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A Molecular Identification and Resistance Evaluation of the Blast Resistance Genes in *Japonica* Rice in Northern China

Zuobin Ma [1,†], Lili Wang [1,†], Liangkun Zhang [2], Shuang Gu [3], Hui Wang [4], Guomin Sui [4,*] and Wenjing Zheng [1,*]

[1] Rice Research Institute of Liaoning Province, Liaoning Academy of Agricultural Sciences, Shenyang 110101, China; mazuo1984@163.com (Z.M.); wll1230510@163.com (L.W.)
[2] Rice Research Institute, Shenyang Agricultural University, Shenyang 110866, China; zhangliangkun0223@163.com
[3] College of Plant Protection, Shenyang Agricultural University, Shenyang 110866, China; gushuang@stu.syau.edu.cn
[4] Liaoning Academy of Agricultural Sciences, Shenyang 110101, China; laaswh@126.com
* Correspondence: guomin666@126.com (G.S.); zwj27@126.com (W.Z.)
† These authors contributed equally to this work.

Abstract: Rice blast is a fungal disease that seriously threatens rice production. It is of great significance to identify blast resistance genes and clarify their functions in rice varieties. In this study, 11 rice blast resistance genes in 80 *Japonica* rice varieties in northern China were investigated, including their resistance to rice blast. The results demonstrated that *Pita*, *Ptr*, *Pib*, *Pik*, and *Piks* were most widely found, accounting for 48.8, 48.8, 41.3, 20.0, and 18.8% of the tested varieties, respectively. *Pi5-G2* at the *Pi5* locus and *Pik-G5* and *Pik-G8* at the *Pik* locus were also commonly found, and these alleles accounted for 30.0, 10.0, and 3.8% of all the tested varieties, respectively. *Pizt* was identified only in two cultivars, and alleles *Pi2*, *Pi9*, and *Pigm* at the *Piz* locus on chromosome 6 were not detected. We found that *Pi5* and *Pita* were relatively conserved, but the alleles of *Pik* were abundant. Besides *Pik*, *Pikm*, and *Piks*, we also found 10 new haplotypes, and *Pikp* and *Pikh* were not found in the japonica rice varieties in northern China. Among the tested varieties, 5 did not carry any of the tested genes, 30 carried only one blast resistance gene, 27 carried two, 14 carried three, and 4 carried four. The resistance of varieties carrying three or four resistance genes was better than those carrying none of the resistance genes or only one or two. There were no significant differences in the resistance characteristics among varieties from different provinces. Our study provided a reference for the molecular breeding of rice blast resistance.

Keywords: *Japonica* rice; gene polymerization; *M. oryzae*; resistance gene

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1. Introduction

Rice blast, caused by *Magnaporthe oryzae* (*M. oryzae*), is a fungal disease that seriously threatens rice production. The most effective method for controlling the disease is via the breeding of rice varieties containing resistance genes. To date, more than 100 R genes or loci related to rice blast have been identified on all chromosomes [1]. Most of the cloned R genes encode proteins containing nucleotide-binding/leucine-rich-repeat (NLR). Many studies have shown that three resistance gene clusters located on chromosomes 6, 11, and 12 can mediate major resistance to rice blast [2]. In addition, the *Pib* and *Pi5* genes have also been shown to have disease-resistant effects [3,4]. *Pi2* [5], *Pi9* [6], *Pigm* [7], and *Piz-t* [5] are alleles located in the resistance gene cluster on chromosome 6. The resistance gene cluster of chromosome 11 includes *Pik* [8,9], *Pi1* [10], *Pik-h/Pi54* [11], *Pikm* [12,13], *Pik-p* [14], *Pik-s* [8], and *Pik-e* [15]. *Pita* [16] and *Ptr* [17,18] are located on the resistance gene cluster of chromosome 12 [3,4].

Despite the numbers of rice blast genes having been cloned, the vital question remains as to how to use these R genes in breeding. Due to the complexity and diversity of *M. oryzae*,

its pathogenicity in different regions varies significantly. Therefore, an accurate evaluation of the resistance genes and resistance phenotypes of different rice varieties is of great importance for the disease control of rice blast. In recent years, through the identification and application of blast-resistant genotypes in breeding resources, great achievements have been made in molecular-marker-assisted breeding. Rice varieties carrying different resistance genes have been applied to different degrees in production [19,20]. However, due to the complexity and variability of *M. oryzae*, the genes that play resistance effects are different in different areas. In addition, some alleles of rice blast resistance genes are abundant and difficult to distinguish. Therefore, common challenges faced by the majority of breeders include the selection of effective genes, the identification of resistant genotypes, and the evaluation of the effects of resistance genes in breeding.

In this study, 80 varieties from eight regions in northern China were used as test materials. The molecular markers of resistance genes were used in combination with PCR amplification and sequencing. *Pib*, *Pi2*/*Pi9*/*Pizt*/*Pigm*, *Pi5*, *Pik*/*Pik*m/*Piks*, *Pita*, *Ptr*, and their alleles in 80 northern *Japonica* rice varieties were systematically evaluated. The test materials were evaluated for their field resistance in Tonghua of the Jilin Province and Fengcheng of the Liaoning Province. At the same time, eight physiological races of blast isolated from the ZA group in the Liaoning Province were used to evaluate the in vitro resistance phenotypes of the tested materials. In order to provide a reference for blast resistance in the breeding of northern *Japonica* rice, the effects of different resistance genes and gene polymerization were analyzed based on an evaluation of the two different resistance phenotypes.

2. Materials and Methods

2.1. Plant Materials and Cultivation

In this study, 80 rice varieties were selected from 14 breeding units in eight provinces or municipalities including Liaoning, Jilin, Heilongjiang, Beijing, Henan, Shandong, Ningxia, and Xinjiang. Rice-blast-inducing nurseries were set up in Dandong, Liaoning Province (N 40°07′, E 124°23′) and Tonghua, Jilin Province (N 40°52′–43°3′, E 125°10′–126°44′) in 2015 and 2016, respectively (Table S1). Each plot was 2 m long, with 4 rows in total and plant spacings of 30 cm × 13.3 cm. Mongolian rice was used as the inducible variety. One inducible variety plot was set in every ten plots, and two lines of inducible varieties were planted outside the plots. The incidence of leaf blast was investigated in mid-July and neck blast in late September. The experimental materials for in vitro inoculation were planted in 10 × 7.0 × 8.5 cm^3 seedling boxes and placed in the potted field of the Liaoning Academy of Agricultural Sciences.

2.2. Fungal Materials and Disease Evaluation

Eight *M. oryzae* strains were identified and provided by Professor Songhong Wei (Shenyang Agricultural University), belonging to the ZA group and numbered J1–J8. *M. oryzae* was inoculated on potato dextrose agar (PDA, Solarbio Cat# P8931) medium and cultured under light at 25 °C for 5–7 days. The active mycelia of *M. oryzae* were inoculated on tomato oatmeal agar (TOA, Oatmeal: SEAMILD NY/T 1510; Fresh tomato; Agar: Coolaber Cat# CA1331-500G) medium and further cultured under light at 25 °C for 2–3 days. Following the incubation period, the aerial mycelia on the TOA medium were gently erased with cotton swabs and cultured under light at 25 °C for 2–3 days. The spores of *M. oryzae* were collected and prepared as 10^5/mL spore suspensions using Tween-20 (Coolaber Cat# CT11551-100 mL). Three 5 cm leaves were taken from each test variety of the three-and-a-half leaves stages and inoculated with three equal punch inoculations. A total of 5 µL of sporosporine suspension was placed at each point on the leaves and placed in 0.1% 6-benzylaminopurine (Coolaber Cat# PH104-100 mL) [21]. The incidence was observed 5–7 days following inoculation. Seven days after inoculation, disease symptoms were evaluated using a standard 0–9 scale [22], where 0–1 indicated highly resistant (HR),

2–3 = resistant (R), 4 = moderately resistant (MR), 5–6 = moderately susceptible (MS), 7 = susceptible (S), and 8–9 = highly susceptible (HS).

2.3. DNA Extraction and PCR Amplification

Rice leaf DNA was extracted using the CTAB method [23]. Primers of the corresponding genes were used for PCR amplification. The PCR reaction system was as follows (25 µL total volume per reaction): 10 µL of 2× Taq PCR Master Mix (Servicebio Cat# G3304), 0.5 µL of 10 µm primer, 1 µL of 50 µg/mL template DNA, and 8 µL of ddH_2O. A total of 4 µL of each of the PCR products was run on 1% agarose gel (Coolaber Cat# PH104-100 mL) and stained by Goldview (Servicebio Cat# G3304). The results were recorded using a gel imaging system. The PCR products were sent to Tsingke Biotechnology Co., Ltd. (Beijing, China) for sequencing. The DNAMAN software (DNAMAN 6.0.3.99) was used for a comparative analysis of the sequencing results.

2.4. Molecular Identification of Pi2/Pi9/Piz-t/Pigm

In order to better distinguish the genotypes of *Pi2/Pi9/Piz-t/Pigm*, we used different primers to distinguish them. PCR amplification was performed with *Pi9* Pro F/R primers. Band typing was performed using 10% polyacrylamide gel to obtain 111 bp for the *Pi2/Piz-t* allele, 128 bp for the *Pi9* allele, and 138 bp for the non-*Pi2/Piz-t* and non-*Pi9* alleles (Table S2). The samples with 111bp bands were amplified with PCR using primer *Pi2* F/R and detected using agarose gel electrophoresis. The bands were *Pi2* with 322bp and *Piz-t* with no bands [24].

2.5. Molecular Identification of Ptr

The rice samples in this study were amplified with PCR using the primer Z12 F/R (Table S2), which can distinguish the susceptibility alleles of *Ptr*. Strip typing could be performed using 4% agarose gel electrophoresis or 10% polyacrylamide gel electrophoresis, and 226 bp was the susceptibility alleles of *Ptr* and 214 bp was the resistance alleles of *Ptr* [18].

2.6. Molecular Identification of Pita

Two pairs of primers, YL155/YL87 and YL183/YL87, can clearly distinguish the alleles of *Pita*. The primers YL155/YL87 were used for the PCR amplification and detection of *Pita* (Table S2). The 1042 bp product was the *Pita* resistance allele, while the absence of a band indicated the *Pita* susceptible allele. In order to further determine the accuracy of the detection, the primers YL183/YL87 were used to detect *Pita* via PCR amplification (Table S2). The product was a 1042 bp *Pita*-susceptible allele, and no band was a disease-resistant allele [25,26].

2.7. Molecular Identification of Pi5

The rice samples tested in this study were amplified with PCR using the *Pi5* F /R primer to detect whether they carried the *Pi5* resistance gene, and then tested using 1% agar-gel electrophoresis (Table S2). The product size of 1105 bp was sequenced and analyzed. The obtained sequence was consistent with that of the *Pi5* gene, indicating that it contained *Pi5* resistance alleles [4].

2.8. Molecular Identification of Pib

Pib F/R primers can clearly distinguish the rice blast resistance gene *Pib*. The rice samples in this study were amplified with PCR using the primer *Pib* F/R to detect whether they carried *Pib*, and then tested using 1% agarose gel electrophoresis (Table S2). The product size was 915 bp. The product sequence was the same as the *Pib* gene sequence, which meant that the Pib disease resistance allele was contained [18].

2.9. Molecular Identification of Pikh/Pik-ku/Pikm/Piks/Pikp/Pi1

The primers RGA4 were used for the PCR amplification of the samples. The amplified product was 1326 bp and sequenced (Table S2). The results were compared with the *Pikh*/*Pik-ku*/*Pikm*/*Piks*/*Pikp* gene sequence, and the resistance gene was determined according to the sequencing results [9]. The trees were constructed based on the whole predicted DNA sequences using neighborhood linkage algorithms. The bootstrap values corresponding to the number of branch order (1000 replicates) matches are displayed on the nodes at each branch point. The unit branch length is 0.0025.

3. Results

3.1. Distribution of Blast Resistance Genes in Japonica Rice in Northern China

3.1.1. *Pi5*

Pi5 consists of two NBS LRR genes (*Pi5-1* and *Pi5-2*). In total, 75 varieties were successfully identified using functional markers of the *Pi5-2* LRR region and 25 amplified the target fragment. Two haplotypes, *Pi5-G1* and *Pi5-G2*, were found by sequencing with detection rates of 30 and 1.25%, respectively. Zhengdao20 carried *Pi5-G1* with a stop codon on the sixth exon of *Pi5-2*. The other 24 varieties were *Pi5-G2* genotypes with 4 SNPs, which were all missense mutations. The 24 varieties were collected in all the provinces, except for Shandong. The sequence alignment results of the *Pi5* alleles are presented in Figure 1.

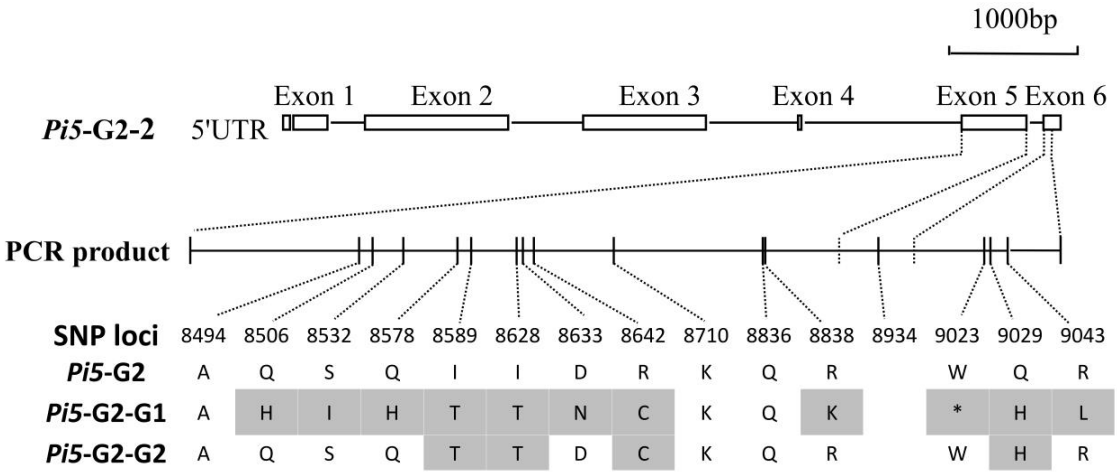

Figure 1. Comparison of amino acid sequences of the *Pi5* allele. SNP loci indicate the base sequence difference sites; the gray square represents the missense mutation site; and * represents the termination codon.

3.1.2. *Pib*

A pair of primers was designed to detect the differences in the *Pib* alleles according to the variations in the functional regions of the third exon of *Pib*. The PCR results showed that 37 varieties amplified the target bands. The sequencing results showed that there were only two genotypes, *Pib* and *Pib-G1*, with detection rates of 41.25 and 2.5%, respectively. Tiejing17 and Jingjing3 were the *Pib-G1* genotypes and there were 60 SNPs within *Pib* (Figure 2). The PCR product sequences of the other 33 varieties that were distributed throughout all eight provinces were identical with *Pib*.

3.1.3. *Pita* and *Ptr*

The functional molecular markers of Pita were designed based on the SNP differences between the alleles at the *Pita* locus, and the functional molecular markers of *Ptr* were designed based on the Indel differences between the *Ptr*-resistant alleles [16,17]. In this study, these two functional markers were used to identify the presence of *Pita* and *Ptr* in the rice varieties. Thirty-nine of the varieties carried the *Pita* and *Ptr* disease resistance alleles.

The two genes were closely linked in all the varieties, with a detection rate of 48.75%. These alleles were distributed in all the provinces except Ningxia (Figure 3).

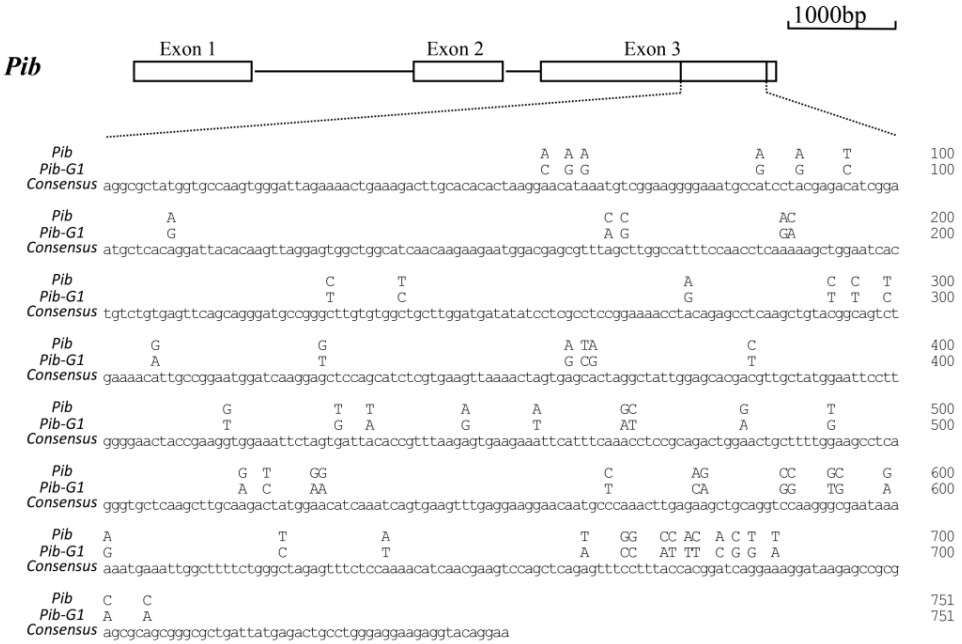

Figure 2. Comparison of the *Pib* allele sequences. The bases shown in the figure are the sites where *Pib* and *PibG1* differ.

3.1.4. *Pi2/Pi9/Piz-t/Pigm*

Pi2/Pi9/ Piz-t/Pigm were cloned at the *Piz* locus on chromosome 6. These alleles have been widely used in the southern rice regions of China. However, no northern *Japonica* rice varieties carrying the *Pi2/Pi9/Pigm* alleles were identified from the tested varieties. Only Longjing 31 and Liaokai 79 carried the *Pizt* locus, with a detection rate of 2.5%. The results showed that the alleles at this locus were less distributed in the main *Japonica* rice varieties in northern China (Figure 3).

3.1.5. *Pikh/Pik-ku/Pikm/Piks/Pikp/Pi1*

The Pik locus is highly polymorphic and six alleles (*Pikh/Pik-ku/Pikm/Piks/Pikp/Pi1*) were cloned at this locus. According to the sequence differences in the *Pik* locus resistance genes, one pair of primers was developed to distinguish different alleles. In this study, 28 out of the 80 varieties did not have *PCR* amplification products, suggesting that they did not carry known resistance alleles at the *Pik* locus. In addition to the known alleles for disease resistance, ten different haplotypes were detected in the Pik locus, including *Pik-G1*, *Pik-G2*, *Pik-G3*, *Pik-G4*, *Pik-G5*, *Pik-G6*, *Pik-G7*, *Pik-G8*, *Pik-G9*, and *Pik-G10*. Among these genotypes, *Pik*, *Piks*, and *Pik-G5* were most widely distributed, with 16, 15, and 8 genotypes detected, respectively. *Pikm* detected two genotypes, while the other genotypes were detected with a low frequency.

These alleles were divided into three subgroups according to the sequence differences in the Pik functional loci (i.e., *Pik* subgroup, *Piks/Pikm/Pi1* subgroup, and *Pikh/Pikp* subgroup) (Figure 4). The *Pik* subsets included *Pik*, *Pik-G1*, *Pik-G2*, *Pik-G3*, and *Pik-G4*, among which, *Pik-G1* was closest to the *Pik* gene sequence, but *Pik-G4* was relatively distant. The largest number of *Pik* alleles was 16 from the Liaoning, Jilin, and Heilongjiang Provinces. The *Piks/Pikm/Pi1* subgroup included seven alleles (*Piks*, *Pikm*, *Pi1*, *Pik-G7*, *Pik-G8*, *Pik-G9*, and *Pik-G10*), among which, *Pik-G7*, *Pik-G9*, and *Pik-G10* had the closest genetic distances with *Piks*. *Pikm* and *Pi1* had the closest genetic distances. The *Pik-G8* sequence was different from that of the other alleles. Fifteen rice varieties carrying *Piks* were from the Liaoning, Jilin, Heilongjiang, Henan, Beijing, and Ningxia Provinces. Only

two rice varieties were carrying *Pikm*, one from Liaoning and one from Xinjiang. The *Pikh*/*Pikp* subgroup contained *Pikh*, *Pikp*, *Pik-LTH*, *Pik-IR64*, *Pik-G5*, and *Pik-G6*. Among these, the genetic distance of *Pikh* and *Pikp* was relatively close, while *Pik-G5* and *Pik-G6* were relatively close. Eight varieties carrying *Pik-G5* originated from the Liaoning, Beijing, and Shandong Provinces. No rice varieties carrying *Pikh* and *Pikp* were detected in this study.

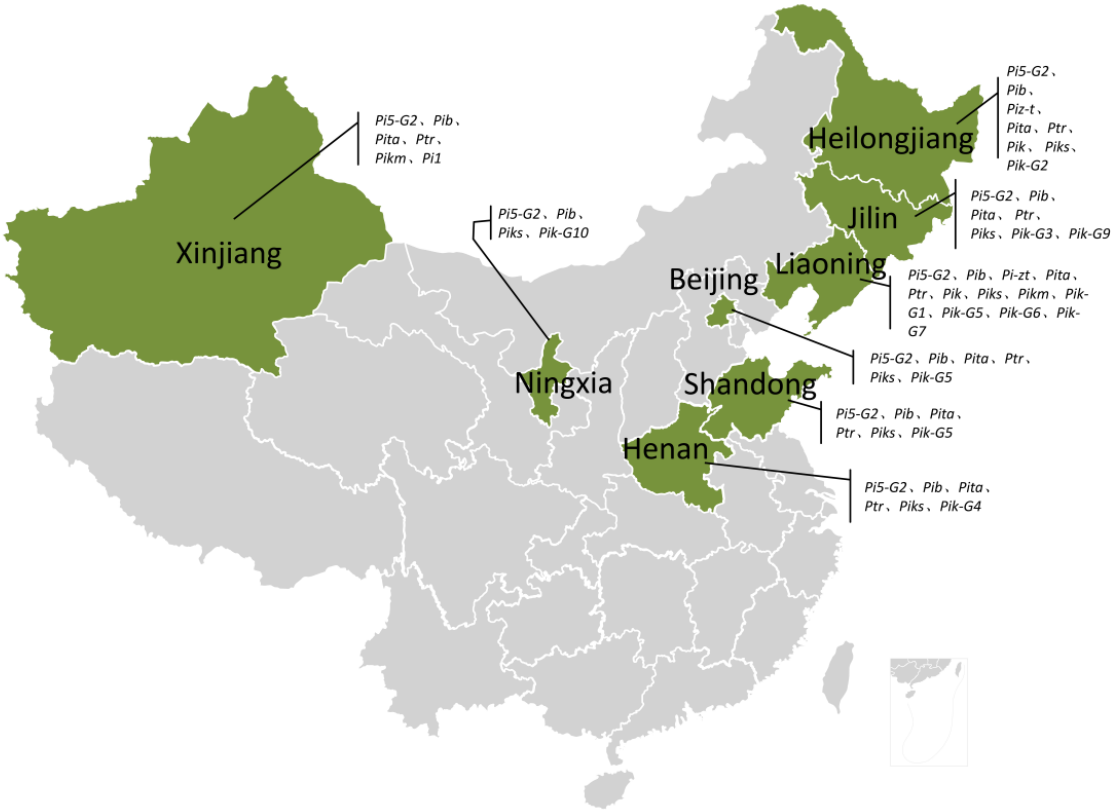

Figure 3. Distribution of rice blast resistance genes throughout China. The green color shows the distribution of blast resistance genes investigated in this study.

3.2. Aggregation of Resistance Genes and Their Contribution to Rice Blast Resistance

There were 39 rice varieties carrying *Pita*, 39 carrying *Ptr*, 33 carrying *Pib*, 16 carrying *Pik*, 15 carrying *Piks*, 2 carrying *Pikm*, and 2 carrying *Pizt* (Figure 5A). In addition, some new alleles were found in *Pi5-G2*, *Pik-G5*, and *Pik-G8*, consisting of 23, 8, and 3 varieties, respectively, accounting for 30.0%, 10.0%, and 3.8% of the total tested varieties. However, further verification is needed to determine whether these three alleles are new disease-resistant alleles. In total, 5 varieties did not carry any of the tested genes, 30 carried only one blast resistance gene, 27 carried two blast resistance genes, 14 carried three blast resistance genes, and 4 carried four blast resistance genes. Except for a few varieties, the incidence of disease was lower when inoculated in vitro than when identified in the field (Figure 5B).

The varieties carrying three or four disease-resistant genes were generally more resistant than those carrying none or only one or two disease-resistant genes (Figure 6A). There were no significant differences in the resistance among varieties in different regions (Figure 6B). Twenty-five rice varieties were artificially inoculated with eight strains and the susceptibility grades were all below the three in three field identification tests. The species used in this study were no disease resistance genes, *Pita*, *Pik*, *Pi5-G2*, *Piks*, *Pik-G8*, *Pi5-G2* + *Ptr*, *Pi5-G2* + *Piks*, *Piks* + *Pita*, *Piks* + *Pizt*, *Pik* + *Pib*, *Pib* + *Pita*, *Pib* + *Pik-G5* + *Pita*, *Pi5-G2* + *Pib* + *Piks*, *Pita* + *Pizt* + *Pik*, *Pi5-G2* + *Pik*, *Pi5-G2* + *Pib* + *Pikm* + *Pita*, and *Pi5-G2* + *Pib* + *Piks* + *Pita* (Figure 6C). When the varieties contained only a single re-

sistance gene, *Pita*, *Pi5-G2*, *Pik*, *Piks*, and *Pik-G8* were overall more resistant than the *Pib* varieties (Figure 6C).

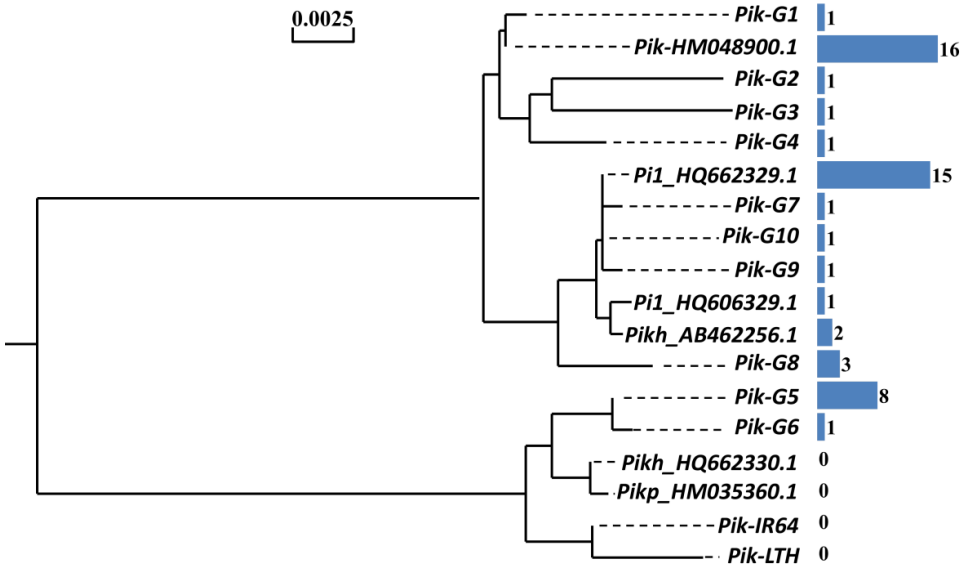

Figure 4. Cluster analysis of Pik functional loci genotypes. The trees are constructed based on the whole predicted DNA sequences using neighborhood linkage algorithms. The bootstrap values corresponding to the number of branch order (1000 replicates) matches are displayed on the nodes at each branch point. The unit branch length is 0.0025.

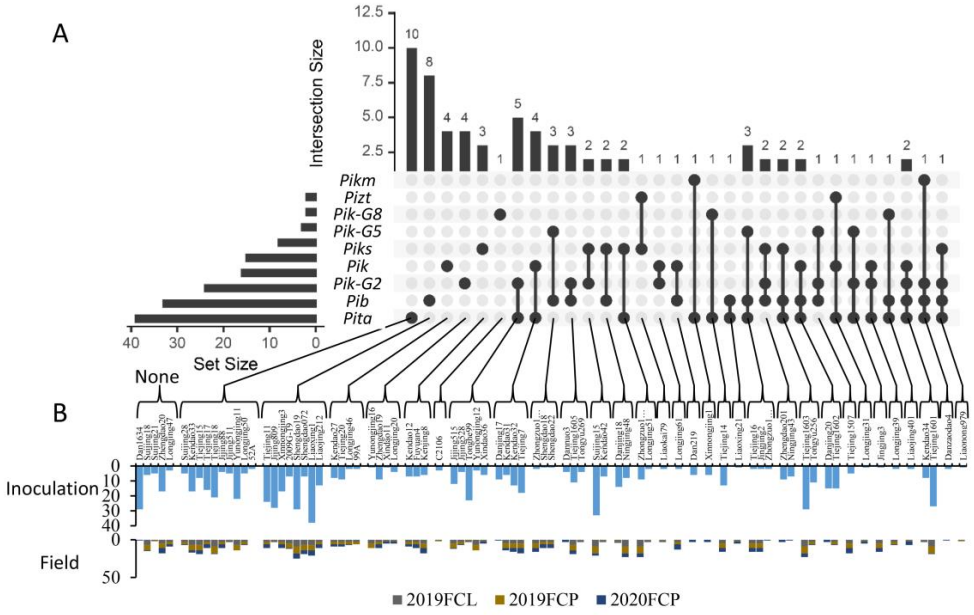

Figure 5. Disease resistance gene aggregation and disease response. (**A**) The number of varieties carrying different blast resistance genes is presented in the upper column, the number of rice blast resistance genes is in the lower column, and the left bar shows the total number of cultivars carrying this resistance gene. (**B**) Identification of *M. oryzae* artificially inoculated with different varieties and field phenotypes. The figure (**B**) above shows the phenotypic identification of artificial inoculation of *M. oryzae.* The bottom graph shows the field phenotypic identification, where 2019 FL (Field Leaf Blast Resistance) displays the incidence of Leaf Blast in 2019, 2019 FP (Field Panical Blast Resistance) shows the incidence of Panical Blast in 2019, and 2020 FL is the incidence of Leaf Blast in 2020.

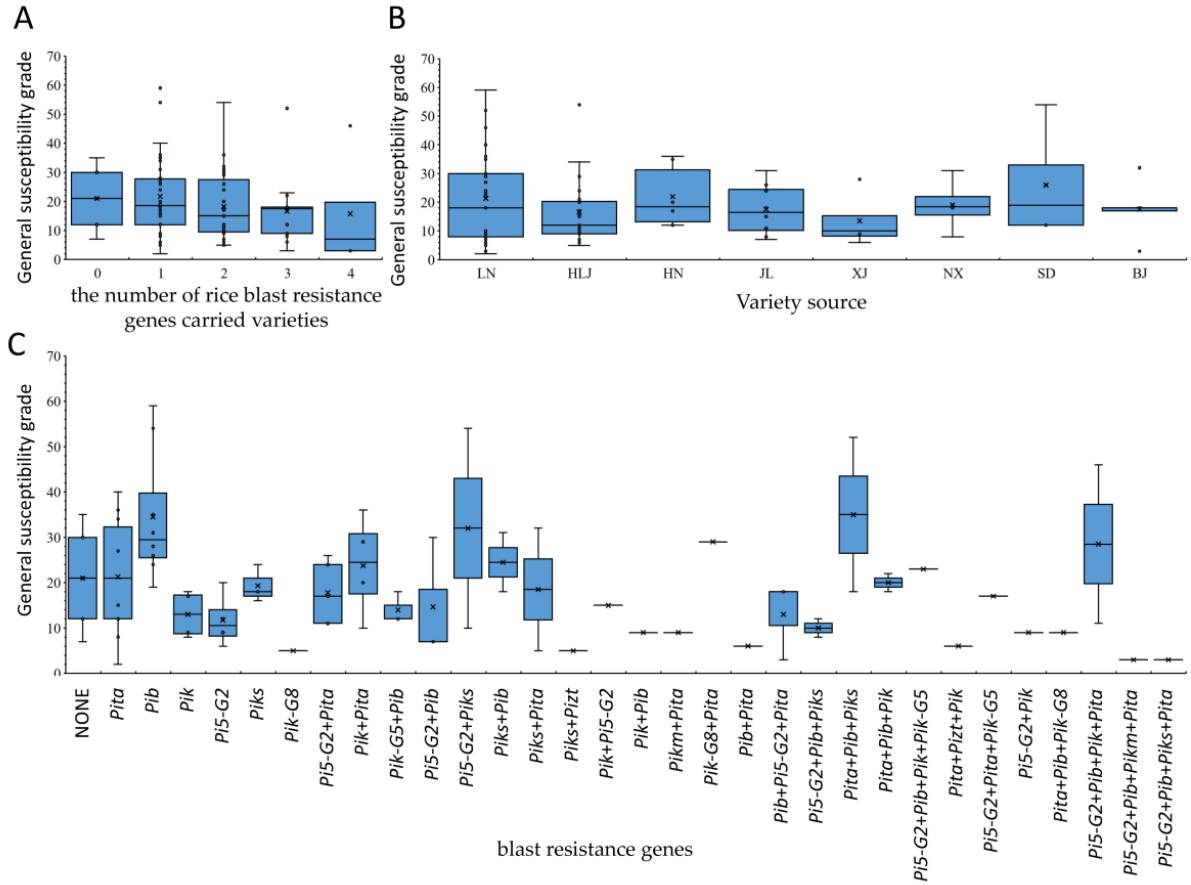

Figure 6. Comprehensive disease resistance of rice blast resistance varieties with different gene combinations. (**A**) Relationships between the number of rice blast resistance genes carried and the comprehensive performance of rice resistance. The letters on the abscissa represent the provinces where rice varieties come from, LN is Liaoning Province, HLJ is Heilongjiang Province, HN is Henan Province, JL is Jilin Province, XJ is Xinjiang Uygur Autonomous Region, NX is Ningxia, SD is Shandong Province, and BJ is Beijing. × Represents the average value, • Represents outliers. (**B**) Resistance of rice blast in different regions of northern China. × Represents the average value, • Represents outliers. (**C**) Relationships between different disease resistance genotypes and overall performance of disease resistance. × Represents the average value, • Represents outliers.

4. Discussion

Based on the need for screening resistant parents in disease resistance breeding, many breeders have carried out the identification of blast resistant genes in germplasm resources. However, comprehensive analyses have found that there were significant differences in the results. For example, some research found that, in the Heilongjiang Province, *Pi9* had the highest distribution frequency, followed by *Pita* and *Pikm*, while *Pib* showed a low distribution frequency [27]. However, other research found that *Pikh* and *Pi54* had the widest distribution [28]. The difference in these identification results was related to the experimental materials and identification methods used. Compared with previous studies, we summarized the identification methods for blast-resistant genes and found a set of effective methods for identifying 18 blast-resistant genes.

The degree of differentiation of the resistance genes was closely related to their positions on chromosomes. For example, *Pik* is located at the end of chromosome 11, which is prone to recombination and exchange in the process of hybridization. Therefore, there are abundant haplotypes of *Pik* in the cultivated species, with 13 types detected in this study alone. For such genes, it is not enough to use only a certain molecular marker in the genotyping of variety blast resistance. The accurate identification of the genotypes at

this locus should be combined with PCR product sequencing, or, according to the reported results, multiple markers should be developed in the mutation-prone area to increase the accuracy of the genotyping. In this study, we identified one mutation hotspot region that can distinguish different alleles at the *Pik* locus. In addition to the known alleles, 10 unknown alleles were identified in the varieties of this study.

Northern China is the primary production area of *Japonica* rice, with an annual cultivated area of ten million hectares. In recent years, rice blast has become more common. The accurate identification of blast resistance genes is of great significance for the breeding of *Japonica* rice. In this study, functional molecular markers of *Pib*, *Pi2*/*Pi9*/*Pizt*/*Pigm*, *Pi5*, *Pik*/*Pikm*/*Piks*, *Pita*, and *Ptr* were used to detect the resistance genes carried by the northern *Japonica* rice from 14 breeding units in eight provinces or municipalities. It was found that *Pib*, *Pita*, *Ptr*, *Piks*, and *Pi5-G2* were most widely distributed in these *Japonica* rice varieties. There were four SNPs between them, however, further research is needed to determine whether *Pi5-G2* and *Pi5* perform the same function. *Pik* was only detected in the varieties from the Heilongjiang and Liaoning Provinces, *Pikm* was only found in the varieties from the Liaoning and Xinjiang Provinces, *Pikh* was not detected on the allele at the *Piz* locus on chromosome 6, and *Pizt* was detected only in two varieties, while *Pi2*, *Pi9*, and *Pigm* were not detected. These results suggest that the genes at the *Piz* locus were less used in northern *Japonica* and would be beneficial for future breeding.

In the field investigation, many varieties often carried known resistance genes, but their field resistance is not stable at different times and regions, due to the fact that the interaction between rice blast fungus and rice conforms to the "gene to gene" hypothesis. Only when the resistance genes carried by rice varieties can recognize the effector of *M. oryzae* can the rice varieties exhibit disease resistance. However, *M. oryzae* is complex and unpredictable. Once the pathogenic gene of the pathogen mutates into a resistance gene that cannot be recognized, the resistance gene cannot play its resistance function, resulting in a loss of resistance. Therefore, an accurate assessment of the main resistance genes in a certain area can be a challenge. The genes that can affect resistance will change with the variation in the endemic rice *M. oryzae* races in this area. In this study, eight physiological races of *M. oryzae* were inoculated onto the experimental materials. The results showed that three varieties carrying *Pib* + *Pik-G5* were resistant to the eight artificially inoculated strains, but showed moderate resistance in the field identification. Not were the results of the artificial inoculation and field induction significantly different, but the resistance effects of the same gene in different genetic backgrounds also varied significantly. For example, three of the ten varieties carrying only *Pita* and *Ptr* showed resistance to the disease during the artificial inoculation and field identification, while the other seven were susceptible to different degrees of the disease. In addition, for *Pita* + *Pib* + *Piks* carriers, Tiejing 1603 was significantly more sensitive to *M. oryzae* than Tongyu256. Kendao3 and Tiejing1601 both carried the *Pi5-G2* + *Pib* + *Pik* + *Pita* gene, but Kendao34 had a significantly better resistance than the Tiejing1601 variety.

The results showed that the aggregation of multiple resistance genes in the same variety not only broadened the resistance spectrum, but also improved the resistance level to some physiological races. In this study, the susceptibility degree of the test materials carrying only *Ptr* was generally higher, while, when *Ptr* and other resistance genes coexisted, *Pik*-G5, *Pik*-G2, *Piks*, *Pik*, and *Pita* could all improve the resistance. Furthermore, the materials carrying three or four disease-resistant genes were generally more resistant than the materials carrying no disease-resistant genes or only one or two disease-resistant genes. However, there were exceptions. In this study, the resistance of Tiejing1603 and Liaonong979 to *Pib* + *Pita* + *Piks* was inferior to Liaoxing21 carrying *Pib* + *Pita* alone. In conclusion, the resistance of rice to blast was very complex, and the aggregation of the resistance genes was not the effect of $1 + 1 \geq 2$. Therefore, the number of genes should not be blindly pursued when carrying out resistance polymerization breeding. In other words, more genes may not be necessarily better. According to the combination of the genotypes and the resistance phenotypes of the parents, the materials carrying the major

blast resistance genes and showing a relatively stable resistance to rice blast in many years should be selected. Taken together, the introduction of different types of blast resistance genes on different chromosomes should be strengthened to avoid the yield loss caused by the decline in disease resistance caused by the large-scale application of single blast resistance genes.

5. Conclusions

In conclusion, *Pib*, *Pita*, *Ptr*, and *Piks* were most widely distributed in northern *Japonica* rice varieties, while *Pi2*/*Pi9*/*Pizt*/*Pigm* alleles at the *Piz* locus were less distributed and require an urgent introduction. In terms of disease resistance, among the tested genes, the two varieties carrying *Pizt* had strong resistance, which deserves further research. *Pik* had abundant loci, among which, *Pikm* demonstrated a better resistance. Many varieties were carrying the *Pita* and *Ptr* loci. Although some of them were not resistant, the resistance contribution of *Pita* and *Ptr* cannot be ignored. Further identification of the resistance of antagonistic parents and selection for application in disease-resistant molecular breeding is needed.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy13102662/s1, Table S1. The rice varieties used in this study. Table S2. Primer sequences for markers used in this study.

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References

1. Wang, G.L. Novel Insights into the Rice Innate Immunity Against Pathogens. *Annu. Rev. Phytopathol.* **2014**, *52*, 213–241.
2. Wang, L.L.; Ma, Z.B.; Zhao, J.M.; Gu, S.; Gao, L.J.; Mukhina, Z.; Ma, D.R.; Zheng, W.J. Progress on mapping, cloning and application of rice blast resistance genes. *Pak. J. Bot* **2022**, *54*, 2363–2375. [CrossRef] [PubMed]
3. Wang, Z.X.; Yano, M.; Yamanouchi, U.; Iwamoto, M.; Monna, L.; Hayasaka, H.; Katayose, Y.; Sasaki, T. The *Pib* gene for rice blast resistance belongs to the nucleotide binding and leucine-rich repeat class of plant disease resistance genes. *Plant J.* **1999**, *19*, 55–64. [CrossRef]
4. Lee, S.K.; Song, M.Y.; Seo, Y.S.; Kim, H.K.; Ko, S.; Cao, P.J.; Suh, J.P.; Yi, G.; Roh, J.H.; Lee, S.; et al. Rice *Pi5*-mediated resistance to Magnaporthe oryzae requires the presence of two coiled-coil-nucleotide-binding-leucine-rich repeat genes. *Genetics* **2009**, *181*, 1627–1638. [CrossRef]
5. Zhou, B.; Qu, S.H.; Liu, G.F.; Dolan, M.; Sakai, H.; Lu, G.D.; Bellizzi, M.; Wang, G.L. The eight amino-acid differences within three leucine-rich repeats between *Pi2* and *Piz-t* resistance proteins determine the resistance specificity to *Magnaporthe grisea*. *Mol. Plant-Microbe Interact.* **2006**, *19*, 1216–1228. [CrossRef] [PubMed]

6. Qu, S.H.; Liu, G.F.; Zhou, B.; Maria, B.; Zeng, L.R.; Dai, L.Y.; Han, B.; Wang, G.L. The broad-spectrum blast resistance gene *Pi9* encodes a nucleotide-binding site-leucine-rich repeat protein and is a member of a multigene family in rice. *Genetics* **2006**, *172*, 1901–1914. [CrossRef] [PubMed]
7. Deng, Y.W.; Zhu, X.D.; Shen, Y.; He, Z.H. Genetic characterization and fine mapping of the blast resistance locus *Pigm(t)* tightly linked to *Pi2* and *Pi9* in a broad-spectrum resistant Chinese variety. *Theor. Appl. Genet.* **2006**, *113*, 705–713. [CrossRef]
8. Fjellstrom, R.; Conawaybormans, C.A.; Mcclung, A.M.; Marchetti, M.A.; Shank, A.R.; Park, W.D. Development of DNA markers suitable for marker assisted selection of three genes conferring resistance to multiple pathotypes. *Cropence* **2004**, *44*, 1790–1798. [CrossRef]
9. Zhai, C.; Lin, F.; Dong, Z.Q.; He, X.Y.; Yuan, B.; Zeng, X.S.; Wang, L.; Pan, Q. The isolation and characterization of *Pik*, a rice blast resistance gene which emerged after rice domestication. *New Phytol.* **2011**, *189*, 321–334. [CrossRef]
10. Yu, Z.H.; Mackill, D.J.; Bonman, J.M.; McCouch, S.R.; Guiderdoni, E.; Notteghem, J.L.; Tanksley, S.D. Molecular mapping of genes for resistance to rice blast (*Pyricularia grisea* Sacc.). *Theor. Appl. Genet.* **1996**, *93*, 859–863. [CrossRef]
11. Rai, A.K.; Kumar, S.P.; Gupta, S.K.; Gautam, N.; Singh, N.K.; Sharma, T.R. Functional complementation of rice blast resistance gene *Pi-k h (Pi54)* conferring resistance to diverse strains of Magnaporthe oryzae. *J. Plant Biochem. Biotechnol.* **2011**, *20*, 55–65. [CrossRef]
12. Li, L.Y.; Wang, L.; Jing, J.X.; Li, Z.Q.; Lin, F.; Huang, L.F.; Pan, Q.H. The *Pikm* gene, conferring stable resistance to isolates of *Magnaporthe oryzae*, was finely mapped in a crossover-cold region on rice chromosome 11. *Mol. Breed.* **2007**, *20*, 179–188. [CrossRef]
13. Ashikawa, I.; Hayashi, N.; Yamane, H.; Kanamori, H.; Wu, J.; Matsumoto, T.; Ono, K.; Yano, M. Two adjacent nucleotide-binding site-leucine-rich repeat class genes are required to confer *Pikm*-specific rice blast resistance. *Genetics* **2008**, *180*, 2267–2276. [CrossRef] [PubMed]
14. Yuan, B.; Zhai, C.; Wang, W.J.; Zeng, X.S.; Xu, X.K.; Hu, H.Q.; Lin, F.; Wang, L.; Pan, Q.H. The *Pik-p* resistance to *Magnaporthe oryzae* in rice is mediated by a pair of closely linked CC-NBS-LRR genes. *Theor. Appl. Genet.* **2011**, *122*, 1017–1028. [CrossRef] [PubMed]
15. Chen, J.; Pei, P.; Tian, J.S.; He, Y.G.; Zhang, L.P.; Liu, Z.X.; Yin, D.D.; Zhang, Z.H. *Pike*, a rice blast resistance allele consisting of two adjacent NBS–LRR genes, was identified as a novel allele at the *Pik* locus. *Mol. Breed.* **2015**, *35*, 117. [CrossRef]
16. Bryan, G.T.; Wu, K.S.; Farrall, L.; Jia, Y.; Hershey, H.P.; McAdams, S.A.; Faulk, K.N.; Donaldson, G.K.; Tarchini, R.; Valent, B. A single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene *Pi-ta*. *Plant Cell* **2000**, *12*, 2033–2045. [CrossRef]
17. Jia, Y.; Martin, R. Identification of a new locus, *Ptr(t)*, required for rice blast resistance gene Pi-ta-mediated resistance. *Mol. Plant-Microbe Interact. MPMI* **2008**, *21*, 396. [CrossRef]
18. Zhao, H.J.; Wang, X.Y.; Jia, Y.L.; Minkenberg, B.; Wheatley, M.; Fan, J.B.; Jia, M.H.; Famoso, A.; Edwards, J.D.; Wamishe, Y.; et al. The rice blast resistance gene *Ptr* encodes an atypical protein required for broad-spectrum disease resistance. *Nat. Commun.* **2018**, *9*, 2039. [CrossRef]
19. Wang, F.; Wang, L.G.; Pan, M.Y.; Niu, Z.Y.; Zhou, Y.; Liang, G.H. Marker-assisted selection and application of blast resistant gene *Pigm(t)* in rice. *North China Agric. J.* **2016**, *31*, 51–56.
20. Cao, N.; Chen, Y.; Ji, Z.J.; Zeng, Y.X.; Yang, C.D.; Liang, Y. Recent Progress in Molecular Mechanism of Rice Blast Resistance. *Chin. J. Rice Sci.* **2019**, *33*, 489–498.
21. Li, W.T.; Zhu, Z.W.; Chern, M.S.; Yin, J.J.; Yang, C.; Li, R.; Cheng, M.P.; He, M.; Wang, K.; Wang, J.; et al. A natural allele of a transcription factor in rice confers broad-spectrum blast resistance. *Cell* **2017**, *170*, 114–126.e15. [CrossRef] [PubMed]
22. International Rice Research Institute (IRRI). *Standard Evaluation System for Rice (SES)*, 5th ed.; International Rice Research Institute: Manila, Philippines, 2002.
23. Lan, B.; Lin, W.; Wu, Z.; Jiang, B.; University, G. Rapid Miniprep Extraction of Genomic DNA from Micro-endosperm Maize with Modified CTAB Method. *Genom. Appl. Biol.* **2015**, *34*, 190–194.
24. Tian, D.G.; Chen, Z.J.; Chen, Z.Q.; Zhou, Y.C.; Wang, Z.H.; Wang, F.; Chen, S.B. Allele-specific marker-based assessment revealed that the rice blast resistance genes *Pi2* and *Pi9* have not been widely deployed in Chinese *indica* rice cultivars. *Rice* **2016**, *9*, 19. [CrossRef]
25. Jia, Y.L.; Wang, Z.H.; Pratibha, S. Development of Dominant Rice Blast *Pi-ta* Resistance Gene Markets. *Crop Sci.* **2002**, *42*, 2145–2149. [CrossRef]
26. Wang, Z.; Jia, Y.; Rutger, J.N.; Xia, Y. Rapid survey for presence of a blast resistance gene *Pi-ta* in rice cultivars using the dominant DNA markers derived from portions of the *Pi-ta* gene. *Plant Breed.* **2007**, *126*, 36–42. [CrossRef]
27. Zhou, Y.L.; Zhang, Y.L.; Zhao, H.S.; Jin, X.H. Molecular Detection and Analysis of Rice Blast Resistance Genes in Main Rice Varieties in Heilongjiang Province. *Crops* **2019**, *35*, 172–177.
28. Wang, D.Y.; Zhang, H.; Wang, L.; Jia, C.H.; Zhao, B.; Zuo, N.; Tong, X.P.; Zhao, F.; Pei, Z.Y. Molecular Detection and Analysis of Blast Resistance Genes and Restorer Genes in 107 *Japonica* Rice. *Mol. Plant Breed.* **2021**, *19*, 16.