g. *Lr21* from *Aegilops tauschii*; Rowland and Kerber 1974), the secondary gene pool (e.g. *Lr28* from *Ae. speltoides*; McIntosh et al. 1982), and the tertiary gene pool (e.g. *Lr9* from *Ae. umbellulata*; Sears 1956). Approximately 40 % of the named Lr genes are derived from the secondary and tertiary gene pools and are often accompanied by some degree of deleterious linkage drag (McCallum et al. 2012) and a reduction or elimination of recombination in neighbouring chromosomal regions thereby limiting gene combinations in breeding material. Thus, broadly effective Lr genes from the primary gene pool are valuable genetic resources, since they normally do not have the same constraints, and measures should be taken to avoid commercial deployment of these genes singly to slow the evolution of pathogen virulence.

A common wheat (*Triticum aestivum* L.) accession from the Kyoto University collection, KU3198, showed resistance to an array of *Pt* races during preliminary tests at Winnipeg, MB, Canada. However the genetics of resistance, including the number and identities of Lr genes, was unknown. The objectives of this study were to determine the number of Lr genes present in KU3198, and to identify their chromosomal locations and map positions using DNA markers.

Materials and methods

Plant material and testing with *P. triticina*

KU3198 is a common wheat accession with a spring growth habit that was collected in Khoshyailagh, Iran in 1956. Preliminary testing with *Puccinia triticina* (*Pt*) showed that KU3198 carried broad spectrum leaf rust resistance. HY644 (Alpha 16//Alpha*4/BgBSR/3/Sceptre/Ning 8331) is a Canadian spring wheat breeding line that is susceptible at the seedling stage to *Pt* isolates used in this study, although it does carry leaf rust resistance genes *Lr1*, *Lr17a* and *Lr34* (McCallum and Seto-Goh 2010). Breeding line, JBT05-714 (HY644*3/KU3198), was developed to introduce additional leaf rust resistance into the Canadian prairie spring (CPS) class of Canadian spring wheat.

A cross was made between HY644 and KU3198 and the F_2 generation was tested at the seedling stage with *Pt* race MBDS isolate 12-3 (McCallum and Seto-Goh 2003) following the nomenclature described by Long and Kolmer (1989). F_3 families from 68 F_2 plants that showed intermediate infection types (1^+2–2) were selected for further testing because they were considered more likely to be heterozygous for a single Lr gene. Twenty-four individuals from each F_3 family were inoculated with race MBDS and a family showing segregation for one of the two genes was selected based on the phenotypic ratio and marker haplotype.(see below) This F_3 family was treated as an "F_2-equivalent" population for the purpose of mapping one of the Lr genes from KU3198. More individuals from the selected F_3 family were inoculated with *Pt* race MBDS for phenotyping to increase the size of the mapping population.

JBT05-714 (HY644*3/KU3198) was crossed to the leaf rust susceptible wheat cultivar Thatcher. The F_1 was used to make a doubled haploid (DH) population using the maize pollination method (Thomas et al. 1997). The population was inoculated at the seeding stage with *Pt* race MBDS.

All inoculations with *Pt* were performed as described by McCallum and Seto-Goh (2003). Briefly, urediniospores suspended in a light mineral oil were sprayed onto seedlings that had two leaves fully emerged. The seedlings were incubated in a dew chamber overnight and then transferred to a greenhouse kept at approximately 20 °C (±4 °C) using supplemental light (approximately 16 h light and 8 h darkness). Plants were rated for infection type (IT) 12 days post-inoculation using a 0–4 scale as described by Stakman et al. (1962). ITs 0–2 were classified as resistant and ITs 3–4 were classified as susceptible. Genetic ratios in each population were assessed for goodness-to-fit to theoretical ratios using the Chi squared (χ^2) test.

Locating Lr genes and mapping with DNA markers

Leaf tissue was collected from the parental lines, the initial F_2 population, the F_2-equivalent sub-population, and the DH population and lyophilized. The lyophilized tissue was macerated and DNA was extracted using a CTAB extraction method described by Kleinhoffs et al. (1993) except that phenol was not used. SSR markers were analyzed using an ABI 3100xl Genetic Analyzer (Applied Biosystems, Streetsville, ON). PCR and analysis with the ABI 3100xl Genetic Analyzer were performed as described by Somers et al. (2004).

Multiple bulk segregant analysis (MBSA) was used to determine the chromosomes carrying the Lr genes inherited from KU3198 following the procedures described by Ghazvini et al. (2013). MBSA utilizes 14 "mini-bulks" of DNA that each represent four gametes from the segregating population under study; in the present case each mini-bulk consisted of DNA from two F_2 progeny. The genotype(s) at the locus/loci of interest must be known for individuals included in the bulks. In the present study, leaf rust resistance showed dominant inheritance (see "Results") thus individuals from the HY644/KU3198 F_2 population that

were susceptible would be homozygous for the susceptible allele for each Lr gene detected by *Pt* race MBDS. A set of 423 SSR markers (Röder et al. 1998; Somers et al. 2004; Song et al. 2005) were previously selected for MBSA based on profile simplicity, apparent polymorphism frequency, and distribution across the genome (Ghazvini et al. 2013). The 14 bulks and parents were tested with the selected set of SSR markers. Markers with the highest frequencies of HY644 (susceptible parent) alleles were considered as possible locations for the Lr genes.

SSR markers that showed putative linkage to the Lr genes were used to test the susceptible F_2 progeny to estimate linkage with the Lr genes (i.e. linkage to the susceptible allele at each locus). These markers and neighbouring SSR markers were used to assess which Lr gene from KU3198 was present in JBT05-714. The SSR markers putatively linked to a second Lr gene from KU3198 as determined by MBSA were used to select an F_3 family from the HY644/KU3198 population that was segregating for a single gene that differed from the gene in JBT05-714. The selected F_3 family was treated as an F_2-equivalent population for mapping the segregating Lr gene. The Thatcher/JBT05-714 DH population and the selected F_2-equivalent sub-population were phenotyped with *Pt* race MBDS (above) and mapping was performed with SSR markers in the chromosome regions identified by MBSA. Maps were constructed using MapDisto (Lorieux 2007) and the Kosambi mapping function (Kosambi 1944) was applied.

Results

The HY644/KU3198 F_2 population inoculated with *Pt* race MBDS 12-3 showed a range of infection types (ITs) from 1 to 4. There were 366 progeny classified as resistant (IT 2 or less), and 25 progeny classified as susceptible (IT 3 or greater), which fitted a two-gene ratio ($\chi^2_{15:1} = 0.014$, $p = 0.91$). Using the susceptible progeny to construct "mini-bulks", MBSA was performed and SSR markers on chromosomes 5BS (cfd20) and 5DS (gwm190 and wmc233) showed the highest frequencies of alleles from HY644, the susceptible parent. Individual susceptible F_2 progeny (i.e. not bulks) were tested with these markers to get a preliminary assessment of linkage to the Lr gene loci inherited from KU3198 by estimating linkage to the susceptibility alleles. Linkage between susceptibility and SSR markers wmc233 and gwm190 on chromosome 5DS were 6 and 12 cM respectively. On chromosome 5BS linkage between susceptibility and SSR marker cfd20 was 2 cM.

The DH population from the cross JBT05-714/Thatcher and parents were tested at the seedling stage with *Pt* race MBDS. JBT05-714 and the resistant DH lines showed an IT;1 whereas Thatcher and the susceptible DH lines showed ITs 3–4. There were 96 resistant and 91 susceptible DH lines which fitted a single gene segregation ratio ($\chi^2_{1:1} = 0.134$, $p = 0.71$). SSR markers located on chromosomes 5DS and 5BS were assessed for linkage with resistance to race MBDS. Markers on chromosome 5BS showed linkage to the Lr gene detected by MBDS and linkage mapping placed this gene (temporarily designated as *LrK1*) distal to the terminal SSR marker (Fig. 1). The closest markers to the gene were cfd20 and gwm234 which cosegregated at 0.6 cM from *LrK1*.

An F_3 family was selected from the HY644/KU3198 population based on segregation for single gene resistance to race MBDS and possession of SSR markers fixed for HY644 alleles on chromosome 5BS and heterozygous on 5DS. This family was treated as an F_2-equivalent population to map the Lr gene putatively located on chromosome 5DS. One-hundred and ninety-six individuals were inoculated with *Pt* race MBDS at the seedling stage. The resistant individuals had ITs 1–22^+ and susceptible individuals had ITs 33^+–4. There were 158 resistant and 38 susceptible plants in the F_2-equivalent population which fitted a single gene segregation ratio ($\chi^2_{3:1} = 3.293$, $p = 0.06$). The Lr gene mapped 5.6 cM distal to the terminal SSR marker barc130 on the genetic map of chromosome 5DS (Fig. 2). As no other Lr genes have been mapped to chromosome 5DS, this represents a novel Lr gene and was designated *Lr70*.

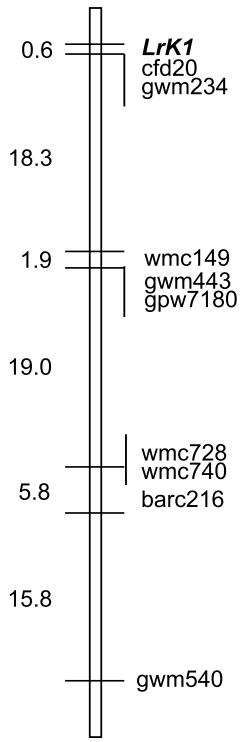

Fig. 1 Genetic map of *LrK1* on chromosome 5BS constructed using the Thatcher/JBT05–714 DH population. Genetic distances are in cM

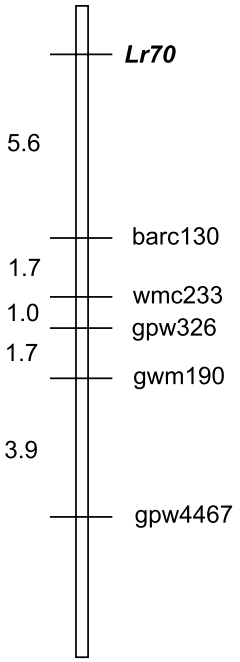

Fig. 2 Genetic map of *Lr70* on chromosome 5DS constructed using the F_2-equivalent population derived from the selected single-gene HY644/KU3198 F_3 family. Genetic distances are in cM

Discussion

KU3198 carries two resistance genes that confer resistance to *Pt* race MBDS 12-3. One of these Lr genes, *LrK1*, mapped to the terminal region of chromosome 5BS. There is one other Lr gene, *Lr52*, that has also been mapped to chromosome 5BS (Hiebert et al. 2005). The map published for *Lr52* (Hiebert et al. 2005) and the map of *LrK1* presented here share two SSR markers in common, gwm443 and wmc149. The map positions of *LrK1* and *Lr52* are similar and it is possible that *LrK1* is *Lr52* or an allele of *Lr52*.

The second Lr gene from KU3198 was mapped to the terminal region of chromosome 5DS. There is one other Lr gene on chromosome 5D, *Lr1*, however it was mapped at the terminal region of the long arm (Cloutier et al. 2007). As this Lr gene from KU3198 has a unique map position, it is a new gene and was designated *Lr70*.

KU3198 is a common wheat accession and as such, any desirable genes targeted for use in breeding programs should not be encumbered by linkage drag that often accompanies genes derived from the secondary and tertiary gene pools of wheat. Thus, KU3198 can be used, and to a very limited extent has been used as a donor of leaf rust resistance in wheat breeding programs. It is more likely that *Pt* populations can evolve virulence or increase the frequency of virulence if Lr genes are deployed singly in cultivars (Kolmer 1999). Important Lr genes such as *Lr16* and *Lr21* from the primary gene pool have been overcome by *Pt* in Canada (McCallum and Seto-Goh 2004; McCallum et al. 2011). To avoid this, stacks or pyramids of Lr genes mutually protect each other and improve the stability of resistance. Gene stacks can enhance adult-plant resistance (APR) when combined with race-nonspecific APR genes like *Lr34* even if the individual genes are not broadly effective (McCallum and Thomas 2014). Marker-assisted selection (MAS) is an efficient approach to select gene combinations in breeding programs. The DNA markers identified as being linked to *LrK1* and *Lr70* in this study are useful for understanding the relationship between these and other Lr genes but are not ideal for MAS because (a) the linkage between the *Lr70* and closest markers is not particularly tight (5.6 cM), and (b) there are no distal markers to flank *Lr70* or *LrK1*. Moreover, there are other types of markers and detection platforms that are more cost effective and amenable to high-throughput genotyping compared to SSRs. For example, the KASPar SNP marker assay (LCG Genomics, http://www.lgcgenomics.com) has proven to be well suited for breeding purposes (Semagn et al. 2014). Thus, future work on *Lr70* and *LrK1* should include the development of improved markers that would allow more reliable selection in breeding programs.

The map position of *LrK1* suggests that it could be allelic to *Lr52*. This could be resolved be either testing for allelism by intercrossing stocks possessing *LrK1* and *Lr52* or by high-resolution comparative mapping. Allelism tests become difficult for closely linked genes due to the rarity of individuals carrying only susceptible alleles, although the frequency of these individuals is also dependent on the type of population used. As an alternative, using high-resolution mapping populations (e.g. studying 5,000–10,000 gametes per population) to fine map each resistance separately may be a better approach to compare the precise map positions of *Lr52* and *LrK1* and may be more informative than an allelism test. This approach would provide the additional benefit of developing improved markers for MAS and may lead to map-based cloning of the resistance gene(s).

The breadth of resistance is an important consideration when choosing Lr genes for inclusion in a breeding program. Simply testing KU3198 with a suite of *Pt* races is an unsuitable approach to determine how broadly useful these genes due to epistasis and the possibility of additional Lr genes that may be present in KU3198 but not detected with *Pt* race MBDS 12-3. If *LrK1* is *Lr52* it will be broadly effective (Hiebert et al. 2005). However, *LrK1* has not been tested as a single gene in a susceptible background. Thus, even if *LrK1* is allelic to *Lr52*, it is unknown whether they differ functionally (i.e. *LrK1* could be a new allele of *Lr52*). Similarly, the breadth of resistance conferred by *Lr70* is presently unknown. Thus, a single gene stock should also be produced to assess *Lr70*. Efforts have been initiated to generate Thatcher near-isogenic lines (NILs) possessing

both *LrK1* and *Lr70*, adding to the valuable series initiated by P.L. Dyck (McCallum et al. 2012). In addition to allowing a direct comparison between these Lr genes and previously described genes in the Thatcher background, it will be possible to monitor changes in virulence to these genes and thus determine their usefulness in different geographic regions.

Author contributions CWH wrote the manuscript, developed segregating populations and performed genetic analyses, BDM performed disease testing and contributed to the manuscript, JBT initiated the study, developed segregating populations and performed genetic analyses.

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Ethical standards The authors declare that all experiments complied to the ethical standards in the country in which they were performed.

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

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