Combining QTL Mapping with Genome Resequencing Identifies an Indel in an *R* Gene that is Associated with Variation in Leaf Rust Disease Resistance in Poplar

Tashbek Nvsvrot,^{1,2} Wenxiu Xia,^{1,3} Zheng'ang Xiao,¹ Chang Zhan,¹ Meifeng Liu,¹ Xiaoqing Yang,¹ Yan Zhang,¹ and Nian Wang^{1,2,†}

¹ College of Horticulture and Forestry Sciences, Huazhong Agricultural University, Wuhan, 430070, China

² Hubei Engineering Technology Research Center for Forestry Information, Huazhong Agricultural University, Wuhan, 430070, China

³ Logistics Service Group, Wuhan University, Wuhan, 430070, China

Accepted for publication 16 January 2020.

ABSTRACT

Poplar trees (*Populus* spp.) are important and are widely grown worldwide. However, the extensive occurrence of leaf rust disease caused by *Melampsora* spp. seriously inhibits their growth and reduces their biomass. In our previous study, a high-quality genetic map was constructed for the poplar F1 population I-69 × YYY by using next-generation sequencing-based genotyping-by-sequencing. Here, we collected phenotypic data on leaf rust disease resistance on three different dates for all 300 progenies of the F1 population. Combining a high-quality genetic map and phenotypic data, we were able to detect 11 major quantitative trait loci (QTLs) for leaf rust disease resistance. Among these 11 QTLs, two pairs were detected on at least two dates. In the corresponding genomic sequence, we found that resistance (*R*) gene clusters were located in these

two QTL regions. By using genome resequencing, PCR confirmation and statistical analysis, a 611-bp deletion within an *R* gene in one QTL region was found to be associated with variation in leaf rust disease resistance. A PCR-based examination of this 611-bp deletion was performed. This 611-bp deletion was also found to affect mRNA splicing and form a new protein with the loss of some key protein domains. Based on this study, we were able to determine the genetic architecture of variation in poplar leaf rust disease resistance, and the 611-bp deletion in the *R* gene could be used as a diagnostic marker for future poplar molecular breeding.

Keywords: genetics and resistance, genome resequencing, leaf rust disease resistance, poplar, QTL mapping, *R* gene indel

Poplar (*Populus* spp.), one of the most important trees, is widely grown worldwide. There are almost 30 species in the *Populus* genus, and most of them can be used as wood, pulp, paper, and fuel due to their fast-growing characteristics. In 2006, a high-quality genomic sequence was released for *Populus trichocarpa* (Tuskan et al. 2006). During the past decade, several genomes of different species—that is, *P. euphratica* (Ma et al. 2013), *P. alba* (Ma et al. 2019), and *P. tremula* × *alba* 717-1B4 (Xue et al. 2015)—were also sequenced, benefiting from high-throughput and low-cost next-generation sequencing (NGS) technology. Additionally, it is easy to produce transgenic lines for some poplar species. All of these characteristics enable poplar trees to become model tree species. Therefore, significant progress has been made in poplar genomics, genetics, and breeding in recent decades. However, the extensive occurrence of diseases in poplar largely inhibits poplar cultivation. Among these diseases, leaf rust is the primary disease affecting poplar growth and is caused by several species of *Melampsora*. After infection by *Melampsora* spp., the photosynthetic efficiency and growth rate of poplar decrease significantly (Royle and Ostry 1995; Widin and Schipper 1981); moreover, their resistance to other pests

and diseases is also largely weakened. Therefore, understanding the leaf rust pathogenic mechanism and uncovering disease resistance resources are major tasks for poplar breeding.

Recently, a number of studies have focused on poplar leaf rust disease. The genome of *Melampsora larici-populina*, the major causal agent of poplar leaf rust, was sequenced, and 16,399 gene models were predicted (Duplessis et al. 2011). Benefiting from this genomic information, some candidate effectors that are likely involved in establishing a compatible poplar–pathogen interaction (Lorain et al. 2015; She et al. 2017). The candidate effector MLP12407 from *M. larici-populina* was found to interact with poplar TOPLESS-related protein 4 by in planta co-immunoprecipitation in a subsequent study (Petre et al. 2015). In addition to the above studies mainly focusing on the leaf rust pathogen, there have also been a number of studies on the pathogen hosts of poplar. The transcription factor PtWRKY23 was found to play a role in resistance to rust infection (Levee et al. 2009). With the development of NGS technology, genome-wide identification of genes or other genetic elements for leaf rust resistance was also performed. In our previous studies, long noncoding RNAs and genes that respond to *M. larici-populina* infection were comprehensively investigated (Luo et al. 2019; Wang et al. 2017; W. Xia et al. 2017). A number of candidate genes responsible for disease defense were predicted. Other studies were also performed to identify genes that played roles in susceptibility to pathogen infection or plant defense during leaf rust infection (Hacquard et al. 2013; Rinaldi et al. 2007). Based on these studies, we were able to understand more about poplar leaf rust disease; however, the knowledge is still insufficient to generate poplar trees with high resistance to this disease.

Quantitative trait locus (QTL) analysis, also known as QTL mapping, has proven to be a powerful method for uncovering and screening genetic factors for a given trait in plants. Usually, two

†Corresponding author: N. Wang; wangn@mail.hzau.edu.cn

Funding: Financial support for this work was provided by the National Natural Science Foundation of China (NSFC grant number 31670651) and the Fundamental Research Funds for the Central Universities (number 2662019PY047).

T. Nvsvrot and W. Xia contributed equally to this work.

*The e-Xtra logo stands for “electronic extra” and indicates that one supplementary figure and four supplementary tables are published online.

The author(s) declare no conflict of interest.

types of information are required in a QTL mapping study: genotypic and phenotypic data. Benefiting from newly developed NGS technology, large amounts of genotypic data can be obtained in a short time at low cost. This has made QTL mapping more powerful in recent years. This advantage is very clear for crop studies. A number of QTLs were identified for key trait variations in rice (D. Xia et al. 2018), wheat (Kage et al. 2017), maize (Dell'Acqua et al. 2015), rapeseed (Wang et al. 2016), and so on. Compared with studies in these major crops, there have been fewer studies on QTL mapping in trees due to the difficulty of generating large populations and trait records in multiple environments. In our previous studies, NGS-based high-density genetic maps were constructed for grapevines and poplar trees, and QTL mapping was also performed for the traits of fruit sugar content and leaf morphology (Chen et al. 2015; Wang et al. 2012; W. Xia et al. 2018). A number of other, similar studies were conducted for tree species; for example, QTL mapping of bud flush, bud set, and height growth in white spruce (Pelgas et al. 2011); QTL mapping of stem height in oil palm (Lee et al. 2015); and wood quality dissection via QTL mapping in *Eucalyptus nitens* (Thumma et al. 2010). All of these studies suggested that QTL mapping is very suitable for uncovering the genetic mechanisms underlying complex quantitative traits in plants.

For poplar leaf rust, some studies identified molecular markers associated with variations in resistance to this disease. Restriction fragment length polymorphism and amplified fragment length polymorphic markers were used to map QTLs for resistance to *M. larici-populina* in 1996 (Cervera et al. 1996; Villar et al. 1996). By using a *P. deltoides* × *P. trichocarpa* F1 population, nine QTLs were also detected with 391 PCR-based molecular markers (Jorge et al. 2005). Two major poplar leaf rust resistance QTL regions—namely, MXC3 and MER—were carefully investigated for their flanking genomic sequence (Jorge et al. 2005). By investigation of the genetic and physical maps, qualitative and quantitative resistances to leaf rust were finely mapped within two nucleotide-binding site leucine-rich repeat (NBS-LRR)-rich genomic regions of chromosome 19 in poplar (Bresson et al. 2011). Several other similar studies were also conducted for screening novel QTL regions for leaf rust resistance or proving previously reported QTLs (Dowkiw and Bastien 2005; La Mantia et al. 2013; Martin et al. 2016; Tabor et al. 2000). In the above studies, a small number of molecular markers or population sizes were employed for leaf rust disease resistance QTL mapping. Most studies were not able to provide effective candidate genes or closely linked molecular markers for the detected QTLs. Therefore, this information was difficult to apply in molecular marker-assisted breeding (MAS).

In our previous study, a high-quality genetic map was constructed for the poplar F1 population I-69 × XYY by using NGS-based genotyping-by-sequencing (GBS) (W. Xia et al. 2018). Here, we collected phenotypic data on three different dates for leaf rust disease resistance. Combining a high-quality genetic map with phenotypic data, genome-wide QTL mapping was conducted for leaf rust disease resistance, and 11 QTLs showed logarithm of odds (LOD) values above the thresholds. Among these 11 QTLs, two pairs were detected on at least two dates. In the corresponding genomic regions, we found that resistance (*R*) gene clusters were located within these two QTL regions. By using genome resequencing, PCR confirmation, and statistical analysis, a 611-bp deletion within one *R* gene was found to be associated with variation in leaf rust resistance. Based on this study, we were able to determine the genetic architecture of the variation in poplar leaf rust disease resistance, and the 611-bp deletion in the *R* gene could be used as a diagnostic marker for future poplar MAS.

MATERIALS AND METHODS

Plant materials and growth conditions. The F1 population was used in our previous study (W. Xia et al. 2018). The female

parent was I-69 (*P. deltoides*), while the male parent was Xiaoyeyang (*P. simonii* Carr., designated as XYY). All 300 F1 progenies were planted in the Poplar Germplasm Repository of Huazhong Agricultural University in Wuhan, China (N114°20', W30°28'). For each progeny, there were at least three lines in the field. The plants were pruned, and a 10-cm branch was left for each individual plant in the winter.

Genetic map construction. There were three types of genetic maps for the F1 population: ll × lm (male map), np × nn (female map), and ab × ab (F2 map). In our previous study, we reported the male and female maps. High-quality single-nucleotide polymorphism (SNP) markers for ab × ab were employed for F2 map construction by using the software Joinmap 4.1 (Van 2006). All of the parameters and strategies for the F2 map construction were the same as those for the male and female maps (W. Xia et al. 2018). The procedures for SNP generation, quality control, and filtration were also identical to our previous report (W. Xia et al. 2018). Briefly, 300 GBS libraries for the 300 progenies were constructed and were employed for subsequent high-throughput sequencing by the HiSeq 2500 platform. The clean data were mapped onto *P. trichocarpa* genome version 3.0 and SNPs were called by GATK and SAMtools (Li 2011; McCormick et al. 2015). The concordance SNPs were filtered by an in-house-programmed Python package. The parameters used for SNP filtering were as follows: at least three reads support one allele and five reads support one SNP; genotype frequency for a SNP in the 300 plants should be above 0.05; and SNPs should have a missing rate of <15%.

Scoring of leaf rust disease symptoms and QTL mapping. In order to investigate the phenotypic data for the 300 I-69 × XYY progenies, we scored the values of leaf rust disease symptoms on three different dates from 2017 to 2018. Poplar leaf rust disease mainly occurs in July and September and the casual agent is *M. larici-populina* in Wuhan, China (Dai et al. 2019; Liu et al. 2008; Wan 2006; Wan et al. 2013). Therefore, we investigated the leaf rust symptoms in July each year, and then the plants were pruned to the ground (10-cm stems were left). The newly developed leaves for each plant were used for scoring in September. The scoring strategy was similar to that in previous reports, with slight changes (Jorge et al. 2005; La Mantia et al. 2013; Tabor et al. 2000). Briefly, the most seriously infected leaf on an individual plant was used for scoring, and this leaf was photographed by a digital camera. The number or area of uredospores was determined by using Image-Pro Plus software (<https://image-pro-plus.updatestar.com/en>). The leaf rust susceptibility scores were classified into six grades. Grades 1 to 6 corresponded to no leaf rust symptoms, 1 to 10 uredospores, 11 to 50 uredospores, 51 uredospores to 2% leaf rust area, 2 to 5% leaf rust area, and >5% leaf rust on the leaves, respectively. Each progeny was scored for at least three individual plants, and the average score was calculated in one environment. The scores for leaf rust symptoms were then rounded to the nearest whole number.

The R package R/qtl was employed for QTL analysis (Arends et al. 2010). QTL mapping in each environment was performed for the three genetic maps individually. A single-QTL model was first performed to determine the maximum number of QTLs in each environment. Thereafter, the multiple-QTL model (MQM), with parameters including interactions among QTLs, was used to identify true QTLs. The thresholds for QTLs in each environment were determined by 1,000 permutations, and the significance level was set to a P value = 0.05. QTL confidence intervals (CIs) were calculated by the function 'lodint' implemented in MQM, and the parameters were set as 1.5-LOD, which corresponds to 95% confidence. The QTLs were illustrated by using MapChart software (Voorrips 2002).

Parental genome resequencing and bioinformatic analysis. High-quality genomic DNA from the two parents (I-69 and XYY) was isolated by using a standard cetyltrimethylammonium bromide (CTAB) method (Clarke 2009). The DNA was sequenced by using the Illumina HiSeq 10× genomics platform, and the raw

data were filtered by using Trimmomatic software with default parameters (Bolger et al. 2014). The clean reads were mapped onto *P. trichocarpa* genome version 3.0 (Tuskan et al. 2006). Thereafter, SNP and insertion or deletion (indel) variations were identified by standard GATK or indel pipelines with default parameters (McCormick et al. 2015; Ye et al. 2009). The non-synonymous SNPs were identified by using the package of SnpEff (Cingolani et al. 2012). SNP and indel variations were finally filtered and summarized by a custom Python script.

Protein sequence alignment was performed with the online server <https://www.ebi.ac.uk/Tools/msa/>, and protein domains were analyzed by using the SMART database (<http://smart.embl-heidelberg.de/>).

PCR amplification and cDNA cloning. The DNA of all 300 progenies and the two parents was isolated by using a standard CTAB method (Clarke 2009). Young leaves of XYY were used to isolate RNA by using an RNeasy Plant Mini Kit (DP432; Tiangen Biotech [Beijing] Co., Ltd., Beijing, China) according to the manufacturer's protocol. High-quality cDNA free of genomic DNA contamination was prepared by using a One-Step gDNA Removal and cDNA Synthesis SuperMix kit (AU311-02; Trans Biotech, Beijing, China) for amplification of the *R* gene coding DNA sequence. The primers R-3F (TGGAGGAGTTTGATGTAAGTGA) and R-3R (TGTTGCTAAAGGAGTCATGCT) were used to amplify the indel within the *R* gene *Potri.017G104100* for all 300 progenies and the two parents. The primers R-cds-1F (ATGAACTGTTT GATGGTGCTTCGT) and R-cds-1R (TTACCTTTGTACCTT CTCCTTGTT) were used to amplify the full coding sequence of the *R* gene without the indel, while R-cds-1F (ATGAACTGTTTGATG TGCTTCGT) and R-cds-2R (GGGCAGAAGATGGTCGTGGT) were used to amplify the coding sequence of the *R* gene with the 611-bp indel.

Statistical analysis. The statistical analysis in this study was conducted by using R software (<https://www.r-project.org/>). The function 'cor.test' was used for correlation analysis at the significance level of $P = 0.05$.

RESULTS

Variation in leaf rust symptoms in the F1 population.

From 2017 to 2018, we observed leaf rust symptoms in July 2017, September 2017, and September 2018 for the I-69 × XYY population. No leaf rust symptoms were observed in July 2018. According to our six-grade scoring system for leaf rust susceptibility, the data showed that 78, 128, 82, 11, 1, and 0 lines were classified into grades 1 to 6, respectively, in July 2017. In September 2017, 9, 57, 88, 51, 6, and 4 lines were classified into grades 1 to 6, respectively. In September 2018, 64, 75, 87, 38, 6, and 10 lines were

classified into grades 1 to 6, respectively. All of these data are illustrated in Figure 1. Because some lines had no new leaves or died after pruning, the total progeny numbers were less than 300 after July 2017. In order to investigate the repeatability of these three sets of phenotypic data, a correlation analysis was carried out. The correlation coefficients between July 2017 and September 2017, July 2017 and September 2018, and September 2017 and September 2018 were 0.51 ($P < 0.0001$), 0.25 ($P < 0.0001$), and 0.41 ($P < 0.0001$), respectively. According to the correlation coefficients, there were some correlations between the phenotypic data among different dates. However, the relatively low correlation scores indicated that leaf rust symptoms would largely be affected by the environment.

QTLs for leaf rust disease resistance. In our previous study, we constructed two parental maps, designated the p1 (I-69) and p2 (XYY) maps. In order to obtain more QTLs for poplar leaf rust resistance, we also constructed an F2 map (for ab × ab SNP markers), and this map was designated the ab map. In total, 1,222 high-quality and representative SNP markers were used to form 19 linkage groups (Supplementary Table S1). The linkage group ID was arranged according to the chromosome ID of *P. trichocarpa* genome version 3.0. After the performance of QTL mapping, 11 significant QTLs were detected (Table 1). There were two QTLs for the ab map and nine QTLs for the p2 map. We were not able to detect any significant QTLs for the p1 map. These QTLs were located on chromosomes 2, 4, 8, 9, 10, 12, 13, 17, and 19. The highest LOD value was 8.61, while the lowest LOD value was 2.76. For the variance of these QTLs, the QTL on chromosome 17 in 201709p2 (September 2017 environment and p2 map) could explain the most leaf rust resistance variation (12.84%). Compared with other QTL studies (La Mantia et al. 2013; Wang et al. 2012; W. Xia et al. 2018), in this study, both the LOD score and explained trait variances were very high. These results might indicate high confidence in the QTL mapping of leaf rust resistance in poplar in this study. Because repeatability is the other main parameter for evaluating the quality of QTL mapping, we focused only on QTLs that could be detected on at least two dates. According to the data shown in Table 1, two pairs of QTLs were clearly repeated in at least two dates (Fig. 2). On chromosome 17, two QTLs with overlapping CI regions at 64.00 to 68.00 were detected for 201709p2 and 201809p2 (Fig. 2A). On chromosome 19, two QTLs with overlapping CI regions at 19.19 to 26.78 were detected for 201707p2 and 201809p2 (Fig. 2B).

In order to conveniently describe these two pairs of QTLs, we labeled the overlapped QTL region on chromosome 17 as LRDR-QTL-17 and we labeled the overlapped QTL region on chromosome 19 as LRDR-QTL-19. Using the SNP molecular markers within LRDR-QTL-17 and LRDR-QTL-19 as references, we found that LRDR-QTL-17 corresponded to 11,257,300 to 12,346,306 bp on

Fig. 1. Scoring of leaf rust resistance in the I-69 × XYY F1 population. Mlp201707 indicates scores of leaf rust disease susceptibility in July 2017, Mlp201709 indicates scores of leaf rust disease susceptibility in September 2017, and Mlp201809 indicates scores of leaf rust disease susceptibility in September 2018.

TABLE 1. Quantitative trait locus mapping results of leaf rust resistance for the I-69 × XYY F1 population^a

Environment	Chr	Pos	LOD	VAR	CI	Sig
201709ab ^b	2	70.49	3.76	6.20	65.49–75.49	***
201707ab	4	76.57	3.82	5.81	71.57–81.57	***
201709p2	8	191.79	2.78	3.94	78.00–210.00	***
201809p2	9	0.00	7.07	9.73	0.00–2.00	***
201809p2	10	228.24	3.21	4.27	226.58–252.00	***
201709p2	12	146.00	5.34	7.74	142.00–150.00	***
201809p2	13	70.00	2.76	3.66	65.00–75.00	***
201709p2	17	65.67	8.61	12.84	64.00–68.00	***
201809p2	17	66.00	4.68	6.31	62.72–72.00	***
201707p2	19	23.19	3.94	4.37	18.19–28.19	***
201809p2	19	21.19	5.51	7.48	19.19–26.78	***

^a Chr = chromosome, Pos = position, LOD = logarithm of odds, VAR = the explained variance, CI = confidence interval, Sig = significance, and asterisks (***) indicate P value < 0.001 .

^b Quantitative trait loci detected for trait data collected in September 2017 using the ab map.

chromosome 17 according to the locations of two SNPs; namely, Chr17-11257300 and Chr17-12346306 (Fig. 2A). However, we could not obtain the exact corresponding region for LRDR-QTL-19 on the reference genome because one SNP marker, Chr19-1553413, within this region was on chromosome 19, and the other SNP marker, Scaffold_25-428363, was on scaffold_25. Within Chr17 (11,257,300 to 12,346,306), we found 15 genes annotated as Toll-interleukin-like receptor (TIR)-NBS-LRR class *R* genes in the *P. trichocarpa* genome version 3.0 (Supplementary Table S3); similarly, there were 5 annotated as TIR-NBS-LRR class and 16 as nucleotide-binding-adaptor shared by APAF-1, *R* proteins, and CED-4 domain-containing *R* genes on scaffold_25, although we were not able to assign an exact region for LRDR-QTL-19 (Supplementary Table S3). These data suggest that a number of *R* genes were also within LRDR-QTL-19. In summary, based on the above results, two major leaf rust resistance QTL regions (LRDR-QTL-17 and LRDR-QTL-19) were identified, and *R* genes might contribute to these two QTL regions.

Identification of indels in an *R* gene within LRDR-QTL-17. To obtain more information on the two QTL regions, the two parents were subjected to genome resequencing. After SNP screening, 2,921 nonsynonymous SNPs were identified for XYY in the two major QTL regions (Supplementary Table S4). There are 6 and 16 *R* genes in LRDR-QTL-17 and LRDR-QTL-19, respectively, with nonsynonymous SNPs. Because SNPs were applied for genetic map construction, we then mainly investigated structural variations (indels) in the two QTL regions within or

between the two parents. With stringent filtering, an *R* gene, *Potri.017G104100*, within LRDR-QTL-17 was found to harbor a 611-bp indel between the two alleles in XYY; the female parent I-69 only had no polymorphism in this gene, and both alleles showed a sequence length similar to that of the large allele in XYY according to the resequencing data. In order to prove these data, the whole genomic sequence for *Potri.017G104100* in XYY was cloned, and the results showed that our bioinformatic analysis was correct (Fig. 3A). Based on the preliminary analysis, we predicted that the 611-bp deletion in one allele would affect the splicing sites for its mRNA generation. The cloning of coding DNA for both alleles in XYY proved that the longer alleles generated mRNA identical to that of the reference genome (Fig. 3A), whereas the short allele lost the second splicing site; therefore, the first intron was not spliced in their mRNA. The retention of the first intron in its mRNA resulted in a premature stop codon at 565 to 567 bp within the mRNA of the short allele (Fig. 3B). When comparing the predicted amino acid sequences between the short (designated as S_R_protein) and long (designated as L_R_protein) alleles for *Potri.017G104100* in XYY, the S_R_protein had only two low-similarity LRR domains, while the L_R_protein had three low-similarity LRR domains and one TIR domain (Fig. 3C). The loss of one low-similarity LRR and one TIR domain in *Potri.017G104100* S_R_protein might affect its function.

Because we detected LRDR-QTL-17 on the p2 map (XYY), the polymorphisms between alleles in XYY could be attributed to this QTL. The indel within *Potri.017G104100* in XYY met this requirement. Using one primer pair, R-3F/R, we developed a

Fig. 2. Leaf rust disease resistance quantitative trait loci (QTLs) on chromosomes 17 and 19. Only repeated QTLs are illustrated in this figure. LOD = logarithm of odds. Bars are drawn to scale for the confidence intervals of the QTLs. **A**, Two repeated QTLs on chromosome 17 and **B**, the other two repeated QTLs on chromosome 19 are shown.

phenotypic data for the tree F1 population are usually not complete because a small number of repeats are conducted for trait observation. The large size and long growth period mean that a small number of plants are grown for each genotype in the tree F1 population. Compared with our previous studies (Chen et al. 2015; Wang et al. 2008, 2011, 2016; W. Xia et al. 2018), more than 30 plants were used for scoring traits in crops, whereas sometimes only 1 plant was used to collected trait data in perennial trees. The insufficient phenotypic data usually result in low repetitiveness, small LOD values, and low explained variance. All three of these parameters are the major indicators for the confidence of QTL mapping. Additionally, some other characteristics such as perennial growth, long juvenile phase, and so on also affect the application of QTL mapping in trees.

Though there are some limitations in the application of QTL mapping in tree quantitative genetic studies compared with that in herbaceous crop plants, we still obtained high-quality QTLs for leaf rust resistance in this study. Summarized from a number of our previous studies and other studies, several strategies could be applied to avoid low-efficiency QTL mapping in tree plants. First, high-quality and dense genetic maps can provide exact genotypic data. Low-cost NGS technology helped us achieve this aim. Second, many tree plants can be propagated by cutting, and more than one plant can be prepared for each genotype. Third, observation of the trait on at least three dates could be the minimum requirement for phenotypic data. The second and third strategies can largely improve the low repetitiveness of tree QTL mapping. Fourth, generating large sequencing datasets by NGS for parents or progenies can help predict candidate genes for major QTLs. In this study, we found an indel in the *R* gene associated with leaf rust resistance by investigating the parental genome resequencing data. In some other studies, the large genome resequencing dataset also sped the discovery of candidate genes (Takagi et al. 2013; Yu et al. 2013). Therefore, although QTL mapping in tree quantitative genetic studies is more difficult than that in herbaceous crop plants, it could still be a powerful methodology for dissecting complex tree traits by applying the above strategies.

The 611-bp indel in the *R* gene is valuable for poplar molecular breeding. With technology development and the increasing requirement for energy worldwide, traditional breeding pipelines have not been able to provide enough plant products for people. Molecular breeding and MAS have been widely applied in crops such as rice, maize, wheat, and rapeseed. Because the MAS strategy can speed the selection of lines with desirable traits, obtaining reliable and easy-to-examine molecular markers is a key issue. In tree breeding programs, the strategy of molecular breeding has only recently started. In poplar, leaf rust disease seriously reduces biomass production (Royle and Ostry 1995; Widin and Schipper 1981); thus, finding breeding lines with high resistance to leaf rust is a major task. In this study, the 611-bp indel showed an association with variation in leaf rust resistance, and this indel can be tested by normal PCR amplification. Therefore, the 611-bp indel in the *R* gene is very valuable for poplar MAS. According to our genome-wide QTL mapping, we found 11 QTLs for leaf rust disease resistance. QTLs on chromosome 19 could also be detected on two different dates. Interestingly, previous studies also pointed out that there was a QTL on chromosome 19 responsible for leaf rust variation, and expression was profiled upon *M. larici-populina* inoculation (Bresson et al. 2011; Kohler et al. 2008). Thus, the QTL on chromosome 19 could be reliable, and development of easy-to-examine molecular markers for this QTL is necessary. Additionally, more phenotyping data could be collected for leaf rust resistance for this F1 population, and the new data could be used to confirm the seven other major QTLs.

The loss of the TIR domain in the *R* protein might be responsible for the variation in leaf rust resistance. In plant genomes, there are many *R* genes, and many of them have been shown to be involved in disease resistance. Usually, the loss of *R* genes or their domains will alter disease resistance. Here, we found

that the loss of the TIR domain in the *R* protein might enhance the resistance to leaf rust disease resistance in poplar. Whether the deletion of the TIR domain in the *Potri.017G104100 R* gene is the direct cause of the variation in leaf rust disease resistance is unknown. However, according to the association result and the function of *R* genes in plants, we were able to speculate that the loss of the TIR domain in the *R* protein might be responsible for variation in leaf rust resistance. Based on a number of *R* genes clustering within LRDR-QTL-17, there is also a possibility that some undetected *R* gene deletions in the *XXY* genome are responsible for this QTL.

In summary, we conducted high-quality QTL mapping for leaf rust resistance by using the I-69 × *XXY* F1 population. There were 11 major QTLs identified, and 4 of them were detected on at least two dates. Combining QTL mapping with parental genome resequencing, a 611-bp indel in the *Potri.017G104100 R* gene was found to be located in two of the major QTL regions. The 611-bp indel resulted in a loss of the TIR domain in the *R* gene, and it was found to be associated with variation in leaf rust resistance in poplar. Based on this study, we were able to provide a valuable molecular PCR-based marker for poplar molecular breeding.

LITERATURE CITED

- Arends, D., Prins, P., Jansen, R. C., and Broman, K. W. 2010. R/qlt: High-throughput multiple QTL mapping. *Bioinformatics* 26:2990-2992.
- Bolger, A. M., Lohse, M., and Usadel, B. 2014. Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114-2120.
- Bresson, A., Jorge, V., Dowkiw, A., Guerin, V., Bourgaït, I., Tuskan, G. A., Schmutz, J., Chalhou, B., Bastien, C., and Rampant, P. F. 2011. Qualitative and quantitative resistances to leaf rust finely mapped within two nucleotide-binding site leucine-rich repeat (NBS-LRR)-rich genomic regions of chromosome 19 in poplar. *New Phytol.* 192:151-163.
- Cervera, M. T., Gusmao, J., Steenackers, M., Peleman, J., Storme, V., VandenBroeck, A., VanMontagu, M., and Boerjan, W. 1996. Identification of AFLP molecular markers for resistance against *Melampsora larici-populina* in *Populus*. *Theor. Appl. Genet.* 93:733-737.
- Chen, J., Wang, N., Fang, L.-C., Liang, Z.-C., Li, S.-H., and Wu, B.-H. 2015. Construction of a high-density genetic map and QTLs mapping for sugars and acids in grape berries. *BMC Plant Biol.* 15:28.
- Cingolani, P., Platts, A., Wang, L. L., Coon, M., Nguyen, T., Wang, L., Land, S. J., Lu, X., and Ruden, D. M. 2012. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff. *Fly (Austin)* 6:80-92.
- Clarke, J. D. 2009. Cetyltrimethyl ammonium bromide (CTAB) DNA miniprep for plant DNA isolation. *Cold Spring Harbor Protoc.* 2009: pdb.prot5177.
- Dai, M., Fang, L., Yin, T., and Li, X. 2019. Development of SNP molecular markers against the foliar rust of poplar using SLAF-seq technique. *J. Nanjing For. Univ.* 2:73-78.
- Dell'Acqua, M., Gatti, D. M., Pea, G., Cattonaro, F., Coppens, F., Magris, G., Hlaing, A. L., Aung, H. H., Nelissen, H., Baute, J., Frascaroli, E., Churchill, G. A., Inze, D., Morgante, M., and Pe, M. E. 2015. Genetic properties of the MAGIC maize population: A new platform for high definition QTL mapping in *Zea mays*. *Genome Biol.* 16:167.
- Dowkiw, A., and Bastien, C. 2005. Characterization of two major genetic factors controlling quantitative resistance to *Melampsora larici-populina* leaf rust in hybrid poplars: Strain specificity, field expression, combined effects, and relationship with a defeated qualitative resistance gene. *Phytopathology* 95:1358-1367.
- Duplessis, S., Cuomo, C. A., Lin, Y. C., Aerts, A., Tisserant, E., Veneault-Fourrey, C., Joly, D. L., Hacquard, S., Amselem, J., Cantarel, B. L., Chiu, R., Coutinho, P. M., Feau, N., Field, M., Frey, P., Gelhaye, E., Goldberg, J., Grabherr, M. G., Kodira, C. D., Kohler, A., Kues, U., Lindquist, E. A., Lucas, S. M., Mago, R., Mauceli, E., Morin, E., Murat, C., Pangilinan, J. L., Park, R., Pearson, M., Quesneville, H., Rouhier, N., Sakthikumar, S., Salamov, A. A., Schmutz, J., Selles, B., Shapiro, H., Tanguay, P., Tuskan, G. A., Henrissat, B., Van de Peer, Y., Rouze, P., Ellis, J. G., Dodds, P. N., Schein, J. E., Zhong, S., Hamelin, R. C., Grigoriev, I. V., Szabo, L. J., and Martin, F. 2011. Obligate biotrophy features unraveled by the genomic analysis of rust fungi. *Proc. Natl. Acad. Sci. U.S.A.* 108:9166-9171.
- Hacquard, S., Delaruelle, C., Frey, P., Tisserant, E., Kohler, A., and Duplessis, S. 2013. Transcriptome analysis of poplar rust telia reveals overwintering adaptation and tightly coordinated karyogamy and meiosis processes. *Front. Plant Sci.* 4:456.

- Jorge, V., Dowkiw, A., Faivre-Rampant, P., and Bastien, C. 2005. Genetic architecture of qualitative and quantitative *Melampsora larici-populina* leaf rust resistance in hybrid poplar: Genetic mapping and QTL detection. *New Phytol.* 167:113-127.
- Kage, U., Karre, S., Kushalappa, A. C., and McCartney, C. 2017. Identification and characterization of a fusarium head blight resistance gene TaACT in wheat QTL-2DL. *Plant Biotechnol. J.* 15:447-457.
- Kohler, A., Rinaldi, C., Duplessis, S., Baucher, M., Geelen, D., Duchaussoy, F., Meyers, B. C., Boerjan, W., and Martin, F. 2008. Genome-wide identification of NBS resistance genes in *Populus trichocarpa*. *Plant Mol. Biol.* 66:619-636.
- La Mantia, J., Klapste, J., El-Kassaby, Y. A., Azam, S., Guy, R. D., Douglas, C. J., Mansfield, S. D., and Hamelin, R. 2013. Association analysis identifies *Melampsora* × *columbiana* poplar leaf rust resistance SNPs. *PLoS One* 8:e78423.
- Lee, M., Xia, J. H., Zou, Z., Ye, J., Rahmadsyah, Alfiko, Y., Jin, J., Lieando, J. V., Purnamasari, M. I., Lim, C. H., Suwanto, A., Wong, L., Chua, N.-H., and Yue, G. H. 2015. A consensus linkage map of oil palm and a major QTL for stem height. *Sci. Rep.* 5:8232.
- Levé, V., Major, I., Levasseur, C., Tremblay, L., MacKay, J., and Séguin, A. 2009. Expression profiling and functional analysis of *Populus WRKY23* reveals a regulatory role in defense. *New Phytol.* 184:48-70.
- Li, H. 2011. A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. *Bioinformatics* 27:2987-2993.
- Liu, L.-L., Cao, Z.-M., Fan, J.-F., Wang, Y.-G., Yu, Z.-D., and Fei, Z.-X. 2008. A study of poplar resistance to *Melampsora larici-populina*. *J. Northwest For. Univ.* 6:137-139.
- Lorrain, C., Hecker, A., and Duplessis, S. 2015. Effector-mining in the poplar rust fungus *Melampsora larici-populina* secretome. *Front. Plant Sci.* 6:1051.
- Luo, J., Xia, W. X., Cao, P., Xiao, Z. A., Zhang, Y., Liu, M. F., Zhan, C., and Wang, N. A. 2019. Integrated transcriptome analysis reveals plant hormones jasmonic acid and salicylic acid coordinate growth and defense responses upon fungal infection in poplar. *Biomolecules* 9:12.
- Ma, J. C., Wan, D. S., Duan, B. B., Bai, Q. X., Chen, N. N., and Ma, T. 2019. Genome sequence and genetic transformation of a widely distributed and cultivated poplar. *Plant Biotechnol. J.* 17:451-460.
- Ma, T., Wang, J. Y., Zhou, G. K., Yue, Z., Hu, Q. J., Chen, Y., Liu, B. B., Qiu, Q., Wang, Z., Zhang, J., Wang, K., Jiang, D. C., Gou, C. Y., Yu, L. L., Zhan, D. L., Zhou, R., Luo, W. C., Ma, H., Yang, Y. Z., Pan, S. K., Fang, D. M., Luo, Y. D., Wang, X., Wang, G. N., Wang, J., Wang, Q., Lu, X., Chen, Z., Liu, J. C., Lu, Y., Yin, Y., Yang, H. M., Abbott, R. J., Wu, Y. X., Wan, D. S., Li, J., Yin, T. M., Lascoux, M., DiFazio, S. P., Tuskan, G. A., Wang, J., and Liu, J. Q. 2013. Genomic insights into salt adaptation in a desert poplar. *Nat. Commun.* 4:2797.
- Martin, T., Ronnberg-Wastljung, A. C., Stenlid, J., and Samils, B. 2016. Identification of a differentially expressed TIR-NBS-LRR gene in a major QTL associated to leaf rust resistance in *Salix*. *PLoS One* 11: e0168776.
- McCormick, R. F., Truong, S. K., and Mullet, J. E. 2015. RIG: Recalibration and interrelation of genomic sequence data with the GATK. *G3: Genes Genomes Genet.* 5:655-665.
- Pelgas, B., Bousquet, J., Meirmans, P. G., Ritland, K., and Isabel, N. 2011. QTL mapping in white spruce: Gene maps and genomic regions underlying adaptive traits across pedigrees, years and environments. *BMC Genomics* 12:145.
- Petre, B., Saunders, D. G., Sklenar, J., Lorrain, C., Win, J., Duplessis, S., and Kamoun, S. 2015. Candidate effector proteins of the rust pathogen *Melampsora larici-populina* target diverse plant cell compartments. *Mol. Plant-Microbe Interact.* 28:689-700.
- Rinaldi, C., Kohler, A., Frey, P., Duchaussoy, F., Ningre, N., Couloux, A., Wincker, P., Le Thiec, D., Fluch, S., Martin, F., and Duplessis, S. 2007. Transcript profiling of poplar leaves upon infection with compatible and incompatible strains of the foliar rust *Melampsora larici-populina*. *Plant Physiol.* 144:347-366.
- Royle, D. J., and Ostry, M. E. 1995. Disease and pest control in the bioenergy crops poplar and willow. *Biomass Bioenergy* 9:69-79.
- She, M., Wang, J., Wang, X., Yin, G., Wang, K., Du, L., and Ye, X. 2017. Comprehensive molecular analysis of arginase-encoding genes in common wheat and its progenitor species. *Sci. Rep.* 7:6641.
- Tabor, G. M., Kubisiak, T. L., Klopfenstein, N. B., Hall, R. B., and McNabb, H. S. 2000. Bulk segregant analysis identifies molecular markers linked to *Melampsora medusae* resistance in *Populus deltoides*. *Phytopathology* 90:1039-1042.
- Takagi, H., Abe, A., Yoshida, K., Kosugi, S., Natsume, S., Mitsuoka, C., Uemura, A., Utsushi, H., Tamiru, M., Takuno, S., Innan, H., Cano, L. M., Kamoun, S., and Terauchi, R. 2013. QTL-seq: Rapid mapping of quantitative trait loci in rice by whole genome resequencing of DNA from two bulked populations. *Plant J.* 74:174-183.
- Thumma, B. R., Southerton, S. G., Bell, J. C., Owen, J. V., Henery, M. L., and Moran, G. F. 2010. Quantitative trait locus (QTL) analysis of wood quality traits in *Eucalyptus nitens*. *Tree Genet. Genomes* 6:305-317.
- Tuskan, G. A., Difazio, S., Jansson, S., Bohlmann, J., Grigoriev, I., Hellsten, U., Putnam, N., Ralph, S., Rombauts, S., Salamov, A., Schein, J., Sterck, L., Aerts, A., Bhaleerao, R. R., Bhaleerao, R. P., Blaudez, D., Boerjan, W., Brun, A., Brunner, A., Busov, V., Campbell, M., Carlson, J., Chalot, M., Chapman, J., Chen, G. L., Cooper, D., Coutinho, P. M., Couturier, J., Covert, S., Cronk, Q., Cunningham, R., Davis, J., Degroove, S., Dejardin, A., Depamphilis, C., Detter, J., Dirks, B., Dubchak, I., Duplessis, S., Ehrling, J., Ellis, B., Gendler, K., Goodstein, D., Gribskov, M., Grimwood, J., Groover, A., Gunter, L., Hamberger, B., Heinze, B., Helariutta, Y., Henrissat, B., Holligan, D., Holt, R., Huang, W., Islam-Faridi, N., Jones, S., Jones-Rhoades, M., Jorgensen, R., Joshi, C., Kangasjarvi, J., Karlsson, J., Kelleher, C., Kirkpatrick, R., Kirst, M., Kohler, A., Kalluri, U., Larimer, F., Leebens-Mack, J., Leple, J. C., Locascio, P., Lou, Y., Lucas, S., Martin, F., Montanini, B., Napoli, C., Nelson, D. R., Nelson, C., Nieminen, K., Nilsson, O., Pereda, V., Peter, G., Philippe, R., Pilate, G., Poliakov, A., Razumovskaya, J., Richardson, P., Rinaldi, C., Ritland, K., Rouze, P., Ryaboy, D., Schmutz, J., Schrader, J., Segerman, B., Shin, H., Siddiqui, A., Sterky, F., Terry, A., Tsai, C. J., Uberbacher, E., Unneberg, P., Vahala, J., Wall, K., Wessler, S., Yang, G., Yin, T., Douglas, C., Marra, M., Sandberg, G., Van de Peer, Y., and Rokhsar, D. 2006. The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray). *Science* 313:1596-1604.
- Van, O. J. 2006. JoinMap 4, Software for the Calculation of Genetic Linkage Maps in Experimental Populations. Kyazma BV, Wageningen, The Netherlands.
- Villar, M., Lefevre, F., Bradshaw, H. D., and duCros, E. T. 1996. Molecular genetics of rust resistance in poplars (*Melampsora larici-populina* Kleb *Populus* sp.) by bulked segregant analysis in a 2x2 factorial mating design. *Genetics* 143:531-536.
- Voorrips, R. E. 2002. MapChart: Software for the graphical presentation of linkage maps and QTLs. *J. Hered.* 93:77-78.
- Wan, X.-Q. 2006. Estimation on resistance to leaf rust in 31 hybrid poplar clones caused by *Melampsora larici-populina*. *For. Sci. Technol.* 5:26-29.
- Wan, Z., Li, Y., Chen, Y., Zhang, X., Guan, H., and Yin, T. 2013. *Melampsora larici-populina*, the main rust pathogen, causes loss in biomass production of black cottonwood plantations in the south of China. *Phytoparasitica* 41: 337-344.
- Wang, N., Cao, P., Xia, W., Fang, L., and Yu, H. 2017. Identification and characterization of long non-coding RNAs in response to early infection by *Melampsora larici-populina* using genome-wide high-throughput RNA sequencing. *Tree Genet. Genomes* 13:34.
- Wang, N., Chen, B. Y., Xu, K., Gao, G. Z., Li, F., Qiao, J. W., Yan, G. X., Li, J., Li, H., and Wu, X. M. 2016. Association mapping of flowering time QTLs and insight into their contributions to rapeseed growth habits. *Front. Plant Sci.* 7:338.
- Wang, N., Fang, L. C., Xin, H. P., Wang, L. J., and Li, S. H. 2012. Construction of a high-density genetic map for grape using next generation restriction-site associated DNA sequencing. *BMC Plant Biol.* 12:148.
- Wang, N., Qian, W., Suppanz, I., Wei, L., Mao, B., Long, Y., Meng, J., Müller, A. E., and Jung, C. 2011. Flowering time variation in oilseed rape (*Brassica napus* L.) is associated with allelic variation in the *FRIGIDA* homologue *BnaA.FRI.a*. *J. Exp. Bot.* 62:5641-5658.
- Wang, N., Wang, Y. J., Tian, F., King, G. J., Zhang, C. Y., Long, Y., Shi, L., and Meng, J. L. 2008. A functional genomics resource for *Brassica napus*: Development of an EMS mutagenized population and discovery of FAEL1 point mutations by TILLING. *New Phytol.* 180:751-765.
- Widin, K. D., and Schipper, A. L. 1981. Effect of *Melampsora medusae* leaf rust infection on yield of hybrid poplars in the north-central United States. *Eur. J. Forest Pathol.* 11:438-448.
- Xia, D., Zhou, H., Liu, R. J., Dan, W. H., Li, P. B., Wu, B., Chen, J. X., Wang, L. Q., Gao, G. J., Zhang, Q. L., and He, Y. Q. 2018. GL3.3, a novel QTL encoding a GSK3/SHAGGY-like kinase, epistatically interacts with GS3 to produce extra-long grains in rice. *Mol. Plant* 11:754-756.
- Xia, W., Xiao, Z., Cao, P., Zhang, Y., Du, K., and Wang, N. 2018. Construction of a high-density genetic map and its application for leaf shape QTL mapping in poplar. *Planta* 248:1173-1185.
- Xia, W., Yu, H., Cao, P., Luo, J., and Wang, N. 2017. Identification of TIFY family genes and analysis of their expression profiles in response to phytohormone treatments and *Melampsora larici-populina* infection in poplar. *Front. Plant Sci.* 8:493.
- Xue, L.-J., Alabady, M. S., Mohebbi, M., and Tsai, C.-J. 2015. Exploiting genome variation to improve next-generation sequencing data analysis and genome editing efficiency in *Populus tremulaxalba* 717-1B4. *Tree Genet. Genomes* 11:82.
- Ye, K., Schulz, M. H., Long, Q., Apweiler, R., and Ning, Z. 2009. Pindel: A pattern growth approach to detect break points of large deletions and medium sized insertions from paired-end short reads. *Bioinformatics* 25:2865-2871.
- Yu, X., Wang, H., Zhong, W. L., Bai, J. J., Liu, P. L., and He, Y. K. 2013. QTL mapping of leafy heads by genome resequencing in the RIL population of *Brassica rapa*. *PLoS One* 8:e76059.