Characterization and Molecular Mapping of Two Novel Genes Resistant to *Pythium* Stalk Rot in Maize

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Accepted for publication 12 October 2018.

ABSTRACT

Pythium stalk rot caused by *Pythium inflatum* is becoming a more and more serious disease in maize, and it has caused severe yield loss in China in recent years. Deployment of resistant maize varieties is the most effective way to control this disease. Searching for the resistant maize germplasm and identifying the resistance genes are the vital processes in the breeding program. The maize inbred line X178 previously showed high resistance to *Pythium* stalk rot. Thus, this study aimed to reveal the gene(s) resistance to *Pythium* stalk rot in X178 by resistance inheritance analysis using the derived F₂ and F_{2,3} genetic populations. The results showed that two independently inherited dominant genes, designated *RpiX178-1* and *RpiX178-2*, carried by X178 are responsible for its resistance relative to the susceptible parent Ye107; they are located on

regions of maize chromosome (chr.) 1 bin 1.09 and chr. 4 bin 4.08, respectively, and flanked by markers umc2047 and bnlgl671 as well as bnlgl444 and umc1313, respectively, by linkage analysis. Subsequently, *RpiX178-1* was finely mapped between markers SSRZ8 and IDP2347, with genetic distances of 0.6 and 1.1 cM, respectively, and the physical distance of the target region was about 700 kb. *RpiX178-2* was also further located between markers bnlgl444 and umc2041, with a genetic distance of 2.4 cM. Moreover, we confirmed that the two genes *RpiX178-1* and *RpiX178-2* were newly identified and different from those genes known on chrs. 1 and 4 according to results of allelism testing. Herein, we newly identified two genes resistant to *P. inflatum*, which provided important genetic information for resistance to *Pythium* stalk rot in maize.

Maize is a major food and feed crop, because it is a high-energy crop, and it is also utilized as an industrial raw material in China. Therefore, it plays an important role in the national economy and agriculture production (Duan et al. 2012). Since 2003, the acreage dedicated to maize cultivation in China has been continuously increasing, with maize being the most highly cultivated crop in China. Therefore, reliable maize production is necessary to maintain China's food security and agricultural stability (Duan et al. 2015b). *Pythium* stalk rot, which is caused by *Pythium inflatum*, is a soilborne disease that severely affects maize production. This disease can occur in diverse environments, but it is more prevalent with excessive humidity, and it can strikingly affect susceptible maize inbred lines and varieties, resulting in severe yield and quality reductions (Duan et al. 2015b; Khokhar et al. 2014). In recent years, changes in climatic conditions, cultivation methods, and the maize varieties utilized have contributed to *Pythium* stalk rot becoming a major disease in the Huang-Huai region and northwestern China. Efforts to control the disease via chemical, agricultural, or biological means have had little success. Therefore, selective breeding and the development of disease-resistant varieties are the most economical, effective, and sustainable measures for controlling *Pythium* stalk rot.

Successfully breeding resistant varieties involves screening for resistant resources, performing genetic studies relating to resistance, and mining and mapping resistance genes. Previous

studies have shown that the inheritance of maize stalk rot resistance depends on quantitative and qualitative traits (Cao et al. 1996; Chen and Song 1999; Kappelman and Thompson 1966). In stalk rot caused by *Gibberella zeae* and *Colletotrichum graminicola*, resistance is mainly associated with quantitative traits, with both additive and dominance effects jointly playing a role (Pe et al. 1993; Yang et al. 2001). Several *Fusarium* stalk rot resistance loci were discovered and mapped, including the major gene *Rfg1* (*qRfg1*) (Wang et al. 2017; Yang et al. 2004, 2010; Ye et al. 2013) and the minor resistance genes *qRfg2* (Zhang et al. 2012), *qRfg3* (Ma et al. 2017), and *Rgsr8.1* (Chen et al. 2017). In addition, the anthracnose stalk rot resistance locus *Rcg1* was also mapped (Frey et al. 2011). However, resistance to *Pythium* stalk rot has been mainly associated with qualitative traits (Cao et al. 1996; Chen and Song 1999; Yang et al. 2005). Only a few *Pythium* stalk rot resistance genes have been mapped and formally named. These genes include the *Pythium* stalk rot resistance gene, *Rpi1* (Yang et al. 2005), *RpiQ1319-1*, and *RpiQ1319-2* (Song et al. 2015). The molecular markers linked with *Pythium* stalk rot resistance genes could assist in selective breeding for resistance to *Pythium* stalk rot.

Because of the coevolution of host and pathogen, many resistance genes do not provide durable resistance, with many superior hybrid varieties losing resistance after a few years of large-scale commercial cultivation. Therefore, a continuous discovery of new *Pythium* stalk rot resistance genes is required to protect maize from stalk rot and minimize yield losses. Simple sequence repeat (SSR) markers, which have advantages such as high reproducibility and codominance, have wide applications in resistance gene mapping. Bulk segregant analysis (BSA) is commonly used to rapidly screen molecular markers that are tightly linked to resistance genes (Michelmore et al. 1991). Maize inbred line X178 exhibits a stable resistance to *Pythium* stalk rot. Genetic analysis has shown that two independently inherited dominant genes carried by X178 are responsible for the resistance relative to the susceptible parent Ye107. The aim of this study was to finely map stalk rot resistance genes in X178, obtain tightly linked molecular markers, and then, perform allelism testing of adjacent genes. The results of this study

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Funding: This project was supported by National Key Research and Development Program of China Grant 2016YFD0100103, Crop Germplasm Conservation and Utilization Program Grant 2017NWB036-12 from the Ministry of Agriculture of China, and the Agricultural Science and Technology Innovation Program from the Chinese Academy of Agricultural Sciences.

C. Duan and F. Song contributed equally to this work.

The author(s) declare no conflict of interest.

provide new molecular markers and genetic resources to aid in the breeding of stalk rot-resistant varieties.

MATERIALS AND METHODS

Plant materials and inoculum. The maize inbred line X178 is highly resistant to *P. inflatum*, which belongs to the PB subgroup (Group B in modern American hybrid) and evolved from the American hybrid P78599 based on genealogy. Resistant X178 and susceptible Ye107 were used as contrasting parents, and they were crossed to generate genetic populations. The derived 90 F₁ individuals, 736 F₂ individuals, and 690 F_{2:3} lines from a cross of X178 × Ye107 were used for phenotypic evaluation and genetic analysis for resistance to *P. inflatum* in X178. The F₂ reciprocal cross-populations were also generated by crossing the resistant lines X178 × 1145 and X178 × Qi319 that were used for allelism tests of resistance genes. Maize germplasm was preserved in the China National Genebank at the Institute of Crop Sciences, Chinese Academy of Agricultural Sciences.

The *Pythium* stalk rot pathogen *P. inflatum* isolate P85-67 was used as inoculum in this study, and it was isolated, purified, and stored in the laboratory of the Institute of Crop Sciences, Chinese Academy of Agricultural Sciences.

Inoculation and phenotypic evaluation. The F₁, F₂, and F_{2:3} populations derived from the same cross between Ye107 and X178 were used to investigate the inheritance of resistance to stalk rot by phenotypic evaluation inoculated with isolate P85-67. All materials were planted at the Changping Experimental Field, Institute of Crop Sciences, Chinese Academy of Agricultural Sciences. The two contrasting parents were planted in the same test field and used as controls.

P. inflatum isolate P85-67 was cultured in maize kernels. The inoculum cultivation procedure was according to the procedure in our previous study (Song et al. 2015). First, maize kernels were soaked in tap water overnight for full expansion, and then, the treated maize kernels were cooked in boiling water for 0.5 h. Second, the cooked kernels were put into a triangular flask and autoclaved at 121°C for 1 h, after which they were inoculated with *P. inflatum*. Third, the inoculated kernels were incubated in a culture room at 25°C with a 0-h photoperiod for 14 days. Fourth, 50 g of maize kernels with *P. inflatum* were embedded into 5-cm-deep soil around each plant at the 10-leaf stage of maize.

The plants of parents, F₁, F₂, and F_{2:3} populations were investigated for phenotypic evaluation three times at weekly intervals. The typical stalk rot symptoms include browning in lower internodes, spongy stem, wilting, lodging, and plant death at the waxy ripe stage. Thus, the stalks of each plant were longitudinally split to observe the severity of stalk rot based on the degree of discoloration in the internodes. The investigation of stalk rot was completed according to published protocols (Song et al. 2015; Yang et al. 2010). The disease severity was scored using a scale from 1 to 6, where a score of 1 indicated no symptom appearance on plants at last scoring, a score of 2 indicated no symptoms at the first two scorings and a few symptoms at the last scoring, a score of 3 indicated no symptoms at the second scoring or some symptoms detected at the last scoring, a score of 4 indicated a few visible symptoms observed at the second scoring and symptoms detected at the last scoring, a score of 5 indicated visible symptoms observed at the second scoring, and a score of 6 indicated severe symptoms observed at the first scoring. The plants with scores of 1, 2, or 3 were classified as highly resistant, resistant, and intermediately resistant, respectively; those with scores of 4, 5, or 6 were classified as intermediately susceptible, susceptible, and highly susceptible, respectively.

DNA extraction and pooling for BSA. Genomic DNA was extracted from healthy young leaves according to a previous description (McCouch et al. 1988). The equal amounts of DNA from 10 homozygous resistant and 10 homozygous susceptible F₂ plants were mixed to produce resistant and susceptible DNA bulks

for BSA (in which equal amounts of DNA from 10 homozygous resistant and 10 homozygous susceptible F₂ plants were separately mixed to produce the resistant and susceptible DNA pools), respectively (Michelmore et al. 1991). DNA bulks were prepared by pooling 1 µg of DNA of each of the selected plants of the F₂ population.

Molecular marker analysis and genetic mapping. A total of 810 SSR markers were selected from the MaizeGDB (<https://www.maizegdb.org/>), and they were used to detect the polymorphism between contrasting parents and two bulks. The BSA polymorphic SSR markers were further tested for the entire F₂ mapping population. The polymerase chain reactions (PCRs) were performed on a GeneAmp PCR System 9700 thermal cycler (ABI, Norwalk, CT) in 20-µl reaction volumes containing 50 ng of genomic DNA, 0.2 µM primer mixture, 1.5 U of Taq DNA polymerase, 0.2 mM deoxy-ribonucleoside triphosphate (dNTPs), and 2.0 µl of 10× buffer. Reactions were performed using the PCR program as follows: 94°C for 4 min followed by 35 cycles of 94°C for 1 min, ~50 to 60°C for 1 min, and 72°C for 1 min and a final extension at 72°C for 10 min. The PCR products were separated on an 8% polyacrylamide gel by electrophoresis and stained with a silver nitrate solution for visualization. To finely map the resistant gene, new SSR markers distributed in the target region were designed based on data in MaizeGDB (<https://www.maizegdb.org/>) with Primer Premier 5.0 (Premier Biosoft International, Palo Alto, CA).

Data analysis and genetic linkage map construction. The segregation patterns of phenotypes and polymorphic SSR markers tested in the genetic mapping population were tested for goodness of fit to Mendelian segregation ratios with chi-squared (χ^2) analysis. Linkage analysis between molecular markers and the resistance gene was performed using Mapmaker/Exp version 3.0b software with the logarithm of the odds (LOD) score threshold of 3.0 (Lincoln et al. 1992). The Kosambi mapping function was used to estimate the map genetic distances (Kosambi 1943). The maize genome database (<https://www.maizegdb.org/>) was used as a reference for the physical mapping of resistance genes to chromosomes. A genetic linkage map was constructed using MapDraw in Microsoft Excel macro.

RESULTS

Genetic analysis of *Pythium* stalk rot resistance in X178.

The parental population (Ye107 and X178) and F₁, F₂, and F_{2:3} progeny were inoculated with *P. inflatum* (P85-67) and examined. The results showed that all 60 X178 individuals were highly resistant (Fig. 1A), that all 60 Ye107 individuals were highly susceptible (Fig. 1B), and that all 90 F₁ hybrid progeny were disease resistant (Table 1). When examining the 736 F₂ progeny, 689 of the individuals were resistant, and 47 were susceptible, fitting to a ratio of 15:1 ($\chi^2 = 0.0232$, degree of freedom (df) = 1, $P = 0.8790$). The 690 F_{2:3} families were identified and classified into 297 homozygous resistant, 348 segregating, and 45 homozygous susceptible types, fitting to a ratio of 7:8:1 ($\chi^2 = 0.1863$, df = 2, $P = 0.9110$) (Table 1). These results suggested that two independently inherited dominant genes were responsible for the resistance to *P. inflatum* in the inbred line X178. The two genes were tentatively designated as *RpiX178-1* and *RpiX178-2*.

Molecular mapping of the stalk rot resistance genes in X178. A total of 810 SSR markers were used for polymorphism analysis between two parents and F₁ hybrids. The results showed that molecular markers umc0041, umc2047, bnlgl671, umc1744, umc1847, bnlgl444, and umc1313 all exhibited polymorphism between the parents and susceptible and resistant pools (10 extremely resistant and susceptible individuals in each pool, respectively). These seven molecular markers distributed on maize chromosomes 1 and 4. Therefore, the *Pythium* stalk rot resistance genes in X178 were likely to be located on chromosomes 1 and 4. These seven pairs of primers were then used for testing in 690 F₂ individuals with explicit phenotype according to their corresponding F_{2:3} lines phenotype.

Linkage analysis between these molecular markers and the resistance genes was performed using Mapmaker/Exp version 3.0b, and it showed that markers umc0041, umc2047, bnlgl671, and umc1744 are linked to the *RpiX178-1* gene flanked by markers umc2047 and bnlgl671, with genetic distances of 2.1 and 6.9 cM, respectively (Fig. 2A). The molecular markers umc1847, bnlgl444, and umc1313 were linked to the *RpiX178-2* gene flanked by markers bnlgl444 and umc1313, with genetic distances of 0.9 and 5.4 cM, respectively (Fig. 2B).

To finely map the gene *RpiX178-1*, all SSR and InDel polymorphism (IDP) markers distributed on maize bin 1.09 to 1.10 were amplified between the parents and the resistant and susceptible pools, indicating that the molecular marker IDP2347 exhibits polymorphism between the resistant and susceptible parents and the resistant and susceptible pools. This marker was then tested in 690 F₂ individuals. Linkage analysis showed that IDP2347 was linked to the *RpiX178-1* gene, and hence, this gene was mapped between the molecular markers umc2047 and IDP2347 at genetic distances of 2.1 and 1.1 cM, respectively. To further narrow the location of the *RpiX178-1* gene, the B73 sequence was used as a reference to design SSR molecular markers. Polymorphic analysis was further tested against both parents and the DNA bulks, and then, the polymorphic markers SSRZ8 were found. The individual testing in 690 F₂ plants and linkage analysis indicated that marker SSRZ33 was at a genetic distance of 0.6 cM from the *RpiX178-1* gene on one side. In summary, the *RpiX178-1* gene was finely mapped between the molecular markers SSRZ8 and IDP2347, and it was refined into an ~700-kb interval flanked by these two markers (Fig. 2 and Table 2).

The same approach was used to map *RpiX178-2*. The molecular markers umc2041 and IDP188 were found to be polymorphic between the resistant and susceptible parents and the resistant and susceptible pools. The two markers were then used for testing in 690

F₂ individuals. Linkage analysis showed that umc2041 and IDP188 were both linked to the *RpiX178-2* gene, with genetic distances of 1.5 and 2.2 cM, respectively, from the *RpiX178-2* gene on one side. Finally, the *RpiX178-2* gene was further located on chromosome 4 flanked by the markers bnlgl444 and umc2041, with genetic distances of 0.9 and 1.5 cM, respectively (Fig. 2 and Table 2). The amplicons for the SSRZ8, IDP2347, and bnlgl444 markers in representative Ye107 and X178 individuals are shown in Figure 3.

Relationships validated by allelism testing. This study shows that the *RpiX178-1* and *RpiX178-2* genes were mapped to chromosome 1 (bin 1.09) and chromosome 4 (bin 4.08). In a

Fig. 2. Genetic linkage map of resistance genes **A**, *RpiX178-1* and **B**, *RpiX178-2*. **A**, Local linkage map of the *RpiX178-1* region on chromosome 1 using reported markers. **B**, Fine-scale mapping of the *RpiX178-2* region on chromosome 4. Loci names are shown on the right side of the map. Genetic distances on the left side of each map are in centimorgans.

Fig. 1. Field performance of maize inbred lines **A**, X178 (healthy plants) and **B**, Ye107 (diseased plants).

TABLE 1. Segregation ratios of the parents, F₁ and F₂ individuals, and F_{2:3} families derived from a cross of Ye107 × X178^a

Parents and crosses	Generation	Total number of plants/families	Observed ratio of R:S			Expected ratio	χ^2	P value
			R	Seg	S			
X178	P _R	60	60		0			
Ye107	P _S	60	0		60			
Ye107 × X178	F ₁	90	90		0			
Ye107 × X178	F ₂	736	689		47	15:1	0.0232	0.8790
Ye107 × X178	F _{2:3}	690	297	348	45	7:8:1	0.1863	0.9110

^a R, highly resistant and resistant; S, susceptible; Seg, segregating.

TABLE 2. Characteristics and sequences of the five markers linked to the two resistance genes in this study

Marker	Primer sequence	Annealing temperature	Linked resistance genes
umc2047	Forward GACAGACATTCCTCGCTACCTGAT	55	<i>RpiX178-1</i>
umc2047	Reverse CTGCTAGCTACCAAACATTCCGAT		
IDP2347	Forward ACCTTGCACTGCATGTTGG	56	<i>RpiX178-1</i>
IDP2347	Reverse AGTCTTCGAAATTTACCGGG		
bnlg1444	Forward AGACGACGAAGCTTTTGCAT	56	<i>RpiX178-2</i>
bnlg1444	Reverse GCATGGATGGAGAAAGAGGA		
umc2041	Forward CTACACAAGCATAGAGGCCTGGAG	58	<i>RpiX178-2</i>
umc2041	Reverse CAGTACGAGACGATGGAGGACAT		
SSRZ8 ^a	Forward GCCGTTCTAATTCCCATC	55	<i>RpiX178-1</i>
SSRZ8 ^a	Reverse GTTCGCCAGTATGTCTCG		

^a Marker developed in this study.

Fig. 3. Amplification patterns of molecular markers **A**, SSRZ8, **B**, IDP2347, and **C**, bnlg1444 linked with resistance genes in this study. B_R, resistant bulk; B_S, susceptible bulk; M, 100-bp DNA ladder; P₁, X178; P₂, Ye107; RR, homozygous resistant plants; Rr, heterozygous resistant plants; and rr, homozygous susceptible plants.

previous study, the resistant inbred line 1145, susceptible Y331, and their F₂ and BC₁F₁ progenies were inoculated with *P. inflatum*, and the resistance gene *Rpi1* was mapped between the molecular markers bnl91937 and agr286OPA-04₈₈₀ on chromosome 1. In another study, the gene *RpiQ1319-1* carried by inbred line Qi319 with resistance to Pythium stalk rot was mapped between the molecular markers SSRZ33 and SSRZ47 on chromosome 1. In addition, using the BC₈F₁ population derived from 1145 and Y331, the minor gene *qRfg2* resistance to Fusarium stalk rot was finely mapped to a 300-kb region on chromosome 1 flanked by CAPSZ459 and SSRZ319 markers. To verify the relationship between the previous stalk rot resistance genes *qRfg2*, *RpiQ1319-1*, and *Rpi1* and the genes *RpiX178-1* and *RpiX178-2*, the inbred maize lines X178, 1145, and Qi-319 were used to construct hybrids. The progeny populations were inoculated with *P. inflatum*, and disease resistance was examined using allelism testing. The resistance determination results showed that all three inbred lines exhibited resistance to Pythium stalk rot. Moreover, the F₁ progeny of the reciprocal crosses between X178 × Qi319 and X178 × 1145 were all disease resistant, whereas the F₂ progeny from the reciprocal crosses had some susceptible individuals (Table 3). Therefore, the X178 resistance genes differ from the resistance genes in the 1145 and Qi319 lines. These findings confirm that the two genes, *RpiX178-1* and *RpiX178-2*, are not identical to previously identified Pythium stalk rot resistance genes. Thus, they are new Pythium stalk rot resistance genes.

DISCUSSION

Extensive monoculturing will often result in a decrease or loss of resistance in the cultivated variety and create a conducive environment for plant pathogens to invade and proliferate. Under artificial selection conditions, resistant resources can be selectively bred to obtain disease-resistant varieties and prevent pathogen invasion. In maize, Pythium stalk rot, which is caused by *P. inflatum*, is an important pathogen with a dearth of research focused on resistance. Resistance identification studies have verified that the inbred maize line X178 exhibits a high to moderate resistance to multiple diseases, such as Southern corn leaf blight, Southern rust, Fusarium ear rot, and Pythium stalk rot (Duan et al. 2015a, 2015b). Currently, little has been reported pertaining to Pythium stalk rot resistance genes in inbred lines. Herein, the X178 line was inoculated with *P. inflatum* under artificial conditions; then, resistance inheritance was performed, and resistance genes were mapped in X178.

When conducting a resistance evaluation, the Ye107 line (susceptible) with no inoculation was utilized as a blank control, and the determination was carried out during the adult stage. The control samples did not show any signs of Pythium stalk rot, thus showing that the experimental field did not contain any Pythium stalk rot contamination. In the inoculated groups, individuals that exhibited a susceptibility to Pythium stalk rot were examined microscopically during the early stage of disease. The examination showed the presence of *P. inflatum* in the pith of the base stem, thus confirming that the inoculation can effectively induce stalk rot, which is consistent with previous studies (Yang et al. 2005). Within the F₂ progeny, the resistance determination identified 689 moderately resistant and 47

susceptible individuals. The segregation ratio of 15:1 suggested two dominant genes in X178. In 690 F_{2,3} families, a segregation ratio of 7:8:1 further confirmed that X178 Pythium stalk rot resistance is controlled by two independent dominant genes denoted *RpiX178-1* and *RpiX178-2*. The *RpiX178-1* gene was mapped to a 700-kb physical interval on chromosome 1. The *RpiX178-2* gene was mapped on chromosome 4 (bin 4.08), located between markers bnl91444 and umc2041, with the genetic interval of 2.4 cM.

Maize stalk rot is a relatively complex disease that is affected by multiple factors, such as the pathogen type, maize genetic background, and environment. In one study, *Fusarium* resistance in the 1145 inbred line was found to be controlled by the dominant gene *Rfg1*, which was mapped to chromosome 6 (Yang et al. 2004). However, in the same line, *P. inflatum* resistance is controlled by *Rpi1*, a dominant gene on chromosome 4 (Yang et al. 2005). These findings suggest that different pathogens may elicit a different stalk rot resistance even within the same maize line. Herein, the two independent dominant genes, *RpiX178-1* and *RpiX178-2*, carried by X178 are responsible for its resistance to *P. inflatum* relative to the susceptible parent Ye107, which is different from the findings in the populations from a cross between 1145 × Y331, where resistance is controlled by a single gene (Yang et al. 2005).

In this study, the *RpiX178-1* gene was found to be tightly linked to the molecular markers SSRZ8 and IDP2347 that are located on chromosome 1. Therefore, these two markers might be used for molecular marker-assisted breeding. Currently, many resistance genes have been found on maize chromosome 1, such as two quantitative trait loci (QTLs) associated with gray leaf spot disease resistance (Maroof et al. 1996) and resistance genes associated with Fusarium ear rot (Maschietto et al. 2017), northern leaf blight (Chung et al. 2011), and Gibberella stalk rot (Zhang et al. 2012). These findings suggest that maize chromosome 1 may be a region where resistance genes are concentrated. In this study, *RpiX178-1* and *RpiX178-2* were located on chromosomes 1 and 4, whereas in the 1145 line, *G. zeae* and *P. inflatum* resistance genes, *Rfg1* and *Rpi1*, are located on chromosomes 6 and 4, respectively (Yang et al. 2004, 2005). Also in the 1145 line, *G. zeae* resistance has been associated with the QTLs *qRfg1* and *qRfg2*, which are located on chromosomes 10 and 1, respectively (Yang et al. 2005; Zhang et al. 2012). Herein, four molecular markers linked with *qRfg2* and *Rpi1* were utilized to carry out linkage analysis of the Ye107 × X178 F₂ population. The results showed that the four markers were not linked to the *RpiX178-1* and *RpiX178-2* genes. To further verify the accuracy of the experimental results, X178 and 1145 hybrids were generated, the F₂ progenies were inoculated, and resistant individuals were discovered. This finding further confirms that the resistance genes in X178 and 1145 differ and shows that the experiment results are accurate and reliable. Therefore, the resistance genes *RpiX178-1* and *RpiX178-2* are novel Pythium stalk rot resistance genes that have not been previously reported.

Information about the genetic mechanisms of *P. inflatum* resistance in maize have been limited, which presents a major bottleneck in the development of new maize varieties with Pythium stalk rot resistance. In this study, the resistance to *P. inflatum* in X178 was examined, and two dominant genes (*RpiX178-1* and *RpiX178-2*) associated with Pythium stalk rot resistance were characterized. To our knowledge, this is the first report on the resistance to *P. inflatum* in inbred lines X178. The molecular markers SSRZ8 and bnl91444 were found to be tightly linked to these two genes and thus, will facilitate breeding new resistant cultivars by marker-assisted selection.

LITERATURE CITED

Cao, R. H., Wang, F. R., Wang, X. L., Fu, Y. H., Chen, Y. X., Ren, J. H., Wang, Z., and Ma, P. Q. 1996. Study on the maize genetic resistance of maize to *Pythium inflatum* Matthews. *Hereditas* 18:4-6.

TABLE 3. The phenotypes of F₁ and F₂ progenies of the reciprocal crosses between resistant inbred lines X178, 1145, and Qi319 used in allelism testing

Hybrids	Generation	Total number of individuals	Resistant	Susceptible
X178 × 1145	F ₁	50	50	0
X178 × 1145	F ₂	458	409	49
X178 × Qi319	F ₁	55	55	0
X178 × Qi319	F ₂	416	373	43

- Chen, Q., Song, J., Du, W. P., Xu, L. Y., Jiang, Y., Zhang, J., Xiang, X. L., and Yu, G. R. 2017. Identification, mapping, and molecular marker development for *Rgsr8.1*: A new quantitative trait locus conferring resistance to Gibberella stalk rot in maize (*Zea mays* L.). *Front. Plant Sci.* 8:1355.
- Chen, S. J., and Song, T. M. 1999. Resistance to maize stalk rot (*Pythium inflatum* Matthews): Simple genetic control of a pair of genes. *J. China Agric. Univ.* 1:56.
- Chung, C. L., Poland, J., Kump, K., Benson, J., Longfellow, J., Walsh, E., Balint-Kurti, P., and Nelson, R. 2011. Targeted discovery of quantitative trait loci for resistance to northern leaf blight and other diseases of maize. *Theor. Appl. Genet.* 123:307-326.
- Duan, C. X., Jiang, K., Qin, Z. H., Sun, S. L., Song, F. J., and Wang, X. M. 2015a. Identification of resistance marker genotype and genetic diversity of maize germplasm with resistance to southern corn rust. *J. Plant Prot.* 42: 899-907.
- Duan, C. X., Wang, X. M., Wu, X. F., Yang, Z. H., Song, F. J., Zhao, L. P., Sun, S. L., and Zhu, Z. D. 2015b. Analysis of maize accessions resistance to *Pythium* stalk rot and *Fusarium* ear rot. *J. Plant Genet. Res.* 16:947-954.
- Duan, C. X., Zhu, Z. D., Wu, X. F., Yang, Z. H., and Wang, X. M. 2012. Screening and evaluation of maize germplasm for resistance to five diseases and Asian corn borer. *J. Plant Genet. Res.* 13:169-174.
- Frey, T. J., Weldekidan, T., Colbert, T., Wolters, P. J. C. C., and Hawk, J. A. 2011. Fitness evaluation of *Rcg1*, a locus that confers resistance to *Colletotrichum graminicola* (Ces.) G. W. Wils. using near-isogenic maize hybrids. *Crop Sci.* 51:1551-1563.
- Kappelman, A. J., and Thompson, D. L. 1966. Inheritance of resistance to diplodia stalk-rot in corn. *Crop Sci.* 6:288-290.
- Khokhar, M. K., Hooda, K. S., Sharma, S. S., and Singh, V. 2014. Post flowering stalk rot complex of maize - present status and future prospects. *Maydica* 59:226-242.
- Kosambi, D. D. 1943. The estimation of map distance from recombination values. *Ann. Eugen.* 12:172-175.
- Lincoln, S., Daly, M., and Lander, E. 1992. Constructing Genetic Maps with Mapmaker/EXP30 Whitehead Institute Tech. Rep., 3rd Ed. Whitehead Institute, Cambridge, MA.
- Ma, C., Ma, X., Yao, L., Liu, Y., Du, F., Yang, X., and Xu, M. 2017. *qRfg3*, a novel quantitative resistance locus against Gibberella stalk rot in maize. *Theor. Appl. Genet.* 130:1723-1734.
- Maroof, M. A., Yue, Y. G., Xiang, Z. X., Stromberg, E. L., and Rufener, G. K. 1996. Identification of quantitative trait loci controlling resistance to gray leaf spot disease in maize. *Theor. Appl. Genet.* 93:539-546.
- Maschietto, V., Colombi, C., Pirona, R., Pea, G., Strozzi, F., Marocco, A., Rossini, L., and Lanubile, A. 2017. QTL mapping and candidate genes for resistance to *Fusarium* ear rot and fumonisin contamination in maize. *BMC Plant Biol.* 17:20.
- Michelmore, R. W., Paran, I., and Kesseli, R. V. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. *Proc. Natl. Acad. Sci. USA* 88: 9828-9832.
- Pe, M. E., Gianfranceschi, L., Taramino, G., Tarchini, R., Angelini, P., Dani, M., and Binelli, G. 1993. Mapping quantitative trait loci (QTLs) for resistance to *Gibberella zeae* infection in maize. *Mol. Gen. Genet.* 241:11-16.
- Song, F. J., Xiao, M. G., Duan, C. X., Li, H. J., Zhu, Z. D., Liu, B. T., Sun, S. L., Wu, X. F., and Wang, X. M. 2015. Two genes conferring resistance to *Pythium* stalk rot in maize inbred line Qi319. *Mol. Genet. Genomics* 290: 1543-1549.
- Wang, C., Yang, Q., Wang, W., Li, Y., Guo, Y., Zhang, D., Ma, X., Song, W., Zhao, J., and Xu, M. 2017. A transposon-directed epigenetic change in *ZmCCT* underlies quantitative resistance to Gibberella stalk rot in maize. *New Phytol.* 215:1503-1515.
- Yang, D. E., Chen, S. J., Wang, Y. G., Zhang, C. L., Li, S. R., and Wang, B. 2001. Genetic analysis of maize stalk rot (*Pythium inflatum* Matthews) resistance gene. *Acta Phytopathol. Sin.* 31:315-318.
- Yang, D. E., Jin, D. M., Wang, B., Zhang, D. S., Nguyen, H. T., Zhang, C. L., and Chen, S. J. 2005. Characterization and mapping of *Rpi1*, a gene that confers dominant resistance to stalk rot in maize. *Mol. Genet. Genomics* 274:229-234.
- Yang, D. E., Zhang, C. L., Zhang, D. S., Jin, D. M., Weng, M. L., Chen, S. J., Nguyen, H., and Wang, B. 2004. Genetic analysis and molecular mapping of maize (*Zea mays* L.) stalk rot resistant gene *Rfg1*. *Theor. Appl. Genet.* 108:706-711.
- Yang, Q., Yin, G., Guo, Y., Zhang, D., Chen, S., and Xu, M. 2010. A major QTL for resistance to Gibberella stalk rot in maize. *Theor. Appl. Genet.* 121:673-687.
- Ye, J., Guo, Y., Zhang, D., Zhang, N., Wang, C., and Xu, M. 2013. Cytological and molecular characterization of quantitative trait locus *qRfg1*, which confers resistance to Gibberella stalk rot in maize. *Mol. Plant-Microbe Interact.* 26:1417-1428.
- Zhang, D., Liu, Y., Guo, Y., Yang, Q., Ye, J., Chen, S., and Xu, M. 2012. Fine-mapping of *qRfg2*, a QTL for resistance to Gibberella stalk rot in maize. *Theor. Appl. Genet.* 124:585-596.