Fine Mapping of the Wheat Leaf Rust Resistance Gene \textit{LrLC10} (\textit{Lr13}) and Validation of Its Co-segregation Markers

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Wheat leaf rust, caused by the fungus \textit{Puccinia triticina} Eriks. (\textit{Pt}), is a destructive disease found throughout common wheat production areas worldwide. At its adult stage, wheat cultivar Liaochun10 is resistant to leaf rust and the gene for that resistance has been mapped on chromosome 2BS. It was designated \textit{LrLC10} and is the same gene as cataloged gene \textit{Lr13} by pedigree analysis and allelism test. We fine-mapped it using recessive class analysis (RCA) of the homozygous susceptible \textit{F$_2$} plants derived from crosses using Liaochun10 as the resistant, male parent. Taking advantage of the re-sequencing data of Liaochun10 and its counterpart susceptible parent, we converted nucleotide polymorphisms in the \textit{LrLC10} interval between the resistant and susceptible parents into molecular markers to saturate the \textit{LrLC10} genetic linkage map. Four indel markers were added in the 1.65 cM map of \textit{LrLC10} flanked by markers CAUT163 and Lseq22. Thirty-two recombinants were identified by those two markers from the 984 \textit{F$_2$} homozygous susceptible plants and were further genotyped with additional ten markers. \textit{LrLC10} was finally placed in a 314.3 kb region on the Chinese Spring reference sequence (RefSeq v1.0) that contains three high confidence genes: TraesCS2B01G182800, TraesCS2B01G182900, and TraesCS2B01G183000. Sequence analysis showed several variations in \textit{TraesCS2B01G182800} and \textit{TraesCS2B01G183000} between resistant and susceptible parents. One KASP marker and an indel marker were designed based on the differences in those two genes, respectively, and were validated to be diagnostic co-segregating markers for \textit{LrLC10}. Our results both improve marker-assisted selection and help with the map-based cloning of \textit{LrLC10}.

**Keywords:** wheat, \textit{LrLC10} (\textit{Lr13}), leaf rust resistance, fine mapping, marker-assisted selection (MAS)

**INTRODUCTION**

Globally, common wheat (\textit{Triticum aestivum}) is one of the most commonly cultivated crops, comprising 20% of human caloric intake and 15% of cultivated area in the world (FAOSTAT, 2015; WAP, 2017). Wheat leaf rust, caused by \textit{Puccinia triticina} Eriks. (\textit{Pt}) is one of the most damaging
diseases of wheat, especially in coastal regions or areas with high temperatures and humidity during the wheat maturing seasons (Kolmer et al., 2018). In China, past widespread wheat leaf rust epidemics have caused severe yield losses (Dong, 2001; Zhou et al., 2013). In the future, due to impending climate change, leaf rust is expected to damage wheat production even more (Jiang et al., 2018). Utilization of wheat resistant cultivars considered a most effective, economical and environmentally-friendly strategy for controlling this disease (Bariana et al., 2007; Singh et al., 2013).

Currently, about 80 leaf rust resistant genes have been reported and formally named in common wheat or its relatives (McIntosh et al., 2017; Qureshi et al., 2018), and by using different types of molecular markers, most of these genes have been mapped on the wheat chromosomes1. Development of robust molecular markers linked to resistance genes is essential in wheat disease resistance breeding, especially for resistance gene pyramiding. Nevertheless, among these designated leaf rust resistance genes, only a few have tightly linked molecular markers for marker-assisted selection2.

Because of limited wheat genomic sequence data, developing molecular markers for wheat genes has been difficult. But now, by combining the T. aestivum ‘Chinese Spring’ (CS) IWGSC RefSeq v1.0 genome3 with annotations of high-quality gene models, these difficulties have been reduced, especially for gene location and markers development (Clavijo et al., 2017). Discovery of the highly abundant, locus-specific wheat nucleotide variations that can be used to identify the relevant genes are now within grasp because of affordable next-generation sequencing (Varshney et al., 2014; Xu et al., 2017). Compared with other kinds of markers, competitive allele-specific PCR (KASP) assays accelerate the conversion of DNA variations into available gene-linked markers. Taking advantage of such whole genomic sequences, Wu et al. (2018b) mapped wheat yellow rust resistance gene Yr26 on a 0.003-cM interval on chromosome 1B near the centromere, Narang et al. (2019) defined wheat leaf rust resistance gene LrP and yellow rust resistance gene YrP on a 15.71 Mb region on 5DS in the CS RefSeq v1.0 genome assembly, and Wu et al. (2019) localized the Pm52 locus within a 5.6 Mb interval on the long arm of chromosome 2B (2BL).

Bulked segregant analysis (BSA) can rapidly identify markers linked to target genes (Michelmore et al., 1991), and it has been improved by bulking homozygous recessive plants and using recessive class analysis (RCA) to map specific genes (Zhang et al., 2014). RCA is highly efficient, with a lower probability of misclassification and more reliability than using a random F2 population. Furthermore, this approach avoids creating F2 populations, thereby reducing the time required for marker development. RCA has been proven to map genes efficiently and reliably (Zhang et al., 1994; Yao et al., 1997; Mei et al., 1999; Chen et al., 2006; Kiswara et al., 2014). In wheat, rapid gene mapping using RCA has been used to map a sterile female gene (Dou et al., 2009) and a stripe rust resistance gene, YrLM168a (Feng et al., 2015), and to successfully map and clone the powdery mildew resistance gene Pm60 (Zou et al., 2018).

In wheat, Lr13, first identified in the Canadian cultivar “Manitou” in 1966, is an important adult-plant leaf rust resistance gene (McIntosh et al., 1995) that is widely found in wheat cultivars (e.g., ‘Frontana,’ ‘FrondoSO,’ and ‘Fronteria’) and used in many breeding programs worldwide (Roelfs, 1988; Pathan and Park, 2006). In China, Lr13 is one of the main resistance genes and confers effective resistance to leaf rust (Singh et al., 1999; Yuan et al., 2007; Yuan and Chen, 2011; Ren et al., 2015; Zhang et al., 2019). Previous studies indicated that Lr13 was located on chromosome 2BS (McIntosh et al., 1995), and Bansal et al. (2008) reported Lr13 was delimited to a 13.8 cM interval flanked by markers Xksm58 and Xstm773b. Recently, using a segregating population of Lr13 near-isogenic lines with simple sequence repeat and KASP markers, Zhang et al. (2016) mapped Lr13 to a small interval of 10.7 cM, and the closest marker was kwh37 (4.9 cM). A morphological marker, hybrid necrosis gene Ne2m, was found linked to Lr13 by Singh and Gupta (1991), but it cannot be used to accurately detect Lr13 (Anand et al., 1991). Therefore, a co-segregated and diagnostic marker for Lr13 in molecular breeding is yet unavailable.

In this study, we confirmed that the leaf rust resistance gene LrLC10 in Liaochun10 is Lr13 by pedigree analysis, and finely mapped it to a close interval with recessive class analysis (RCA) through the markers developed according to the re-sequencing data of the parental lines. We also developed molecular markers that were closely linked to LrLC10 and that can be used to facilitate marker-assisted selection of LrLC10 in wheat resistance breeding.

### MATERIALS AND METHODS

#### Plant Materials

The spring wheat cultivar Liaochun10, is highly resistant to leaf rust, was crossed with two susceptible wheat lines Han 87-1 (87-1 and 7D49 (a wild emmer wheat introgression line created by the crossing IW123/Zheng98/88-1*2)), to construct two F2 segregating populations of 3,908 plants. Wild emmer wheat IW123 was donated by T. Fahima and E. Nevo, University of Haifa, Israel and Lr13-carrier line RL4031 was provided by Zaifeng Li, College of Plant Protection, Hebei Agricultural University, China. The 1,395 F2 plants derived from the Liaochun10 × RL4031 cross were used to test allelism. A total of 35 cultivars with known presence / absence of Lr13 were chosen to validate the co-segregating markers (Table S1). A panel of 524 Chinese wheat accessions/landraces was used to test the Lr13 frequencies (Supplementary Table S1). Through all the experiments, a susceptible, common wheat line, Xuezao, was used to check for successful inoculation.

#### Field Evaluation of Leaf Rust Symptoms at the Adult Stage

Wheat leaf rust isolate PHT (provided by the Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing,

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1. https://shigen.nig.ac.jp/wheat/komugi/
2. https://maswheat.ucdavis.edu/protocols/index.htm
3. https://www.wheatgenome.org/
visible symptoms, 0; indicated hypersensitive flecks, and 1–4, based on an infection type scale of 0–4, where 0 indicated no inoculation when the susceptible control was fully infected, leaf of each individual was evaluated about 1–2 month post-inoculation when the susceptible control was fully infected, Xuezao. The urediniospore was propagated by injecting urediniospore suspended in 0.1% Tween 20 into the leaf (Feekes stage 5) at least one tiller of each plant was inoculated by Agriculture University, Beijing, China. At the late tillering stage (Feekes stage 5) at least one tiller of each plant was inoculated by PHT isolate was avirulent on Liaochun10 and RL4031.

China) was used as the inoculum. PHT isolate was avirulent on Liaochun10 and RL4031.

The populations were sown at the experiment farm of China Agriculture University, Beijing, China. At the late tillering stage (Feekes stage 5) at least one tiller of each plant was inoculated by injecting urediniospore suspended in 0.1% Tween 20 into the leaf bundle with a 10 mL syringe. The urediniospore was propagated in the greenhouse on the susceptible control, Xuezao.

The infection type of the flag leaf and the top second leaf of each individual was evaluated about 1–2 month post-inoculation when the susceptible control was fully infected, based on an infection type scale of 0–4, where 0 indicated no visible symptoms, 0; indicated hypersensitive flecks, and 1–4, indicated small uredinia with necrosis, small- to medium-sized uredinia with green islands and surrounded by necrosis or chlorosis, medium- to large-sized uredinia with chlorosis, and large uredinia without chlorosis, respectively. Values 0–2 were categorized as resistant and 3–4 were classified as susceptible (Roelfs et al., 1992). A second assessment was conducted for each plant 4 days after the first examination.

**Allelism Tests**

We used an F2 population derived from Liaochun10 × RL4031 to determine the allelic relationships between genes Lr13 and LrLC10. The responses of each F2 plant to Pt race PHT was determined by the rust response method described above.

**Development of Molecular Markers**

The sequences of all the markers anchored in the LrLC10 (Lr13) genetic linkage map were used as queries to search against the Chinese Spring reference genome sequence (RefSeq v1.0) to define the genome interval of the resistance gene on chromosome arm 2BS. Near the LrLC10 locus, single-nucleotide polymorphisms (SNPs) or insertion/deletion (indel) polymorphisms were found based on re-sequencing result of the two parents (the concrete method refer to Chai et al., 2018) and the 300 bp flanking sequence of those indel sites which were ≥5 bp that were obtained from the Chinese Spring reference genome sequence4, Primer3 (v.0.4.0)5 was used to design the indel markers. The SNPs or indels (<5 bp) were converted into kompetitive allele-specific PCR (KASP) markers, which were designed using PolyMarker6. The markers used in this study were listed in Table 2.

**Marker Genotyping Assays**

PCR amplification was conducted in a 10 μL reaction volume consisting of 5 μL 2 × Tag PCR StarMix with loading dye, 50–100 ng/μL DNA 1.5 μL, 1.5 μL primer (mixture of forward and reverse primer, 2 μM), and 2 μL H2O. PCR proceeded with initial denaturation at 94°C for 5 min, then 35 cycles at 94°C for 30 s, 30 s at 50–60°C for primer annealing (depending on the specific primers), 72°C for 30 s of extension; and the final extension at 72°C for 5 min. The PCR product was separated in either 8% or 10% non-denaturing polyacrylamide gels (acrylamide: bisacrylamide = 39:1) that were silver stained and photographed. KASP assays were performed following the protocol described in Wu et al. (2018a).

**Construction of the Genetic Linkage Map**

We performed chi-square analysis of the leaf rust test data from the segregating F2 populations to confirm the goodness-of-fit of the observed ratios to theoretical expectations. The recombination frequencies of the resistance gene and the markers were calculated according to Chen et al. (2006). Using the Kosambi mapping function, we converted the recombination

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**TABLE 1 | Phenotype of 35 common wheat cultivars and genotyped with Lseq302 and Lseq102.**

| Accession | Phenotype | Lseq302 | Lseq102 | Progressive necrosis |
|-----------|-----------|---------|---------|----------------------|
| Zhounai30  | Resistant | A       | A       | N                    |
| Apache     | Resistant | A       | A       | Y                    |
| Maris Dove | Resistant | A       | A       | Y                    |
| Gaoyuc2018 | Resistant | A       | A       | Y                    |
| Gaoyuc9628 | Resistant | A       | A       | Y                    |
| Gaoyuc5766 | Resistant | A       | A       | Y                    |
| Kenong2009 | Resistant | A       | A       | N                    |
| Shannong06-278 | Resistant | A   | A       | N                    |
| Cunmai11   | Resistant | A       | A       | N                    |
| Yannong999 | Resistant | A       | A       | N                    |
| Yunmai53   | Resistant | A       | A       | Y                    |
| 16Y2N137   | Resistant | A       | A       | Y                    |
| 16Y2N132   | Resistant | A       | A       | Y                    |
| 16Y2N1688  | Resistant | A       | A       | Y                    |
| Zhounai36  | Resistant | A       | A       | N                    |
| 90214      | Resistant | A       | A       | Y                    |
| Zhouyuan9369 | Resistant | A   | A       | N                    |
| Bainong419 | Resistant | A       | A       | N                    |
| Nongda211  | Resistant | A       | A       | Y                    |
| Nongda212  | Resistant | A       | A       | Y                    |
| Altgold    | Resistant | A       | A       | Y                    |
| Lianping66 | Resistant | A       | A       | N                    |
| CI12633    | Resistant | A       | A       | Y                    |
| Nongda1108 | Resistant | B       | B       | N                    |
| Zhongmai66 | Resistant | B       | B       | N                    |
| Nongda3432 | Susceptible| B       | B       | N                    |
| Lianping99 | Susceptible| B       | B       | N                    |
| Xinmai26   | Susceptible| B       | B       | N                    |
| Shi4185    | Susceptible| B       | B       | N                    |
| Nongda4503 | Susceptible| B       | B       | N                    |
| Jimai229   | Susceptible| B       | B       | N                    |
| Xuezao     | Susceptible| B       | B       | N                    |
| Gao5       | Susceptible| B       | B       | N                    |
| CA1062     | Susceptible| B       | B       | N                    |
| Nongda3753 | Susceptible| B       | B       | N                    |

Y, indicates progressive necrosis; N, indicates the cross was not tested; A, indicates the band is identical with resistant parent; B, indicates the band is identical with susceptible parent.

4https://www.wheatgenome.org/
5http://bioinfo.ut.ee/primer3-0.4.0
6http://www.polymarker.info/
TABLE 2 | Markers used in this study.

| Marker    | Marker type | Forward primer                  | Reverse primer               | Annealing temperature |
|-----------|-------------|---------------------------------|------------------------------|-----------------------|
| Lseq29    | Indel       | CGCTTCTATCCCTGCTGGTG            | AGCATTGGAGGACAGAGA           | 56                    |
| Lseq31    | Indel       | CCAAGCTTGAGCAACGAGTGA           | CTTGGGCTCTGTGCTCATCTTG       | 56                    |
| Lseq35    | Indel       | ACTCAACAGGCTAATCAGGGT           | GAACAAACCTGACATCGGG          | 56                    |
| Lseq54    | Indel       | CCAACCAAGAATGCTAAGAAGC          | CACCAGTACGAGATAAGG           | 56                    |
| Lseq55    | Indel       | CAGTTGGACGAGGGGAGTG             | GAACCAATCGTCGACAGAG          | 56                    |
| Lseq102   | Indel       | CAACCTCAGATGGCTCGCTATC          | GAACACCGAAGTTGCTTGG          | 56                    |
| Lseq85    | Indel       | CAGCAGATGGATGCGAAATA            | GCACAGATCCGACGG              | 56                    |
| Lseq99    | Indel       | TACGACCATGGGGGTACGGTA           | CTACAGTTGTCGCTTAG            | 56                    |
| Lseq100   | Indel       | ATGGGGAATGGCTGCTTGG             | TATTCTGCTACATCGAGG           | 56                    |
| Lseq3     | Indel       | CCAGTTGGACGAGGGGAGTG            | CAGGGTTCGGGGTGGGGGAAG        | 56                    |
| Lseq11    | Indel       | CAGTCAGGAAACGGGTGCCAAC          | GTTGGGACACTGACACGG            | 56                    |
| Lseq302   | KASP        | GAAGGTCAGGAGGTCGAGGGAT          | GCTGAAGTTGAGTGCTGCA          | –                     |

FIGURE 1 | Phenotype of resistant parent Liaochun10, susceptible parent 87-1, and 7D49 30 days after inoculation with Pt race PHT.

RESULTS

Genetic Analysis of Wheat Leaf Rust Resistance Gene LrLC10 in Two Segregating Populations

The parental lines 87-1 and 7D49 were highly susceptible to Pt race PHT, [infection type (IT) = 3], whereas Liaochun10 was highly resistant (IT = 0, Figure 1). We examined the two segregating F2 populations that grew from crossing the susceptible lines with Liaochun10. The 87-1 crossed with Liaochun10 produced the F2 population including 3,057 plants, of which 2,300 were resistant and 757 were susceptible to Pt isolate PHT ($\chi^2_{2.1} = 0.092, P > 0.05$). In the F2 population derived from 7D49 crossed with Liaochun10, 624 plants were resistant and 227 were susceptible to Pt race PHT ($\chi^2_{2.1} = 1.268, P > 0.05$). These results indicated that leaf rust resistance in Liaochun10 is controlled by a single dominant gene.

Allelism Test of LrLC10 and Lr13

Liaochun10, RL4031, and the 1,395 F2 plants from the cross of Liaochun10 × RL4031 were evaluated against Pt race PHT. We found no susceptible plants, thus confirming that LrLC10 in Liaochun10 was on the same locus as Lr13. Since there was Lr13 donor parents Frontanan and UP301 in the Liaochun10 pedigree (Singh and Gupta, 1991; He et al., 2001; Pathan and Park, 2006), we concluded that leaf rust resistance gene LrLC10 in Liaochun10 is Lr13.

Molecular Mapping of Leaf Rust Resistance Gene LrLC10 (Lr13)

We chose 92 extremely susceptible individuals from the 7D49 × Liaochun10 F2 population to be re-genotyped using markers linked to LrLC10 that were established by Lv et al. (2017) (Figures 2A,B). To define the LrLC10 physical interval, we searched the sequences of all markers anchored in the genetic map against the Chinese Spring reference genomic sequence (RefSeq v1.0) and found that the relative physical positions of those markers were generally consistent with the genetic map (Figures 2B,C). Two flanking markers, CAUT163 and Xbarc18, spanned an approximately 100 Mb region (153,676,602–255,348,323) in the reference genome, and here we detected numerous sequence variations between the parents when we analyzed the re-sequencing data. Twenty indel primer pairs were designed based on those insertion/deletion polymorphisms we found between parental lines in the 11 Mb (159,000,000–170,000,000) section that was 6 Mb from marker CAUT163 going toward LrLC10. Among these, 4 markers (Lseq22, Lseq29, Lseq31, and Lseq35) were successfully added to the genetic map and the LrLC10 gene was delimited within a 1.65 cM area between markers CAUT163 and Lseq22, an interval corresponding to a 5.7 Mb (153,676,602–159,302,377) region in the CS reference genome (Figure 2C).
Development of Tightly Linked Markers to LrLC10 (Lr13)

We used those co-dominant flanking markers, CAUT163 and Lseq22, to identify recombinants in the 984 homozygous, susceptible F2 plants. Thirty-two recombinant plants were identified and then used for fine mapping of LrLC10.

Based on the parents' re-sequencing data that corresponded to the 5.7 Mb interval of the CS RefSeq v1.0, we designed 80 indel primers and a KASP marker. They were tested on 3 parental lines and 10 markers were polymorphic between the parents and used to finely map LrLC10 (Figure 3).

Among the 32 recombinant plants, 28 showed recombination between marker CAUT163 and LrLC10, while 4 recombination events were detected between marker Lseq22 and LrLC10. We used the 10 polymorphic markers located between the flanking markers to examine these recombinants and found that the closest flanking markers were Lseq301 (with 1 recombination event) and Lseq85 (with 2 recombination events) and markers Lseq302 and Lseq102 co-segregated with LrLC10 (Figure 3). These results suggest that LrLC10 locus is located in a 314.3-kb region between markers Lseq85 and Lseq301 (157,688,415–158,002,717) in the CS RefSeq v1.0 (Figure 3). Markers Lseq85 and Lseq301 were designed based on the 5 bp and 6 bp deletions, respectively, in Liaochun10 as compared to 7D49. The KASP marker Lseq302 was based on the SNP (A/T) detected in exon 2 of TraesCS2B01G182800 between Liaochun10 and 7D49 (Figure 4A and Table 3), while Lseq102 was developed based on a 9-bp deletion in the TraesCS2B01G183000 coding region between Liaochun10 and 7D49 (Figure 4B and Table 3).

Validation of LrLC10-Co-segregating Markers for Marker-Assisted Selection

We wanted to test if these co-segregating markers (Lseq302 and Lseq102) could be used for marker-assisted selection of LrLC10 in different backgrounds. We tested 25 wheat leaf rust resistant accessions and 10 susceptible cultivars with those 2 markers to evaluate their utility. Twenty-three of the resistant accessions had the same marker genotypes as Liaochun10 and all the susceptible cultivars' genotypes were identical to 87-1 and 7D49 (Figures 5A,B and Table 1). Moreover, we found that the F1 plants of the crosses of those 14 of the 23 resistant accessions with Xuezao, which was proved to carry the hybrid necrosis gene Ne1 (unpublished results), showed progressive necrosis (Table 1). According to Zhang et al. (2016), Lr13 and Ne2m are the same gene; so by inference, the F1 plants' phenotypes indicate that those 14 cultivars have leaf rust resistance gene Lr13. These results suggest that markers Lseq302 and Lseq102 can be used to identify Lr13.

To evaluate the distribution of Lseq302-L and Lseq102-L (the marker alleles of Lseq302/Lseq102 in Liaochun10) in China, a panel of 524 common wheat accessions/landraces from China was tested with these markers. Lseq302-L and Lseq102-L always co-existed in all the cultivars, forming a specific haplotype block. The haplotype of Lseq302-L/Lseq102-L was present in various frequencies in 4 of 10 agro-ecological production zones: I, North China winter wheat region (30.76%); II, Yellow and Huai River valleys winter wheat region (24.56%); III, middle and lower Yangtze River valley winter wheat region (14.81%); and V, South China winter wheat region (33.33%) (Figure 5C and Table 4).
FIGURE 3 | Fine mapping of LrLC10 and three annotated genes localize in the mapping interval on Chinese Spring reference genome. Phenotypes and genotypes of four F2 recombinants including (F2-3531, F2-2704, F2-3369, F2-2104) are showed. The name and phenotype of F2 individuals were labeled in the left and right, respectively. Black, white and gray blocks present genomic region of LC10, 87-1/7D49 and heterozygous, respectively. r indicated the number of recombinants.

FIGURE 4 | Structure of annotated genes [TraesCS2B01G182800 and TraesCS2B01G183000 (A,B)] showing the nucleotide and amino acid sequences polymorphism between resistant and susceptible parents. Introns, exons are shown in lines, blue boxes. Red and black fonts represent resistant and susceptible parents, respectively. The numbers in bracket represent the positions of nucleotide sequences relative to ATG. F.S. represents frame shift, - indicates nucleotide deletion, IN represents amino acid insertion, DE represents amino acid deletion.
### TABLE 3 | Sequence comparison of the annotated genes.

| Gene-ID          | Position on reference | Region of the gene | Position in the gene | Sequence variants (LC10/7D49) | Protein variants (LC10/7D49) |
|------------------|-----------------------|--------------------|----------------------|-------------------------------|-----------------------------|
| TraesCS2B01G182800 | Exon2                 | Exon2              | 462                  | A/T                           | S/C                         |
| 157695820        | Exon2                 | Exon2              | 827                  | A/G                           | –                           |
| 157695441        | Exon2                 | Exon2              | 841                  | C/T                           | N/S                         |
| 157695415        | Exon2                 | Exon2              | 867                  | C/T                           | N/D                         |
| 157695245        | Exon2                 | Exon2              | 1037                 | A/C                           | M/I                         |
| 157695237        | Exon2                 | Exon2              | 1045                 | C/T                           | D/G                         |
| 157695131        | Exon2                 | Exon2              | 1151                 | A/G                           | –                           |
| 157694999        | Exon2                 | Exon2              | 1283                 | C/A                           | –                           |
| 157694868        | Exon2                 | Exon2              | 1414                 | G/A                           | M/T                         |
| 157694755        | Exon2                 | Exon2              | 1527                 | G/C                           | G/R                         |
| 157694710        | Exon2                 | Exon2              | 1572                 | G/A                           | –                           |
| 157694690        | Exon2                 | Exon2              | 1592                 | G/A                           | –                           |
| 157694666        | Exon2                 | Exon2              | 1626                 | T/G                           | Q/K                         |
| 157694599        | Exon2                 | Exon2              | 1683                 | T/G                           | Q/K                         |
| 157694590        | Exon2                 | Exon2              | 1692                 | A/T                           | T/S                         |
| 157694468        | Exon2                 | Exon2              | 1814                 | A/G                           | –                           |
| 157694465        | Exon2                 | Exon2              | 1817                 | G/A                           | –                           |
| 157694447        | Exon2                 | Exon2              | 1835                 | G/A                           | –                           |
| 157694427        | Exon2                 | Exon2              | 1855                 | A/T                           | Y/F                         |
| 157694415        | Exon2                 | Exon2              | 1867                 | G/T                           | E/A                         |
| 157694404        | Exon2                 | Exon2              | 1878                 | C/T                           | S/G                         |
| 157694391        | Exon2                 | Exon2              | 1891                 | G/C                           | C/S                         |
| 157694334        | Exon2                 | Exon2              | 1948                 | C/T                           | K/R                         |
| 157694248        | Exon2                 | Exon2              | 2034                 | T/A                           | S/T                         |
| 157694141        | Exon2                 | Exon2              | 2141                 | T/G                           | –                           |
| 157694095        | Exon2                 | Exon2              | 2187                 | C/T                           | K/E                         |
| 157694089        | Exon2                 | Exon2              | 2193                 | A/G                           | P/S                         |
| 157694084        | Exon2                 | Exon2              | 2198                 | A/-/AAT                       | Frame shift                 |
| 157694082        | Exon2                 | Exon2              | 2200                 | A/-/AT                        | Frame shift                 |
| 157694050        | Exon2                 | Exon2              | 2232                 | T/C                           | G/R                         |
| 157693981        | Exon2                 | Exon2              | 2301                 | C/T                           | K/E                         |
| 157693981        | Exon2                 | Exon2              | 2321                 | A/G                           | –                           |
| 157693922        | Exon2                 | Exon2              | 2360                 | A/G                           | –                           |
| 157693616        | Exon2                 | Exon2              | 2666                 | G/-/GCTA                      | Insertion                   |
| 157693556        | Exon2                 | Exon2              | 2726                 | G/A                           | –                           |
| 157693457        | Exon2                 | Exon2              | 2825                 | C/G                           | L/F                         |
| 157693411        | Exon2                 | Exon2              | 2871                 | G/T                           | H/N                         |
| 157693396        | Exon2                 | Exon2              | 2886                 | C/T                           | A/T                         |
| 157693385        | Exon2                 | Exon2              | 2897                 | A/G                           | –                           |
| 157693336        | Exon2                 | Exon2              | 2946                 | T/G                           | N/H                         |
| 157693331        | Exon2                 | Exon2              | 2951                 | A/C                           | C/W                         |
| 157692236        | Intron2               | Intron2            | 4046                 | T/C                           | –                           |
| 157692035        | Intron2               | Intron2            | 4247                 | C/T                           | –                           |
| 157691555        | Intron2               | Intron2            | 4727                 | A/G                           | –                           |
| 157691227        | Intron2               | Intron2            | 5055                 | A/G                           | –                           |
| 157691178        | Intron2               | Intron2            | 5104                 | C/T                           | –                           |
| 157691106        | Intron2               | Intron2            | 5176                 | C/T                           | –                           |
| 157691012        | Intron2               | Intron2            | 5270                 | G/A                           | –                           |
| 157690905        | Intron2               | Intron2            | 5377                 | T/C                           | –                           |
| 157690902        | Intron2               | Intron2            | 5380                 | T/C                           | –                           |
| 157690874        | Intron2               | Intron2            | 5408                 | A/G                           | –                           |
| 157690808        | Intron2               | Intron2            | 5474                 | T/C                           | –                           |

(Continued)
TABLE 3 | Continued

| Gene-ID | Position on reference | Region of the gene | Position in the gene | Sequence variants (LC10/7D49) | Protein variants (LC10/7D49) |
|---------|-----------------------|---------------------|----------------------|--------------------------------|-----------------------------|
| 157690406 | Intron2               | 5876                | C/T                  | –                              | –                           |
| 157690397 | Intron2               | 5885                | G/A                  | –                              | –                           |
| 157690385 | Intron2               | 5897                | T/C                  | –                              | –                           |
| 157690201 | Intron2               | 6081                | C/T                  | –                              | –                           |
| 157690155 | Intron2               | 6127                | A/G                  | –                              | –                           |
| 157689837 | Intron2               | 6445                | A/G                  | –                              | –                           |
| 157689423 | Intron2               | 6859                | AT/A-                | –                              | –                           |
| 157689364 | Intron2               | 6918                | G/A                  | –                              | –                           |
| TraesCS2B01G183000 | Exon1     | 27                  | G-/GA                | Frame shift                     |                             |
| 157755306 | Exon2                 | 609                 | G-/AAGCGACGAGTC       | Deletion                        |                             |
| 157755888 | Intron2               | 808                 | C-/CA                | –                              | –                           |

Position in the gene represents the positions of nucleotide sequences relative to ATG.

FIGURE 5 | Validation of Lr13 diagnostic markers (A, B) and haplotype distribution of Lseq302 and Lseq102 in 524 common wheat cultivars/landraces from China (C). A co-segregated marker Lseq102 genotyped several Lr13 carriers and susceptible accessions in PAGE gel. The number 1 and 24 present Liaochun10 and 87-1, respectively, 2–23, 25–31 indicate Lr13 carriers and susceptible lines, respectively. M: Marker; B plots of KASP marker Lseq302 tested 23 Lr13 carriers (blue dots), 10 susceptible lines (red dots) and other cultivars with unknown leaf rust resistance. Black dots are water controls; C I, north China winter wheat region; II, Yellow and Huai River valley winter wheat region; III, middle and lower Yangtze River valley winter wheat region; IV, south-western winter wheat region; V, south China winter wheat region; VI, north-eastern spring wheat region; VII, northern spring wheat region; VIII, north-western spring wheat region; IX, Qinghai–Tibet spring–winter wheat region; X, Xinjiang winter–spring wheat region.

DISCUSSION

Seven leaf rust resistance genes (Lr13, Lr16, Lr23, Lr35, Lr48, Lr73, and LrZH22) had been mapped on wheat chromosome 2BS (Seyfarth et al., 1999; Park et al., 2014; Nsabiyera et al., 2016; Wang et al., 2016; Zhang et al., 2016; Chhetri et al., 2017; Kassa et al., 2017). We found that LrLC10 is located in the region 157,688,415–158,002,717 bp (Figure 3) (2BS1-0.53-0.75) on the reference sequence of Chinese Spring (RefSeq v1.0). Of those 7 Lr genes, only Lr13 and LrZH22 were reported to be located in the same region as LrLC10 (Wang et al., 2016; Zhang et al., 2016). LrZH22 confers resistance to leaf rust at both the seedling and adult stages (Wang et al., 2016), whereas resistance gained from LrLC10 is effective only after the four-leaf stage. Up to now, the relationship between LrLC10 and Lr13 was unknown, but we confirmed that LrLC10...
TABLE 4 | Percentage of different alleles in Chinese accessions.

| Agro-ecological region | No. of accessions | Lseq302 | Lseq302-A | Lseq302-B | Lseq302-C | Percentage of the allele |
|------------------------|------------------|---------|-----------|-----------|-----------|------------------------|
| I                      | 89               | 62      | 0.7       | 0.7       | 0.7       | 27                     |
| II                     | 388              | 294     | 0.76      | 0.24      | 0.85      | 94                     |
| III                    | 27               | 23      | 0.85      | 0.15      | 0.65      | 10                     |
| IV                     | 15               | 10      | 0.65      | 0.33      | 0.33      | 4                      |
| IX                     | 4                | 3       | 0.75      | 0.25      | 0.25      | 1                      |
| Total                  | 524              | 394     | 0.75      | 0.75      | 0.75      | 130                    |

I, Northern winter wheat region; II, Yellow and Huai River valley winter wheat region; III, low and middle Yangtze River valley winter wheat region; IV, south-western winter wheat region; IX, Qinghai–Tibet spring-winter wheat region.

The pedigree of Liaochun10 is 1048 (Ke71F4370-10/Mexipak66//UP301) × Liaoj07181-2 (Liaochun6/Jinghong1) and the parents of Liaochun6 are Frontana and Liaochun1 (He et al., 2001). Since the leaf rust resistance gene in Frontana and UP301 is Lr13 (Singh and Gupta, 1991; Pathan and Park, 2006), the leaf rust resistance gene in Liaochun10 may be derived from the Frontana or UP301 parent.

In the process of fine mapping LrLC10, enough polymorphic markers were found to narrow down the genetic interval covering the targeted gene. We accomplished this by re-sequencing the parental lines and developing indel and SNP markers based on sequence information in the targeted region. Four indel markers revealed polymorphisms and localized LrLC10 gene in a 1.65 cM genetic interval, which corresponded to a 5.7 Mbp interval on the Chinese Spring reference genomic sequence (Figures 2B,C). Based on sequence diversities between the parental lines on the candidate interval, we designed 9 indel markers and a KASP marker and used these to test the 32 recombinants that we identified using LrLC10-flanking markers CAUT163 and Lseq22 derived from 984 homozygous, susceptible F2 individuals. LrLC10 was finally delimited into a 314.3-kb genomic interval on the Chinese Spring reference sequence v1.0 by markers Lseq301 and Lseq85 (Figure 3). This mapping of LrLC10 demonstrated our methods efficiently developed molecular markers based from the re-sequencing data of the parents.

Lr13 is one of the most widely distributed leaf rust resistance genes in wheat (McIntosh et al., 1995), but it has become ineffective in some regions, such as Mexico and South America (Singh and Rajaram, 1992). However, it is effective in combination with other resistance genes, such as Lr3ka, Lr34, and Lr16 (Kolmer, 1992). Therefore, we need diagnostic molecular markers for Lr13 to facilitate selection or stacking it with other Lr genes. In our study, KASP marker Lseq302 and indel marker Lseq102 co-segregated with Lr13 and were effective in diverse wheat backgrounds (Figures 5A,B, Table 1, and Supplementary Table S1). Therefore, these diagnostic markers may be used for efficient marker-assisted selection of Lr13, thus enabling researchers to either pyramid it with other adult plant resistant genes to achieve durable leaf rust resistance or stack it with stripe rust, stem rust, and powdery mildew resistance genes (e.g., Yr27, Srp40, and pm42) on chromosome 2BS, to create multi-resistance accessions (McDonald et al., 2004; Hua et al., 2009; Wu et al., 2009).

To date, several leaf rust resistance genes have been isolated in wheat: Lr1, Lr10, Lr21, Lr34, Lr67, and Lr22a (Feuillet et al., 2003; Huang et al., 2003; Cloutier et al., 2007; Krattinger et al., 2009; Moore et al., 2015; Thind et al., 2017). Among those genes, Lr34 encodes an ATP-binding cassette transporter that carries resistance-related metabolites that affect the growth of pathogenic bacteria (Krattinger et al., 2009). Lr67 encodes a hexose transporter, LR67res, that through heterodimerization with the LR67-susceptible functional transporter LR67sus, exerted a dominant-negative effect that restricted the growth of multiple biotrophic pathogens by reducing their glucose uptake (Moore et al., 2015). Lr34 and Lr67 provide wheat with resistance to multiple fungal pathogens. The others are typical resistance
genes with nucleotide-binding site leucine-rich repeat (NBS-LRR) domains (Feuillet et al., 2003; Huang et al., 2003; Cloutier et al., 2007; Thind et al., 2017). However, LrLC10 (Lr13) was shown to be a race-specific resistance gene (Dyck et al., 1966), thus it most likely has the R-gene structure of NLR.

In this study, we delimited LrLC10 to a 314.3 kb region on short arm of chromosome 2B in Chinese Spring reference genome sequence (RefSeq v1.0). Three high confidence genes (TraesCS2B01G182800, TraesCS2B01G182900, and TraesCS2B01G183000) have different functions based on the annotation of Chinese Spring reference genome7, which encode a typical NBS-LRR protein, Ribonuclease, and an F-box domain containing Leucine-rich repeats protein were located in this region. DNA sequence comparison showed that the parents did not differ in TraesCS2B01G182900. Compared to 7D49, Liaochun10 had a 9 bp deletion in its TraesCS2B01G183000 coding region, resulting in a deletion of three amino acids, and another 1-bp deletion that led to a translational frame shift (Figure 4B and Table 3). In TraesCS2B01G182800, we found many sequence variations, including 56 SNPs and 4 indels, between Liaochun10 and its rust-susceptible parent. Among them, we found 18 SNPs and one indel in the intron region, and 38 SNPs and three indels in exon 2. Among those 38 SNPs, 26 caused amino acid substitutions, and two of the indels led to a translational frame shift. A 3-bp insertion in 7D49 resulted in an amino acid insertion that was not found in Liaochun10 (Figure 4A and Table 3). Based on the TraesCS2B01G182800 and TraesCS2B01G183000 sequence polymorphisms between the parental lines, we developed the markers Lseq102 and Lseq302 and found that they co-segregated with LrLC10 (Figure 3). In total, our results suggest that LrLC10 (Lr13) might be one of those two annotated genes. However, there is still a chance that the sequence corresponding to LrLC10 is absent in the CS genomic sequence. Therefore, our analysis of re-sequencing data based on the wheat reference genome sequence is not enough to be absolutely certain of its identity. Because of this, a library of the resistant parent must be constructed so that a physical map would enable the cloning of LrLC10. Recently, some alternative methods (e.g., MutRenSeq, TACCA, and MutChromSeq) have been used to clone wheat disease resistance genes

7 https://urgi.versailles.inra.fr/download/ivgsc/IVGSC_RefSeq_Annotations/ v1.0/

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DATA AVAILABILITY STATEMENT
The datasets generated for this study can be found in the European Variation Archive (EVA) using accession number PRJEB37197.

AUTHOR CONTRIBUTIONS
LQ and CX conceived the project. LQ, HW, WW, YuL, and JM performed the experiments. MG and YiL assisted in revising the manuscript. WG analyzed the re-sequencing data. JMa, ZH, and QS provided materials. LQ wrote the manuscript. CX revised the manuscript.

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SUPPLEMENTARY MATERIAL
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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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