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Validation of CAPS marker WR003 for the leaf rust resistance gene *Lr1* and the molecular evolution of *Lr1* in wheat

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Abstract: The wheat leaf rust resistance gene *Lr1* encodes a typical coiled-coil nucleotide-binding site leucine-rich repeat (CC-NBS-LRR) of resistance protein containing 1 344 amino acids. WR003, a cleaved amplified polymorphic sequence (CAPS) marker is derived from the LRR regions of *Lr1*. In this study, a worldwide collection of 120 *Aegilops tauschii* accessions and 282 hexaploid wheat varieties was screened for *Lr1* alleles using WR003, and the specificity of WR003 for *Lr1* was confirmed by pathogenicity tests and genotype analysis. The sequence alignment and phylogenetic tree analysis of 38 *Lr1* haplotypes provided a further view of the molecular evolution of *Lr1*. The results showed that there were very few polymorphisms between the *Lr1* alleles from *Ae. tauschii* and hexaploid wheat with the same resistance phenotype. The polymorphisms of the *Lr1* haplotypes were mainly between the different resistance lines, rather than between the different ploidy levels. These results indicate that *Lr1* originated from *Ae. tauschii* and differentiated into resistant and susceptible genotypes before its introgression into hexaploid wheat. Therefore, it is likely that wheat *Lr1* has at least two major variants for disease resistance and susceptibility, and except for certain point mutations, few gene conversions and genetic re-combinations occurred during the hexaploid wheat domestication.

Keywords: *Aegilops tauschii*; genetic evolution; leaf rust resistance; *Triticum aestivum* L.

Wheat (*Triticum aestivum* L.) is one of the world's most essential cereal crops (Haas et al. 2019). However, the wheat yield is seriously influenced by various diseases (Keller et al. 2018), of which the most common is leaf rust, a disease caused by the fungal pathogen *Puccinia recondita* f.sp. *tritici*, (Ling et al. 2003; Prasad et al. 2020). Epidemics of this disease can result in both severe yield losses and nutritional quality decreases (Manjunatha et al. 2018). Pesticide applications can not only result in environmental

pollution and ecological damage, but also may accelerate the selection for resistant pathogen strains (Wu et al. 2020b; Garnault et al. 2021). Therefore, the development of cultivars with natural resistance is the most effective, economical, and ecological way to reduce leaf rust disease (Pasam et al. 2017).

Wheat is an allohexaploid species ($2n = 42$, AABBDD). It evolved from the hybridisation of *Triticum turgidum* (a tetraploid wheat, AABB) with *Aegilops tauschii* (*Ae. tauschii*, a diploid progenitor, DD)

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10 000 years ago (Krattinger & Keller 2016; Avni et al. 2017; El Baidouri et al. 2017). Therefore, *Ae. tauschii* (the D-genome donor) is an evolutionarily young progenitor of bread wheat.

Lr1 was first described in the hexaploid wheat cultivar Malakoff (Ausemus et al. 1946) and was then found in a variety of wheat cultivars (Gebrewahid et al. 2020; Wu et al. 2020a). Plants with the *Lr1* resistance genotype display an intense reaction after infection by the avirulent pathogen *Puccinia recondita* f.sp. *tritici* (Ling et al. 2003). Qiu et al. (2007) isolated *LR1RGA1*, a candidate for the resistance gene *Lr1* via a sub-genome map-based cloning strategy, and then Cloutier et al. (2007) cloned the *Lr1* gene and confirmed that *LR1RGA1* was the resistance gene *Lr1*, which is a member of the polygenic family (Cloutier et al. 2007), is 4 036 bp long and encodes a coiled-coil nucleotide-binding site leucine-rich repeat (CC-NBS-LRR) resistance protein of 1 344 amino acids (Cloutier et al. 2007; Qiu et al. 2007). The cleaved amplified polymorphic sequence (CAPS) marker WR003 of 757 bp was developed by Qiu et al. (2007) from the LRR repeats 9–15 of *Lr1*, which showed polymorphisms between the *Lr1* alleles from three susceptible (Thatcher, Xiaoyan54 and TA1704) and two resistant lines (Thatcher*Lr1* and Tr.t213). In this study, a large collection of wheat and *Ae. tauschii* accessions was screened for *Lr1* alleles, and the specificity of WR003 was verified by phenotypic assessment and DNA sequence analysis. In addition, the evolutionary analysis of the *Lr1* alleles provided a further view of the molecular genetics and origin of wheat *Lr1*.

MATERIAL AND METHODS

Plant material. A total of 282 hexaploid wheat varieties (including 262 Chinese wheat mini-core collections) and 120 *Ae. tauschii* accessions from throughout the world were analysed for the presence of *Lr1* alleles (Table S1 in the Electronic Supplementary Material (ESM)). Fifty-seven of the materials were tested for disease resistance against the leaf rust race BRW-90035, an avirulent isolate for resistant *Lr1* (Table 1). Thirty-eight *Lr1* haplotypes from nine hexaploid wheat varieties and 29 *Ae. tauschii* accessions were isolated, and the GenBank accession numbers were provided in Table S2 in the ESM.

Pathogenicity tests. Nine seeds of each material were sown on plastic plates as mentioned by Ling et al. (2004). The susceptible wheat cultivar Thatcher and resistant wheat cultivar Thatcher*Lr1* were sown on the

same plates as the negative and positive controls, respectively. The two-week-old seedlings were artificially inoculated with the spores of the *Lr1* virulent isolate BRW-90035, mixed with a light mineral oil. The spore suspension was sprayed using a sprayer at a pressure of 17 kPa. The infected seedlings were transferred to and maintained in a greenhouse at 18 to 23 °C. The resistance phenotypes of the plants were recorded 10 days after inoculation based on a 0–4 scale for leaf rust (scores: 3–4, susceptible; 0–2, resistant).

Genomic DNA extraction. The genomic DNA was extracted from fresh plant leaves as previously described with some improvements (Graner et al. 1990). The quality of the DNA was examined by electrophoretic separation with a 0.8% agarose gel. The concentration of DNA samples was adjusted to 0.1 µg per µL for the subsequent polymerase chain reaction (PCR) amplification.

CAPS analysis. The CAPS marker WR003 developed by Qiu et al. (2007) was used in this study. The PCR analyses were performed in a reaction system of 25 µL with 50 ng of genomic DNA templates, 1 × PCR buffer, 0.6–1 U of *rTaq* DNA polymerase, 400 nM PCR primers (Table 2), and 200 µM dNTPs. The programme for the amplification was as follows: first, 95 °C for 5 min; then 36 cycles of 95 °C for 1 min, 57 °C for 1 min, and 72 °C for 1 min; and finally, 10 min for the elongation reaction at 72 °C. The enzyme digestion reaction was performed at 37 °C for 5 h; the reaction mixtures were 25 µL, containing 10 µL of the PCR products and 1 U of *Bgl*II (New England Biolabs, Beijing, China). The digested products were then fractionated using 2% standard agarose gel electrophoresis.

PCR isolation of *Lr1* alleles. *Lr1* allele sequences were isolated from the genomic DNA by PCR amplification using four primer pairs *Lr1*-1, *Lr1*-2, *Lr1*-3, and *Lr1*-4 (Table 2). Some primer pairs were designed by Qiu et al. (2007) and Cloutier et al. (2007). Each overlapped fragment of the *Lr1* alleles was amplified by two independent PCRs. The amplification products excised from the agarose gels were purified and ligated into the vector pMD18-T, and then transformed into *Escherichia coli* cells (DH10B) following the method described by Froger and Hall (2007). Three individual clones of each PCR product were sequenced. The complete sequence of each *Lr1* allele was spliced by the software DNAMAN (Ver. 8.0; LynnonBiosoft, USA).

Sequence analysis and construction of the phylogenetic tree. The nucleotide and amino acid se-

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Table 1. Evaluation of 38 *Aegilops tauschii* accessions and 19 hexaploid wheat cultivars

No.	Accessions/cultivar names	Species	Genome	Alleles	Phenotype
1	LA213	<i>Ae. tauschii</i>	DD	<i>Lr1</i>	R
2	LA1704	<i>Ae. tauschii</i>	DD	<i>lr1</i>	S
3	Aus18913	<i>Ae. tauschii</i>	DD	<i>Lr1</i>	R
4	LA309	<i>Ae. tauschii</i>	DD	<i>Lr1</i>	R
5	RL5688	<i>Ae. tauschii</i>	DD	nd	S
6	LA2397	<i>Ae. tauschii</i>	DD	<i>lr1</i>	S
7	SX-4	<i>Ae. tauschii</i>	DD	nd	S
8	SX-5	<i>Ae. tauschii</i>	DD	nd	S
9	SX-6	<i>Ae. tauschii</i>	DD	nd	S
10	SX-11	<i>Ae. tauschii</i>	DD	nd	S
11	SX-14	<i>Ae. tauschii</i>	DD	nd	S
12	SX-18	<i>Ae. tauschii</i>	DD	nd	S
13	SX-24	<i>Ae. tauschii</i>	DD	nd	S
14	SX-27	<i>Ae. tauschii</i>	DD	nd	S
15	SX-28	<i>Ae. tauschii</i>	DD	nd	S
16	SX-30	<i>Ae. tauschii</i>	DD	nd	S
17	SX-31	<i>Ae. tauschii</i>	DD	nd	S
18	SL-1	<i>Ae. tauschii</i>	DD	nd	S
19	SL-4	<i>Ae. tauschii</i>	DD	nd	S
20	SC-4	<i>Ae. tauschii</i>	DD	nd	S
21	T002	<i>Ae. tauschii</i>	DD	nd	S
22	T005	<i>Ae. tauschii</i>	DD	nd	S
23	T008	<i>Ae. tauschii</i>	DD	nd	S
24	T014	<i>Ae. tauschii</i>	DD	nd	S
25	T023	<i>Ae. tauschii</i>	DD	nd	S
26	T028	<i>Ae. tauschii</i>	DD	nd	S
27	T033	<i>Ae. tauschii</i>	DD	nd	S
28	T036	<i>Ae. tauschii</i>	DD	nd	S
29	T040	<i>Ae. tauschii</i>	DD	nd	S
30	T056	<i>Ae. tauschii</i>	DD	nd	S
31	T063-2	<i>Ae. tauschii</i>	DD	nd	S
32	T074-2	<i>Ae. tauschii</i>	DD	nd	S
33	T075-4	<i>Ae. tauschii</i>	DD	nd	S
34	T079	<i>Ae. tauschii</i>	DD	nd	S
35	T081-1	<i>Ae. tauschii</i>	DD	nd	S
36	T085	<i>Ae. tauschii</i>	DD	nd	S
37	T107	<i>Ae. tauschii</i>	DD	nd	S
38	T117	<i>Ae. tauschii</i>	DD	nd	S
39	Thatcher <i>Lr1</i>	<i>Triticum aestivum</i>	AABBDD	<i>Lr1</i>	R
40	Thatcher	<i>T. aestivum</i>	AABBDD	<i>lr1</i>	S
41	Xiaoyan54	<i>T. aestivum</i>	AABBDD	<i>lr1</i>	S
42	Frisal	<i>T. aestivum</i>	AABBDD	nd	S
43	Lumai14	<i>T. aestivum</i>	AABBDD	<i>Lr1</i>	R
44	Xuzhou21	<i>T. aestivum</i>	AABBDD	<i>Lr1</i>	R

Table 1 to be continued

No.	Accessions/cultivar names	Species	Genome	Alleles	Phenotype
45	Jinmai62	<i>T. aestivum</i>	AABBDD	<i>Lr1</i>	R
46	Jinchun13	<i>T. aestivum</i>	AABBDD	<i>Lr1</i>	R
47	Bainong64	<i>T. aestivum</i>	AABBDD	<i>Lr1</i>	R
48	Wenmai4	<i>T. aestivum</i>	AABBDD	<i>Lr1</i>	R
49	Fengkang8	<i>T. aestivum</i>	AABBDD	nd	S
50	Kanzler	<i>T. aestivum</i>	AABBDD	nd	S
51	Zhengzhou9023	<i>T. aestivum</i>	AABBDD	<i>lr1</i>	S
52	Luofulin10	<i>T. aestivum</i>	AABBDD	<i>lr1</i>	S
53	Youbailan	<i>T. aestivum</i>	AABBDD	<i>lr1</i>	S
54	Xiaobaidong	<i>T. aestivum</i>	AABBDD	<i>Lr1</i>	R
55	Fuzhuang30	<i>T. aestivum</i>	AABBDD	<i>Lr1</i>	R
56	Nongda15	<i>T. aestivum</i>	AABBDD	<i>Lr1</i>	R
57	Chinese Spring	<i>T. aestivum</i>	AABBDD	nd	S

R – resistant; S – susceptible phenotype upon leaf rust inoculation, respectively; *Lr1* and *lr1* indicated the presence of resistance-conferring and susceptible-conferring alleles at the *Lr1* locus, respectively; nd – not detected, which indicates no *Lr1* haplotype identified using the CAPS marker WR003

quences were aligned using the software DNAMAN (Ver. 8.0) and ClustalW (Ver. 2.0.10) (Larkin et al. 2007). Phylogenetic trees were constructed using the neighbour-joining (NJ) method by the MEGA (Ver. 7.0.14) program (Kumar et al. 2016).

RESULTS

Validation of CAPS marker WR003 specificity for *Lr1*. More than 400 accessions containing 120 *Ae. tauschii* lines and 282 hexaploid wheat varieties were screened for *Lr1* alleles by the CAPS marker WR003. In 100 out of 282 wheat varieties (35.5%) and 36 out of 120 *Ae. tauschii* lines (30%), a single band (757 bp) in each material was amplified using WR003 primer pairs, indicating that these accessions might carry *Lr1* alleles (Figure 1). For 24% (24 of 100) of the wheat varieties and 11.1% (4 of 36) of the *Ae. tauschii* lines with a target band of 757 bp,

the restriction patterns of *Bgl*II were in accordance with the resistant line Thatcher*Lr1* (the two bands of 236 and 521 bp). These accessions were postulated to carry resistant *Lr1* alleles, whereas the rest were in accordance with the susceptible line Thatcher (two bands of 329 and 428 bp), indicating that these accessions might carry susceptible *Lr1* alleles (Figure 2). These data indicated that resistant *Lr1* alleles were present in 8.5% (24 of 282) of the wheat varieties and 3.3% (4 of 120) of the *Ae. tauschii* accessions.

The leaf rust resistance identification of 57 materials indicated that the materials displaying the resistance restriction patterns showed resistance after artificial infection with the isolate BRW-90035, whereas the rest showed susceptibility (Table 2). Therefore, the CAPS marker pattern analysis highly agreed with the phytopathology tests. A subsequent DNA sequence analysis also revealed the presence or absence of the resistant or susceptible *Lr1* alleles

Table 2. The PCR primers used in this work

Primer pairs	Forward primers (5'→3')	Reverse primers (5'→3')	T (°C)
WR003	GGGACAGAGACCTTGGTGGA	GACGATGATGATTTGCTGCTGG	57
<i>Lr1</i> -1	ATGGCGGCGGCTCTCG	GGTGTTCGACGCTGAAGA	60
<i>Lr1</i> -2	GCGGAGGATCTGCTGGAT	AACCTCATTCCCCGTCAA	57
<i>Lr1</i> -3	GAGTATTATTTGGATGATCGATGC	TCTGTAGTTGGTCCACCAAGG	57
<i>Lr1</i> -4	GGGACAGAGACCTTGGTGGA	GTTAGCAATACGAGACGGATAAAATCTG	57

T – temperature

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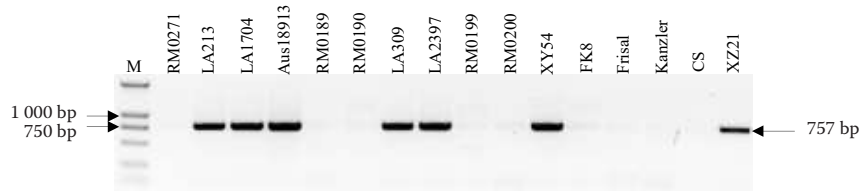


Figure 1. The *Lr1*-specific marker WR003 amplified the specific fragments in the partial *Aegilops tauschii* lines and bread wheat varieties

The names of the materials are indicated above the electrophoretograms; the materials with the amplified bands are indicated to contain *Lr1* alleles; M – 2 kb DNA ladder marker; the molecular weights are marked with arrows

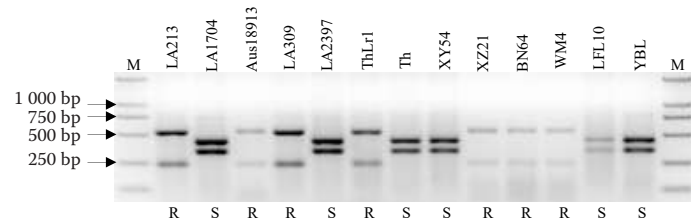


Figure 2. The restriction enzyme patterns of the *Lr1* allele-specific marker WR003 in partial materials

The names of the materials are indicated above the electrophoretograms; the sizes of the digested fragments from the resistant materials (R) were 236 and 521 bp, whereas the sizes of the digested fragments from the susceptible materials (S) were 329 and 428 bp, respectively; the analysis was conducted on 2% agarose gels; M – 2 kb DNA ladder marker; the molecular weights are marked with arrows

in these accessions. In conclusion, WR003 was highly specific for *Lr1* within the set of accessions screened.

Sequence comparison of *Lr1* alleles. To elucidate the molecular evolution of the *Lr1* gene, 38 allelic sequences of *Lr1* were acquired by PCR amplifi-

cation from wheat and the *Ae. tauschii* genomic DNA (Figure 3). The full-length sequence alignment of the *Lr1* alleles from susceptible and resistant lines manifested a divergence in the DNA sequence block of 570 bp (2 479–3 048 bp, corresponding to a se-

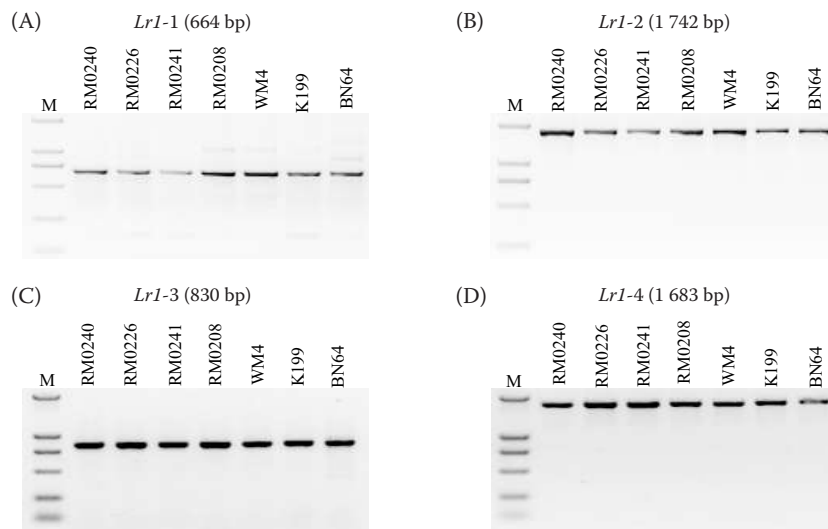


Figure 3. PCR amplifications of the first (A), second (B), third (C), and fourth (D) fragments of the *Lr1* gene, respectively

The names of the materials and sizes of the PCR amplified fragments are indicated above the electrophoretograms; M – 2 kb DNA ladder marker

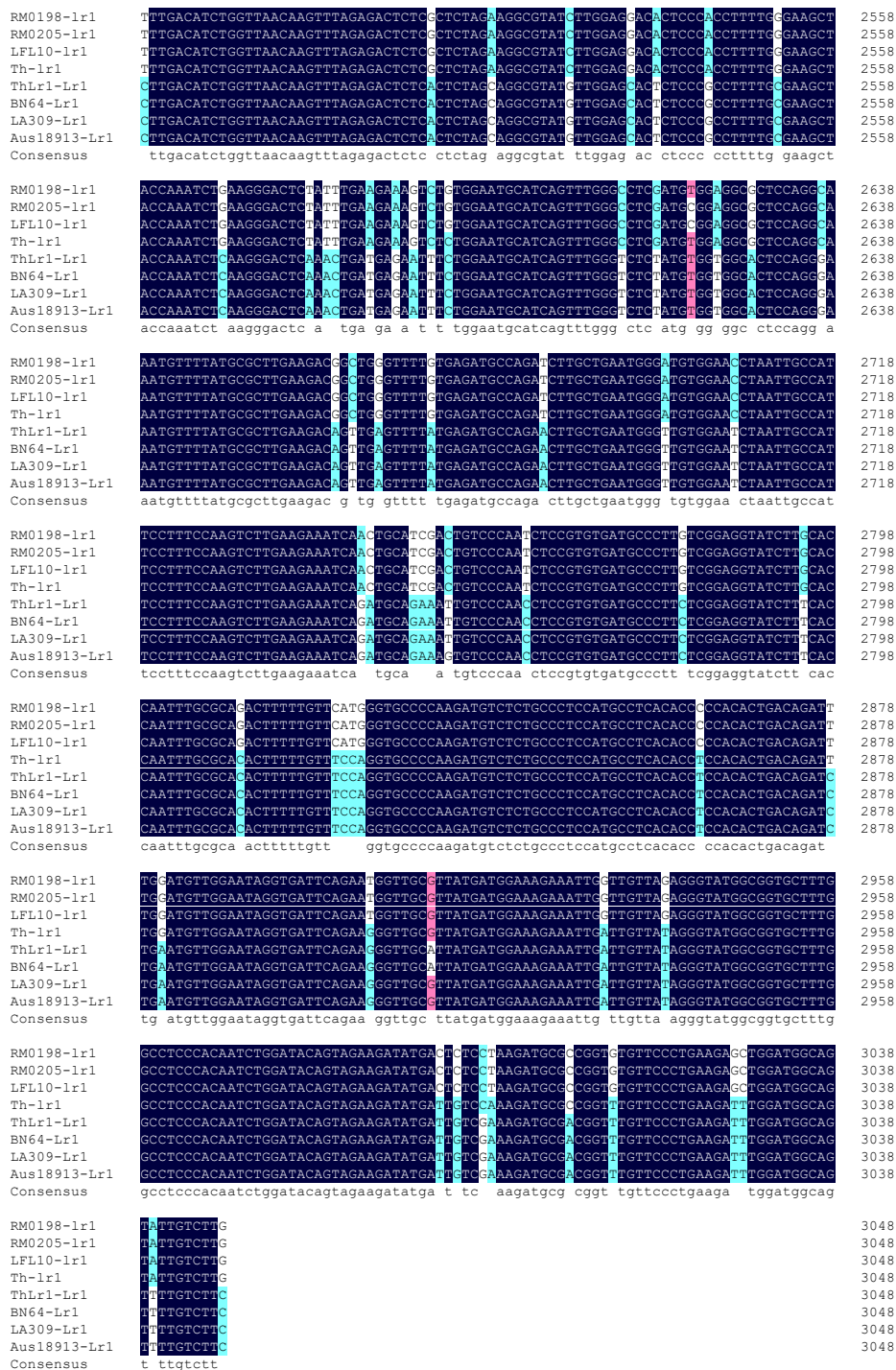


Figure 4. The sequence alignment of the 570 bp divergent sequence block of the *Lr1* alleles from eight lines (the block sequence alignment of the *Lr1* alleles from 38 lines is available as Figure S1 in the ESM)

LA309 and Aus18913 – the resistant *Ae. tauschii* lines; BN64 and ThLr1 – the resistant hexaploid wheat varieties; Th and LFL10 – the susceptible hexaploid wheat varieties; RM0205 and RM0198 – the susceptible *Ae. tauschii* lines; the blue and white nucleotide sites represent the differences between the resistant and susceptible genotypes; the purple-red and white nucleotide sites represent only two point mutations between the hexaploid wheat and *Ae. tauschii* with the same resistance in the divergent sequence block: one point mutation (at 2 622 bp) occurred in the partial alleles from the susceptible *Ae. tauschii* lines, then introgressed into the susceptible hexaploid wheat; another one (at 2 912 bp) was a non-synonymous mutation that only occurred in the alleles of the resistant hexaploid wheat

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quence of 827 to 1 017 amino acids), encoding the 9–15th LRR repeats of *Lr1*. This block of polymorphism clearly differentiated between susceptible and resistant accessions (Figure 4). Thatcher (susceptible wheat line) showed a conservative mosaic pattern in this sequence block with either susceptible or resistance alleles; however, this pattern was not detected in the *Ae. tauschii* lines. The results indicate that a small gene conversion or recombination might occur in this region after the hexaploid wheat domestication. Except for Thatcher, the allelic sequences of *Lr1* of the hexaploid wheat and *Ae. tauschii* with the same resistance were mostly identical in the sequence block – only two point mutations were detected. At 2 622 bp from the start codon, one point mutation existed in the partial *Lr1* alleles from the susceptible *Ae. tauschii* lines, which then introgressed into the susceptible hexaploid wheat. At 2 912 bp, one non-synonymous mutation appeared only in the

Lr1 alleles of the resistant hexaploid wheat (G→A, corresponding to amino acids R→H); in contrast, the remaining alleles were more conserved at this locus. The result suggests that the mutation at 2 912 bp only occurred in the resistant alleles during the evolution of the hexaploid wheat. In addition to these two point mutations, the *Lr1* allelic sequences of *Ae. tauschii* and the hexaploid wheat with the same resistance were completely identical in the sequence block. The flanking sequences (a total of 3 466 bp) of this sequence block were relatively conservative, as only 26 point mutations were detected. These 26 point mutations occurred in accessions with the same resistance rather than having different resistance. This suggests that these mutations might not affect the *Lr1* function.

The *Lr1* alleles of *Ae. tauschii* and the hexaploid wheat with the same resistance were more identical (99.7% identity at the nucleotide level). There was only

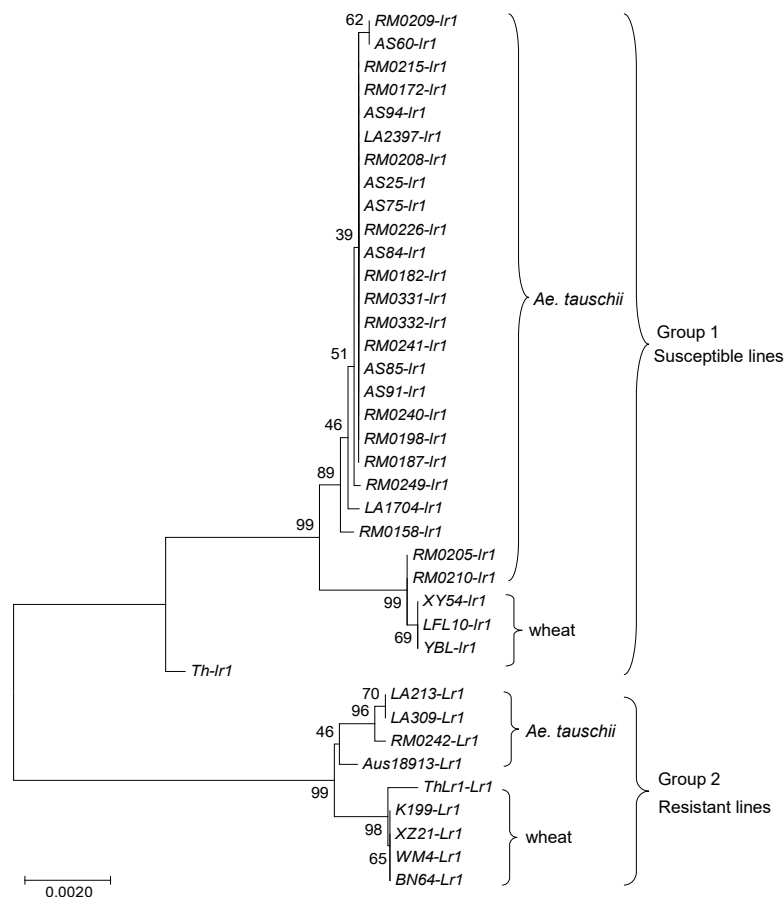


Figure 5. The neighbour-joining phylogenetic tree of the *Lr1* alleles at the nucleic acid level

The 38 nucleotide sequences can be classified into two clear groups, corresponding to the susceptible and resistant alleles; the lengths of the branches, which reflect the degrees of similarity between the sequences, indicate high conservation among the sequences of the *Lr1* alleles with the same resistance; the numbers on the branches indicate the bootstrap values

one point mutation between the susceptible hexaploid wheat and *Ae. tauschii* (at 2 460 bp, a synonymous mutation); nine point mutations were detected between the resistant hexaploid wheat and *Ae. tauschii* (two synonymous mutations and seven non-synonymous mutations), one in the 570 bp block and eight in the rest of the sequence. These results show that these point mutations possibly occurred after the formation of the hexaploid wheat. All these data strongly indicate that the wheat *Lr1* gene originated from *Ae. tauschii*, and differentiated into resistant and susceptible genotypes prior to its introgression into hexaploid wheat.

Fourteen synonymous base mutations and 51 non-synonymous base mutations were lined up in tandem in the LRR coding region. Except for three of the synonymous base mutations and one of the non-synonymous base mutations, all these polymorphisms were distributed in the sequence region of 570 bp.

Phylogenetic analysis of *Lr1* alleles. The neighbour-joining phylogenetic tree showed that the 38 *Lr1* allele sequences could be classified into two clear groups based on 98% of the nucleotide identity, corresponding to susceptible and resistant alleles (Figure 5), both of which were highly supported by the bootstrap values. Group 1 was composed of 29 members, all of which were susceptible alleles, while group 2 consisted of 9 resistant *Lr1* alleles. The length of the branches indicates high conservation among the sequences of *Lr1* alleles from the same resistance lines (shared 99.5–100% identity to each other). However, Thatcher (a susceptible cultivar) formed a distinct branch because it showed a conservative mosaic pattern in the LRR coding region with either susceptible or resistance alleles. Each group was divided into two independent clades, corresponding to the sequences from *Ae. tauschii* and the hexaploid wheat. However, RM0205-Lr1 and RM0210-Lr1 (from *Ae. tauschii* lines) were different from the other sequences from the *Ae. tauschii* lines, and shared complete identity with the sequences from the wheat (XY54-Lr1, LFL10-Lr1 and YBL-Lr1) at the amino acid level, because, at the DNA level, only one synonymous mutation occurred at 2 460 bp.

DISCUSSION

Breeding strategies for improving leaf rust resistance have mainly relied on the deployment of the host-plant resistance (Kthiri et al. 2019). However, the use of molecular markers provides a direct aid

in selecting disease-resistant varieties and speeds up the breeding process (Juliana et al. 2018; Rani et al. 2019). CAPS markers are cost-efficient and simple tools and are extensively used by breeders (Michikawa et al. 2019; Matuszczak et al. 2020). In this study, the CAPS marker WR003's specificity for *Lr1* was validated in a set of wheat varieties and their progenitor *Ae. tauschii* accessions. The genotype scores based on WR003 were compared with the *Lr1* score for the phenotypic assessment and DNA sequences analysis, and good agreement was found. Using WR003, we can screen out *Ae. tauschii* and the wheat varieties containing the *Lr1* allele and can also accurately detect the resistance traits of these varieties.

A better understanding of the origin and molecular evolution of the disease resistance gene in wheat is an important prerequisite to improve wheat field resistance (Krattinger & Keller 2016). In this study, a high sequence identity of the *Lr1* alleles was detected between *Ae. tauschii* and the wheat with the same resistance. The sequence alignment and phylogenetic analysis of 38 *Lr1* alleles further showed that *Lr1* in the hexaploid wheat originated from *Ae. tauschii* and infiltrated into the D genome of the wheat during wheat domestication. In addition, the sequence alignment of the *Lr1* alleles revealed a divergent sequence block, which was displayed mainly between the different resistance genotypes rather than at the different ploidy levels. Except for a few point mutations, all the allelic sequences of *Ae. tauschii* and the wheat with the same resistance were completely identical. These results indicate that *Lr1* differentiated into resistant and susceptible genotypes before the formation and evolution of hexaploid wheat. Therefore, it is likely that the leaf rust resistance gene *Lr1* in wheat has at least two origins.

Lr1 is a member of the large gene family (Cloutier et al. 2007). Many studies have shown that duplication, recombination, and gene conversion play important roles during the development and evolution of resistant gene families (Segura et al. 2017; Chen et al. 2020). The sequence alignment of *Lr1* alleles manifested a divergence in the block of 570 bp, while the remaining sequences are highly conservative. The sequence pattern indicated that *Lr1* might be derived from an ancient gene through one or more gene conversion or recombination events.

The polymorphisms between the susceptible and resistant alleles were only detected in the LRR region and represented the only differences between

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the susceptible and resistant alleles, which strongly indicate that the resistance phenotypes may be assigned to the LRR domain. The result is consistent with previous research showing that the LRR region of the resistant gene contains ligand binding sites and controls the resistant gene's specificity (Qiu et al. 2007; Sarkar et al. 2019; Saucet et al. 2021). In addition, 14 synonymous mutations and 51 non-synonymous mutations occurred within the LRR coding region of *Lr1*. The synonymous changes represent a very ancient variation of *Lr1* (Qiu et al. 2007). However, 51 non-synonymous changes between the *Lr1* alleles may have occurred before the formation of the hexaploid bread wheat, as these mutations were detected between the resistant and susceptible alleles of the *Ae. tauschii* accessions rather than the alleles of the diploid and hexaploid lines.

CONCLUSION

The phenotypic assessment and genotype analysis suggest that the CAPS marker WR003 is highly specific for the resistance gene *Lr1*, and has practical applications in wheat breeding. The sequence alignment and phylogenetic analysis of the *Lr1* alleles showed that the wheat *Lr1* gene evolved from *Ae. tauschii* and differentiated into resistant and susceptible genotypes before its introgression into hexaploid wheat. Thus, the wheat leaf rust resistance gene *Lr1* may have at least two major variants, and it is likely that very few gene recombination or conversion events have taken place in the *Lr1* genes after the formation of hexaploid wheat, except for certain point mutations that may not affect the function.

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