Allelic variation of the rice blast resistance gene *Pid3* in cultivated rice worldwide

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In this study, the re-sequencing data from 3,000 rice genomes project (3 K RGP) was used to analyze the allelic variation at the rice blast resistance (*R* *pid3*) locus. A total of 40 haplotypes were identified based on 71 nucleotide polymorphic sites among 2621 *Pid3* homozygous alleles in the 3k genomes. *Pid3* alleles in most *japonica* rice accessions were pseudogenes due to premature stop mutations, while those in most *indica* rice accessions were identical to the functional haplotype Hap_6, which had a similar resistance spectrum as the previously reported *Pid3* gene. By sequencing and CAPS marker analyzing the *Pid3* alleles in widespread cultivars in China, we verified that Hap_6 had been widely deployed in *indica* rice breeding of China. Thus, we suggest that the priority for utilization of the *Pid3* locus in rice breeding should be on introducing the functional *Pid3* alleles into *japonica* rice cultivars and the functional alleles of non-Hap_6 haplotypes into *indica* rice cultivars for increasing genetic diversity.

Rice (*Oryza sativa* L.) is a staple food for nearly half of the world’s population. It also represents a model for functional genome research among the crop plants. Rice was the first crop plant to be fully sequenced and so far, has at least four different reference genomes in two subspecies (*Oryza sativa* subsp. *indica* and *Oryza sativa* subsp. *japonica*)

Rice blast, caused by the filamentous ascomycete *Magnaporthe oryzae* (*M. oryzae*), is the most devastating rice fungus disease worldwide. It has been proven that deployment of cultivars with resistance (*R*) genes is the most effective and eco-friendly approach for the control of rice blast. To date, at least 69 rice blast *R* loci have been identified, of which 16 loci harboring more than 30 *R* genes/alleles have been cloned and functionally analyzed in detail. It is important to note that almost all cloned rice blast *R* genes encode nucleotide-binding site leucine-rich repeats (NBS-LRR) proteins except for *Pid2* and *pid1*; the former encodes a receptor-like kinase and the later a proline-rich protein. Likewise, a number of NBS-LRR genes cloned from maize, sorghum, and brachypodium were also proved being blast resistant in rice. In recent years, a trend has become clear: a significant number of newly cloned rice blast *R* genes have finally been verified as being allelic to one of the
previously cloned rice blast R genes, and fewer represent a new rice blast R locus. Considering that there are more than 400 NBS–LRR gene sequences identified in a rice genome, and that allelic rice blast R genes may confer distinct resistance spectra to M. oryzae isolates, we believe that allele mining of cloned rice blast R genes in germplasms would reveal more favorable alleles for rice blast resistance breeding.

However, the majority of the cloned rice blast R genes are clustered as most of NBS-LRR genes present in diverse multigene families. Moreover, the clustered NBS-LRR genes usually fall into heterogeneous groups based on their structural similarity. For example, in the 76-kb chromosomal region containing the rice blast R gene Pi9 locus, six tandemly arranged NBS-LRR type putative genes were identified. The identities among the six paralogs ranged from 63.8 to 98.6% and only the Nbs2-Pi9 was proved to be the Pi9 gene. The other example is Pi5, whose blast resistance function is actually conferred by two NBS-LRR genes, Pi5-1 and Pi5-2. The ~90-kb sequences of the Pi5 locus are significantly diverged between resistant and susceptible rice cultivars; the susceptible cultivar Nipponbare completely lacks the corresponding allele of Pi5-2. Similar statuses were also found at other rice blast R loci, like Pb1, Pia33, Pia9, Pia37, Pb13, and Pit6. These duplicated sequences have diverged through accumulated mutation, which increase the complexity of NBS-LRR gene sequences. Therefore, it is difficult to identify alleles of cloned NBS-LRR type rice blast R genes through allele mining approach based on either traditional PCR or NGS data analyzing. However, at a few rice blast R loci, the structure of NBS-LRR genes are rather simple, with only single NBS-LRR gene. Allele mining at these loci is feasible.

The rice blast R gene Pid3 was initially identified in the indica variety Digu by performing a genome-wide comparison of paired NBS-LRR genes and their pseudogene alleles between 93-11 (indica) and Nipponbare (japonica) on the premise of the verification of obvious different resistance of indica and japonica varieties to M. oryzae strains collected from south and north China. Pid3 is a typical CC-NBS-LRR protein of 924 amino acids with no intron. Alleles in most japonica varieties were identified as pseudogenes due to the presence of a nonsense mutation at the nucleotide position 2209 starting from the translation initiation site; however, this pseudogene mutation did not occur in tested indica varieties, including African cultivated rice varieties and AA genome-containing wild rice species. Then, a number of Pid3 alleles or orthologs were cloned by map-based cloning and sequencing-based allele mining from indica and wild rice accessions, of which five had been verified to confer differential resistance spectra to a set of M. oryzae isolates. In this study, mainly based on the 3 K RGP sequencing data, a total of 40 haplotypes were identified according to 71 nucleotide polymorphic sites in 2621 Pid3 homoyzogous alleles. Finally, by PCR-based allele mining and gene transformation, we disclosed a functional Pid3 allele, which has been widely deployed in indica rice cultivars and especially in hybrid rice in China. With the above overview, we may propose different strategies in application of the functional Pid3 alleles to indica and japonica rice breeding.

**Results**

**The nonsense mutation of Pid3 alleles at the position 2209.** We previously revealed that Pid3 alleles in 29 out of 32 japonica varieties were identified as pseudogenes due to the presence of a nonsense mutation (CAG to TAG) at the nucleotide position 2209, whereas none of the varieties in 32 indica collection contained this mutation. To figure out the distribution of nonsense mutation of Pid3 alleles in the 3 K RGP sequencing data, we checked the corresponding position 13055819 on chromosome 6, where “G” represents “C” and “A” represents “T”, since Pid3 coding sequence is on the “-” chain of the sequencing data. A total of 2953 Pid3 alleles at this position were identified, of which 22 out of 1732 indica, 715 out of 859 japonica and 40 out of 362 other rice accessions were “A”, indicating Pid3 alleles in most japonica and scarcely in indica rice accessions are nonfunctional due to the nonsense mutation at the position 2209. The detailed information was shown in Fig. 1. To verify the general survey based on the sequencing data, we used the CAPS marker to test nearly 300 varieties, including 149 widely cultivated japonica varieties in north China and 140 indica varieties, most of which were backbone parents of...
hybrid rice cultivars widely used in China. We found the nonsense mutation in 91.9% of the 149 *japonica* cultivars but neither of the 140 *indica* cultivars. The details for these cultivars were listed in Supplementary Table S1. Both of the results further confirmed our previous report that the pseudogenization of *Pid3* has prevailed in *japonica*.

**Nucleotide polymorphisms of *Pid3* alleles.** The coding sequence of *Pid3* is located in a region (13055253-13058027) on chromosome 6 according to the *Nipponbare* genome in the 3K RGP sequencing data. In this region, only 16 *Pid3* alleles showed obvious InDel polymorphisms at one singleton nucleotide site, which is an 8-bp (ATATATTTC) insertion at the position 13055566, corresponding to the nucleotide position 2461 starting from the translation initiation site of *Pid3* gene. Of the 16 *Pid3* allelic loci, three showed heterozygous insertions, and all alleles were from *indica* subpopulation excepting one allele from *japonica* (Supplementary Table S2). After eliminating alleles with heterozygous sequences and ambiguous single site deletion probably caused by insufficient sequencing coverage, we have obtained a total of 2621 *Pid3* alleles for subsequent analyses.

In total, 71 nucleotide polymorphic sites were detected in the 2775-bp coding region of these 2621 *Pid3* alleles (Fig. 2). It was consistent to our previous finding that the *Pid3* gene processed lower nucleotide polymorphism ($\pi = 0.00255$ in this study), belonging to the conserved type of plant NBS-LRR class R genes. Among the surveyed six subpopulations, *indica* has the lowest nucleotide polymorphism ($\pi = 0.00102$), while aromatic basmati/sadri (aro) group has the highest ($\pi = 0.00265$). The values of Tajima’s D were mostly negative but did not significantly deviate from the neutral model (Table 1). To figure out detailed information, three domains including CC, NBS and LRR were defined as those in our former study, and we assigned *Pid3-W5*, a functional *Pid3* ortholog cloned from *Oryza rufipogon* as the reference, and calculated the Ka/Ks ratio between each *Pid3* haplotype and *Pid3-W5* (Supplementary Table S3). On average, the nucleotide polymorphisms in NBS domain were comparable with those in LRR domain, but much higher than those in CC domain. However, most of the ratios of $\pi_{non}/\pi_{syn}$ and Ka/Ks in NBS domain were far less than 1, while they were much greater than 1 in LRR domain, indicating that the nucleotide polymorphisms in NBS domain were affected by purifying selection, while polymorphisms in LRR domain mainly affected by positive selection (Table 1 and Supplementary Table S3).

**Haplotype analysis of *Pid3* alleles.** Based on the 71 nucleotide polymorphic sites, a total of 40 haplotypes of *Pid3* alleles were identified (Fig. 2 and Table 1). The average haplotype diversity (hd) of the *Pid3* coding region was 0.680. The aus/boro subpopulation had the highest hd (0.706), while the *indica* subpopulation has the lowest hd (0.384). The number of rice accessions in each haplotype varied significantly; Hap_6 was the largest haplotype shared by as many as 1376 rice accessions, whereas, in contrast to Hap_6, there were nine haplotypes each was carried by only one accession (Fig. 2). Besides the 14 haplotypes that had the premature stop codon at the above mentioned nucleotide position 2209, Hap_11, Hap_36 and Hap_2 were newly identified pseudogenization types

**Figure 2.** Summary of DNA variations in the 2775-bp coding region of 2621 *Pid3* alleles. Numbers in brackets represent rice accessions belonging to specific haplotypes. Site 1 corresponds to the first position of the start codon. Dots represent nucleotide variants identical to the *Pid3* sequence in Digu. The boxed nucleotide is the premature mutation.
with a premature stop codon at nucleotide position 885, 1088 and 1766, respectively. It is noteworthy that Hap_36 owned both of the premature stop codons at 1088 and 2029 simultaneously.

A haplotype flowchart was constructed to describe the evolutionary relationships and mutational steps of these 31 haplotypes, which were identified in at least two rice accessions (Fig. 3). Meanwhile, the components of each haplotype were also taken into account. The flowchart analysis illustrated that the haplotypes of Pid3 could be roughly divided into three groups. Group I contains Hap_9 and twelve other haplotypes, in which most carriers of this group are diverse. It is notable that although Hap_2, shared mostly by tropical japonica accessions, is not in group I, it is still a pseudogene due to the premature stop codon at 1766 as mentioned above. Indeed, out of the 20 Pid3 alleles belonging to Hap_2, 86 were identified from tropical japonica accessions. All of these haplotypes have premature stop codon at 2209. Accordingly, in Group II, the predominant haplotype is Hap_6 but most of its carriers are indica varieties. Including temp, trop, temp/trop, trop/temp and japx varieties; Aus, aus varieties; Inax, including temp, trop, trop/tem, trop/temp and japx varieties; Admx, all other unassigned varieties. S, number of segregating sites; π, nucleotide diversity; πsyn, average synonymous site diversity; π nonsyn, average nonsynonymous site diversity; π nonsyn/π syn, ratio of nonsynonymous site diversity over synonymous site diversity; Nhap, number of haplotype; Hdp, haplotype diversity; *Statistical significance P < 0.05.

Table 1. Polymorphism, neutral test and haplotype analysis of Pid3 alleles. Indica, including ind1, ind2, ind3 and indx varieties; Japonica, including temp, trop, temp/trop, trop/temp and japx varieties; Aus, aus varieties; Inax, admixed aus and indica varieties; Aro, aromatic varieties; Admx, all other unassigned varieties. S, number of segregating sites; π, nucleotide diversity; π syn, average synonymous site diversity; π nonsyn, average nonsynonymous site diversity; π nonsyn/π syn, ratio of nonsynonymous site diversity over synonymous site diversity; Nhap, number of haplotype; Hdp, haplotype diversity; *Statistical significance P < 0.05.
rice orthologs, a total of 26 SNPs were identified, of which only the SNPs at the position 46 and 994 were not included by the above identified 71 SNPs. In the 20 Pid3 orthologs from wild rice, a total of 101 SNPs were characterized, of which only 35 could be included by the above described 40 haplotypes (Supplementary Table S4).

The closest wild relatives of *O. sativa* are *O. nivara* and *O. rufipogon*, although which of them is the immediate progenitor of the cultivated rice remains controversial. To investigate the domesticated history of Pid3, the 40 cultivated and 20 wild rice haplotypes were aligned (Fig. 4). It could be inferred that the haplotypes Hap_6 and Hap_9 were the ancestral types in cultivated rice, as they existed in all six cultivated rice subpopulations (Fig. 3) and two wild rice accessions, W12 and W11 (Fig. 4). In addition, they could be domesticated independently from different wild rice accessions, and the other haplotypes in group I and group II mentioned above might originate from Hap_9 and Hap_6, respectively. The haplotypes in group III might originate from a third type of wild rice accessions, because these haplotypes were much different from Hap_6 and Hap_9, and most of them were similar to those from wild rice accessions.

Analysis of predicted Pid3 proteins. A total of 44 amino acid variations caused by the 71 nucleotide polymorphic sites, leads to 32 different predicted proteins (the original Pid3 haplotype Hap_9 = Hap_24 = Hap_31 = Hap_33 = Hap_38 and Hap_6 = Hap_12 = Hap_16 = Hap_17 = Hap_34). Of them, 20 encode complete CC-NBS-LRR proteins with 924 amino acids, and 12 show premature transcription termination at the position 295, 363, 589 and 737, respectively (Fig. 5). Most predicted proteins encoded by Pid3 alleles are different from Pid3 itself at nine positions, including 44, 259, 571, 577, 625, 815, 856, 894 and 896. It is noteworthy that besides the premature site between full length and truncated proteins at the position 737, there are five other completely different sites (153, 204, 515, 669 and 670) among them (Fig. 5), but this phenomenon was not found in other truncated proteins, which were premature at the position 295 and 589.

We also compared Pid3 protein sequences in different cultivated rice growing areas, including indica/japonica growing areas in East Asia and Southeast Asia, and indica/japonica/aus/aro growing areas in South Asia. The sequence comparison revealed that most amino acid variations were found in the LRR region, and indica subgroups had lower diversity in all three areas (Supplementary Figure S2). For indica and japonica subgroups in East Asia and Southeast Asia, Pid3 haplotypes had no obvious difference. However, two stop codons at the position 589 and 737, five common variant amino acids, T153M, G204S, R515H in NBS domain, V669F, G670D in LRR region were found between indica and japonica subgroups in these two areas. We can infer that these variants are
related to indica - japonica differentiation and play an important role in Pid3 function. Most aus and aro rice cultivars were found in South Asia. In this area, except for the 15 aus cultivars belonging to the japonica predominant Hap_9, most of the remaining haplotypes were extremely similar to indica haplotypes. The Pid3 ortholog (Hap_6) present a similar resistance spectrum as Pid3 gene. In a previous study, we evaluated the resistance of 11 Pid3 orthologs by rice genetic transformation and blast inoculation, and found that five Pid3 orthologs were functional rice blast R genes, including Pid3-I1(Hap_14), Pid3-I3 (Hap_20/Pid3/Pi25) from indica varieties and Pid3-W3, Pid3-W4, Pid3-W5 (Hap_13/Pid3-A4) from wild rice accessions29. However, although it is the most popular haplotype in cultivated rice accessions, the rice blast resistance of the Pid3 ortholog (Hap_6) had not been verified yet. In this study, Pid3-I2 from the indica variety 93-11, a widely used inbred cultivar and backbone parent of hybrid rice in China, was chosen as the representative of Hap_6 for blast resistance testing. First, the entire 2775-bp coding region of Pid3-I2 was inserted into the binary vector pZH01 under the (CAMV) 35 S promoter control and transformed into the susceptible rice variety TP309, which was the same recipient used for 11 Pid3 orthologs in our previous study 29. Next, we performed a genetic complementation test of Pid3-I2 as previously described 44. A 6236-bp 93-11’s DNA fragment, including the Pid3-I2 coding region, 3010-bp upstream region, and 451-bp downstream region, was sub-cloned into the binary vector pMNDRBBin6, which was then introduced into TP309 as well. Finally, we obtained nine and eleven independent primary transgenic plants (T0) for these two constructs, respectively. All 20 transgene-positive plants were confirmed to be resistant to the M. oryzae isolate Zhong-10-8-14, which was the same isolate employed in our previous study (Fig. 6). Co-segregation of the transgene and the blast resistance was confirmed in selfed progenies (T1) of the two types of T0 lines, respectively. The results suggested that Pid3-I2/Hap_6 was indeed functional rice blast R gene. We then inoculated Pid3 and Pid3-I2/Hap_6 homozygous T2 transgenic plants, respectively, with 125 M. oryzae isolates collected from China. The testing revealed that compared to the susceptible recipient TP309, Pid3-I2/Hap_6 transgenic lines conferred resistance to 28 isolates, with a resistance frequency 22.4%, which is the same as that of the Pid3 transgenic plants (Supplementary Tables S5 and S6).
Geographic distribution of the known functional Pid3 alleles in cultivated rice accessions. At present, of the total of 42 haplotypes of Pid3 identified in cultivated rice accessions, only four haplotypes, including Hap_6, Hap_13, Hap_14, and Hap_20 were confirmed to be functional in rice blast resistance. Of the remaining 38 haplotypes, 16 were identified as pseudogenes due to the presence of premature stop codons at different positions (Fig. 2), while the other 22 remained to be further elucidated. Based on the information of the 3 K RGP, the worldwide geographic distributions of the three types of Pid3 haplotypes were presented in Table 2. Obviously, Hap_6 is the most common haplotype with the widest geographic distribution in the indica-cultivated area, whereas in most japonica-cultivated area, such as Japan, South Korea and Europe, functional haplotypes of Pid3 are almost nonexistent. In Southeast Asia countries, such as Thailand, Vietnam, Cambodia and Myanmar, Hap_20 distributes widely, while Hap_14 is only found in four South Asia countries: India, Pakistan, Nepal and Bangladesh. Finally, Hap_13, the rarest functional haplotype in cultivated rice accessions, is only found in China, India and Bangladesh. Although the number of haplotypes (whose functions have not been determined yet) is up to 22, these haplotypes are distributed scarcely in most countries and areas.

Hap_6 has been widely employed in hybrid rice breeding in China. To investigate the distribution of the known functional Pid3 haplotypes in cultivated rice varieties in China, we first sequenced the respective allelic Pid3 coding regions of the 12 widely cultivated japonica varieties in China, which would not contain the premature mutation at the position 2209 as testified by the CAPS marker (Supplementary Table S1). Sequence comparison confirmed that in these japonica varieties Pid3 alleles were identical to Hap_6, suggesting that Hap_6 of Pid3 might be introduced into minor japonica varieties by rice breeders. Next, we investigated whether Pid3 alleles in widely cultivated indica varieties in China were identical to Hap_6 or not. We focused on backbone parental lines of hybrid rice varieties in China. We chose nine restorer lines (Minghui 63, Shuhui 527, Gui 99, Fuhui 838, Xianhui 207, Miyang 46, CDR22, IR24, Mianhui 725) and nine male sterility lines (II-32A, Zhenshan 97A, Jin23A, Tianfeng A, V20A, Gang 46 A, Peiai64S, Y58S, Guangzhan 63-4S) to fully sequence their Pid3 alleles because they are most frequently used parents of hybrid rice in China (http://www.ricedata.cn/variety/). For example, the most popular male sterility line in hybrid rice breeding in China is II-32A, from which more than 200 hybrids have been released in recent 20 years (http://www.ricedata.cn/variety/). The results showed that out of the 18 lines, 16 have Pid3 alleles identical to Hap_6, while the alleles of remaining two lines, Tianfeng A and
V20A, were identical to Hap_21. In addition, these backbone parental lines all conferred resistance to the *M. oryzae* strain Zhong-10-8-14, and the transcripts of the *Pid3* alleles could be obviously detected in these lines (Supplementary Figure S3). These results demonstrated that Hap_6 of *Pid3* has been widely utilized for hybrid rice breeding in China.

Moreover, by using re-sequencing data of hybrid rice 12, we investigated nucleotide polymorphisms of *Pid3* in 1495 hybrid rice varieties, which included 1,439 hybrid varieties from *indica-indica* crosses, 18 from *indica-japonica* crosses, and 38 from *japonica-japonica* crosses12. A total of 11 nucleotide polymorphism sites were identified in these hybrid rice varieties, all of which were included in the 71 sites (Supplementary Table S7). Only 88 hybrid rice varieties were found containing heterozygous sequences of *Pid3*; the remaining 1407 *Pid3* alleles belonged to four haplotypes, of which Hap_H1 was the most common haplotype shared by 1392 hybrid rice varieties. Because of low sequencing coverage (approximate 2×)12, it was impossible to get full sequences of *Pid3* in these 1407 hybrid rice varieties, though all variations of the Hap_H1 at the 11 nucleotide polymorphism sites were identical to Hap_6. As a result, we have reason to believe that the *Pid3* alleles of Hap_6 have prevailed in hybrid rice varieties in China.

### Discussion

So far, a great quantity of rice blast *R* genes have been identified and cloned, and almost all the cloned blast *R* genes have been applied to rice blast resistance breeding via *R* gene- self based or -tightly linked markers48–51. However, considering the possibility of a variety of functional alleles of the known blast *R* genes in rice populations, before a specific *R* gene is used for introgression, an accurate evaluation of its alleles in recurrent parental lines is in need. Moreover, some superior alleles, if any, could be identified by precise evaluation of the cloned *R* loci in rice germplasms29, 46, 52. Usually, there are three ways can be taken to evaluate a cloned *R* locus: first, certain markers which are always used in the MAS procedure for *R* loci can be applied. Then, the coding sequence fragment(s) amplified based on the cloned *R* gene should be examined. Finally, the complete *R* gene coding sequence(s) of every donor should be evaluated. However, due to the complicated and variable structure of these *NBS-LRR* type *R* genes, it is impossible to obtain accurate distribution of these cloned *R* genes just by markers and CDS fragments since single SNP could lead to the loss of function44, 53. Therefore, it is necessary to get the entire *R* gene sequences for their function assessment.

For the majority of rice blast *R* genes, it is rather difficult to obtain full sequences of alleles/orthologs by allele mining approach, due to the complexity of gene structure and vulnerable variations. However, the *Pid3* locus, as mentioned above, is a typical *NBS-LRR* type *R* gene, and it is relatively uncomplicated, because it is single-copy and intronless. Moreover, our former study29 has revealed that alleles/orthologs of *Pid3* in other rice germplasms, even from wild rice lines, contained no InDel or structure variations (SVs), so it is practical for evaluation of *Pid3* in rice resources by exploring the existing NGS data. In this study, we analyzed nearly 3,000 *Pid3* alleles in cultivated rice accessions mainly based on the 3 K RGP sequencing data, in which each genome had an average sequencing...
depth of 14× with averaged genome coverage and mapping rates of 94.0% and 92.5%, respectively. Except for 16 alleles with an 8-bp insertion at the position 2461, the remaining alleles revealed no obvious InDel polymorphisms. In the coding region of the 2621 homozygous Pid3 alleles, a total of 71 polymorphic sites were identified. By comparing sequences of Pid3 alleles obtained from the PCR-based allele mining approach 29, we found that most polymorphisms of Pid3 in cultivated rice accessions were included in these 71 sites. Recently, in another study, the sequence variations of Pid3 in 80 Yunnan rice landraces were analyzed by PCR-based allele mining approach54, in which a total of 39 nucleotide variations were found in the coding region of Pid3 alleles and no InDel or SV variations were identified. By comparing the positions of nucleotide variation, we found in that study, except for 8 sites, the remaining 31 were all involved in the 71 sites. Moreover, the haplotype 8 with the highest frequency (28.8%) in that study is identical to the most common Hap_6 in our work. These results demonstrated that it was feasible to analyze the sequence variations of Pid3 locus by utilization of the 3 K RGP sequencing data.

Some studies have shown that rice blast resistance is also correlated with the changes of some R gene expressions42, 43. In this study, our analyzing was only focused on the Pid3 coding sequences. Because most sequence variations existed in the promoter regions, they are difficult to be further judged for their relationship with the expression changes of the corresponding Pid3 alleles. Nevertheless, in some cultivars we checked the expression levels of Pid3 alleles (Supplementary Figure S3) since it is possible that the loss of blast resistance in certain haplotypes might be caused by sequence variations in their promoter regions.

Of note, in this work, a total of 2953 Pid3 alleles were tested for the premature mutation at the nucleotide position 2209. It was found that 22 (1.3%) of 1732 indica, 715 (83.2%) of 859 japonica and 40 (11%) of 362 other

| Continents | Countries/ Regions | Total rice accessions | Functional haplotypes | Non-functional haplotypes | Function not determined haplotypes |
|------------|--------------------|-----------------------|-----------------------|--------------------------|-----------------------------------|
| Asia       | China              | 439                   | 265                   | 13                       | 0                                 | 2                                 | 112                               | 47                                 |
|            | India              | 351                   | 241                   | 6                        | 3                                 | 6                                 | 43                                | 52                                 |
|            | Indonesia          | 210                   | 112                   | 9                        | 0                                 | 0                                 | 84                                | 5                                  |
|            | Philippines        | 195                   | 110                   | 3                        | 0                                 | 0                                 | 72                                | 10                                 |
|            | Bangladesh         | 142                   | 80                    | 0                        | 12                                | 1                                 | 2                                 | 47                                 |
|            | Thailand           | 131                   | 77                    | 28                       | 0                                 | 0                                 | 19                                | 7                                  |
|            | Laos               | 114                   | 51                    | 5                        | 0                                 | 0                                 | 54                                | 4                                  |
|            | Malaysia           | 63                    | 25                    | 4                        | 0                                 | 0                                 | 19                                | 15                                 |
|            | Myanmar            | 57                    | 38                    | 12                       | 0                                 | 4                                 | 3                                 | 3                                  |
|            | Cambodia           | 51                    | 28                    | 17                       | 0                                 | 0                                 | 3                                 | 3                                  |
|            | Japan              | 51                    | 3                     | 0                        | 0                                 | 0                                 | 44                                | 4                                  |
|            | Vietnam            | 47                    | 30                    | 7                        | 0                                 | 0                                 | 7                                 | 3                                  |
|            | Sri Lanka          | 45                    | 34                    | 0                        | 0                                 | 0                                 | 3                                 | 8                                  |
|            | Nepal              | 43                    | 31                    | 0                        | 2                                 | 0                                 | 6                                 | 4                                  |
|            | Taiwan             | 34                    | 22                    | 0                        | 0                                 | 0                                 | 12                                | 0                                  |
|            | South Korea        | 32                    | 5                     | 0                        | 0                                 | 0                                 | 27                                | 0                                  |
|            | Pakistan           | 29                    | 11                    | 0                        | 4                                 | 0                                 | 4                                 | 10                                 |
|            | Bhutan             | 16                    | 6                     | 0                        | 0                                 | 0                                 | 9                                 | 1                                  |
| Africa     | Madagascar         | 66                    | 38                    | 0                        | 0                                 | 0                                 | 27                                | 1                                  |
|            | Senegal            | 22                    | 14                    | 1                        | 0                                 | 0                                 | 1                                 | 6                                  |
|            | Ivory Coast        | 21                    | 5                     | 0                        | 0                                 | 0                                 | 15                                | 1                                  |
|            | Sierra Leone       | 18                    | 6                     | 0                        | 0                                 | 0                                 | 3                                 | 9                                  |
|            | Liberia            | 12                    | 4                     | 1                        | 0                                 | 0                                 | 7                                 | 0                                  |
|            | Nigeria            | 12                    | 8                     | 0                        | 0                                 | 0                                 | 3                                 | 1                                  |
| Europe     | Italy              | 37                    | 2                     | 0                        | 0                                 | 0                                 | 35                                | 0                                  |
|            | Portugal           | 22                    | 0                     | 1                        | 0                                 | 0                                 | 21                                | 0                                  |
|            | France             | 10                    | 1                     | 0                        | 0                                 | 0                                 | 9                                 | 0                                  |
|            | Spain              | 10                    | 0                     | 0                        | 0                                 | 0                                 | 10                                | 0                                  |
| South America | Colombia         | 24                    | 12                    | 2                        | 0                                 | 0                                 | 8                                 | 2                                  |
|            | Brazil             | 22                    | 8                     | 0                        | 0                                 | 0                                 | 10                                | 4                                  |
|            | Argentina          | 11                    | 1                     | 0                        | 0                                 | 0                                 | 10                                | 0                                  |
| North America | United States     | 47                    | 3                     | 0                        | 0                                 | 0                                 | 43                                | 1                                  |
|            | Australia          | 13                    | 4                     | 0                        | 0                                 | 0                                 | 9                                 | 0                                  |

Table 2. Geographic distributions of three types of haplotypes of Pid3. Only county/region containing more than 10 rice accessions were included; Non-functional haplotypes, including Hap_2, Hap_7, Hap_8, Hap_9, Hap_11, Hap_24, Hap_30, Hap_31, Hap_32, Hap_33, Hap_35, Hap_36, Hap_37, Hap_39, Hap_40; Function not determined haplotypes, including Hap_1, Hap_5, Hap_10, Hap_14, Hap_13, Hap_15, Hap_17, Hap_18, Hap_19, Hap_21, Hap_22, Hap_25, Hap_26, Hap_29.
types carried the premature mutation. Moreover, haplotype analysis demonstrated that although Hap_2 (which was carried by 95 rice accessions and made up mostly of japonica lines) did not contain the premature mutation at the position 2209, it was still a pseudogene due to the premature stop codon at the position 1766. In addition, we checked the premature mutation at the position 2209 in 149 widely cultivated japonica varieties in north China with CAPS marker. The results showed that except for 12 accessions, all the other japonica cultivars carried the premature mutation. These results clearly demonstrated that Pid3 alleles in most of these japonica rice cultivars were non-functional, leaving a great opportunity for utilization of functional Pid3 alleles to improve their blast resistance. For example, the japonica variety Kongyu 131, the most important cultivar in north China (http://www.ricedata.cn/variety/), has not contained a functional Pid3 allele yet (Supplementary Table S1).

Similarly, in 2621 cultivated rice lines, merely 40 haplotypes of Pid3 were identified, and most haplotypes in group I and group II were similar to Hap_9 and Hap_6, respectively, and distinguished only by one to two SNPs. Until now, we have verified four functional Pid3 alleles (Hap_6, Hap_13, Hap_14 and Hap_20) from cultivated rice. Of them, Hap_14 has the broadest resistance spectrum. For the remaining 16 haplotypes which encode until now, we have verified four functional Pid3 alleles (Hap_6, Hap_13, Hap_14 and Hap_20) from cultivated rice. Of them, Hap_14 has the broadest resistance spectrum. For the remaining 16 haplotypes which encode.

Materials and Methods

Re-sequencing data of Pid3 alleles. Data of SNPs and InDels at the Pid3 locus in 3,000 rice accessions were downloaded from the Rice SNP-Seek Database (http://oryzasn.org/irc-portal/index.zul) and the RMB database (http://www.rmbreeding.cn/snp3k). The 2775-bp coding sequence of Pid3 corresponds to the region (13055256-13058027) on chromosome 6 of the Nipponbare genome in the 3,000 rice genome project sequencing data. Sequence variations at the Pid3 locus in 1495 hybrid rice varieties were obtained from the RiceHap4 database (http://202.127.18.228/Rice/Hap4/index.php).

Plant materials. A set of 289 cultivated varieties including 140 indica varieties and 149 japonica varieties (Supplementary Table S1) were selected from China for detection of the nonsense mutation of Pid3 at the nucleotide position 2209 by the CAPS marker. All of the rice varieties were kept in our lab. The susceptible recipient TP309 was used for transformation of the Hap_6 of Pid3. The varieties were cultivated in the experimental field of the Hunan Hybrid Rice Research Center in Changsha under normal growing conditions.

M. oryzae isolates. 125 M. oryzae isolates used in this study were collected from rice fields around China, and were kindly provided by Dr. Yulin Peng of Sichuan Academy of Agricultural Sciences and by Dr. Cailin Lei of Institute of Crop Sciences, Chinese Academy of Agricultural Sciences. The diagnostic isolate of M. oryzae Zhong-10-8-14 was used for the phenotypic evaluation of the backbone parental lines of hybrid rice varieties in China, and the remaining isolates were used to assay the resistance spectra of Pid3 and Hap_6.

Detection of the nonsense mutation by the CAPS marker. Genomic DNA were extracted from fresh leaves of the 289 rice varieties using modified CTAB method of DNA isolation. A 658-bp fragment was amplified using the primer pair (Pd3CF: 5′-TACTACTCATGGAGCTATTCTC-3′ and Pd3CR: 5′-ACGTACAAATCTTGGCCT-3′). PCR amplification was carried out using the following profile: initial DNA denaturation at 95 °C for 4 min; followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 30 s; and final extension at 72 °C for 5 min. PCR products were digested with the restriction endonuclease BamHI. The absence of a 506-bp restriction fragment was considered to represent the nonsense mutation at the position 2209.

Sequence analysis. Sequences were aligned using CLUSTAL X version 2.0 and adjusted manually with Microsoft office excel 2010. Nucleotide diversity π (average number of nucleotide differences per site), πNS/πSYN (average ratio of non-synonymous site diversity over synonymous site diversity) and haplotype diversity analysis were calculated using DNASP v5.0. Haplotype flowchart was constructed with the computer program Network 5.0 (http://www.fluxus-engineering.com/sharenet.htm). DNASP v5.0 was also used to perform Tajima’s D test and sliding-window analysis of Pid3 alleles.

DNA sequencing. DNA was extracted from fresh leaves of the 18 indica and 12 japonica rice varieties. Primers (Pd3CF: 5′-AGTAACACCCAAAGGATAGTATAG-3′ and Pd3SR: 5′-GAAGCACAAGTGGCACTGATT-G-3′) that amplified the full coding sequence of Pid3 were designed according to Pid3 sequence in rice variety Digu.
PCR amplification was carried out using the following profile: initial DNA denaturation, 95 °C for 4 min; followed by 30 cycles of denaturation, 95 °C for 30 s; annealing, 58 °C for 30 s; extension, 72 °C for 30 s; and final extension at 72 °C for 5 min. The PCR products were sequenced by TsingKe Biology Technology.

**Vector construction and Rice transformation.** For the Hap_6 overexpression test, primer pair (Pid3F: 5′-TTTCTAGAAGTAAACCAACGGAATGATG-3′ and Pid3R: 5′-CTGTGCGAACCAGCAATGGGACATGATTG-3′) were designed to amplify the coding sequence of Pid3 allele from genome DNA of cultivar 93-11. An XbaI and an SalI recognition site (underlined) with two protecting bases (TT and CT) were added to their 5′ ends, respectively, then the PCR product was cloned into the binary vector pZH01 through the XbaI and SalI cloning sites. For the Hap_6 complementation test, the 6236-bp genomic sequence of Pid3 allele containing the promoter region and the full coding region was amplified from genomic DNA of 93-11 using the primer pair (Pid3FF: 5′-GGGTACCCACACATTGTACAACAGACCACAC-3′ and Pid3FR: 5′-CCCCGGGGAGAACAGAHTGCCGACATGATTG-3′), and then cloned into the binary vector pMNDRB-Bin62 through the KpnI and Xmal cloning sites (underlined). After sequence verification the final constructs were introduced into Agrobacterium tumefaciens LBA4404. The callus of susceptible japonica variety TP309 was transformed according to published methods83. The resistance of the primary transgenic lines (T0) was challenged by inoculation with the M. oryzae strain Zhong-10-8-14.

**Expression analysis of Pid3 alleles.** RNA was isolated from leaf tissue with the TRizol reagent (Invitrogen, Carlsbad, CA), and cDNA was synthesized from poly(A) + RNA using a cDNA synthesis kit (Transgen, Beijing). RT-PCR was performed with the specific primer pair Pid3C for 30 cycles of amplification. PCR amplification was as follows: 95 °C for 4 min; followed by 30 cycles of denaturation, 95 °C for 30 s; annealing, 58 °C for 30 s; extension, 72 °C for 30 s; and final extension at 72 °C for 5 min. Transcription of the actin gene was used to normalize the cDNA levels with the primer pair 5′-ACGAACTGGAATATGATTGGA-3′ and 5′-CAGGGCGATGTAGGAAGAC-3′. Amplification of the actin gene was conducted for 27 cycles and the annealing temperature was 57 °C.

**Fungal inoculation.** Six to eight plants were tested for each cultivar. Disease reaction to blast followed the modified standard pathogenicity assay as previously described84. Specifically, Rice seedlings at the four-leaf stage were inoculated by spraying a spore suspension (5 × 10⁴ spores/ml) of the M. oryzae isolates onto the leaves in a plastic bag. After inoculation plastic bags were sealed to maintain at 25 °C and 100% humidity in the dark for 24 h. Subsequently, plants were moved to the greenhouse (the humidity was maintained 70–85%, the temperature was 23/28 °C, and the lighting was 14/10 h for light/dark). and were allowed to grow to permit the development of expected disease symptoms. The disease reaction was examined one week after inoculation with the susceptible variety, TP309, as a control. The disease reaction was rated as 0–5, 0–3 as resistance and 4–5 as susceptible based on visual number and amount of lesions at the second youngest leaf85.

**References**

1. Goff, S. A. et al. A draft sequence of the rice genome (Oryza sativa L. ssp. japonica). Science 296, 92–100 (2002).
2. Yu, J. et al. A draft sequence of the rice genome (Oryza sativa L. ssp. indica). Science 296, 79–92 (2002).
3. International Rice Genome Sequencing, P. The map-based sequence of the rice genome. Nature 436, 793–800 (2005).
4. Zhang, J. et al. Extensive sequence divergence between the reference genomes of two elite rice varieties Zhenshan 97 and Minghui 63. Proc Natl Acad Sci USA 113, E163–71 (2016).
5. Jacquemin, J., Bhatia, D., Singh, K. & Wing, R. A. The International Oryza Map Alignment Project: development of a genus-wide comparative genomics platform to help solve the 9 billion-people question. Curr Opin Plant Biol 16, 147–56 (2013).
6. Sakai, H. et al. Construction of pseudomolecule sequences of the aus rice cultivar Kasalath for comparative genomics of Asian cultivated rice. DNA Res 21, 397–405 (2014).
7. Schatz, M. C. et al. Whole genome de novo assemblies of three divergent strains of rice, Oryza sativa, document novel gene space of aus and indica. Genome Biol 15, 506 (2014).
8. Xu, X. et al. Resequencing 50 accessions of cultivated and wild rice yields markers for identifying agronomically important genes. Nat Biotechnol 30, 105–11 (2011).
9. Huang, X. et al. A map of rice genome variation reveals the origin of cultivated rice. Nature 490, 497–501 (2012).
10. Subbaian, G. K. et al. Genome-wide DNA polymorphisms in elite indica rice inbreds discovered by whole-genome sequencing. Plant Biotechnol J 10, 623–34 (2012).
11. Yang, W. et al. Combining high-throughput phenotyping and genome-wide association studies to reveal natural genetic variation in rice. Nat Commun 5, 5087 (2014).
12. Huang, X. et al. Genomic analysis of hybrid rice varieties reveals numerous superior alleles that contribute to heterosis. Nat Commun 6, 6258 (2015).
13. Leung, H. et al. Allele mining and enhanced genetic recombination for rice breeding. Rice (N Y) 8, 34 (2015).
14. Zhao, H. et al. RiceVarMap: a comprehensive database of rice genomic variations. Nucleic Acids Res 43, D1018–22 (2015).
15. McCouch, S. R. et al. Open access resources for genome-wide association mapping in rice. Nat Commun 7, 10532 (2016).
16. Li, J. Y., Wang, J. & Zeigler, R. S. The 3,000 rice genomes project: new opportunities and challenges for future rice research. Gigascience 3, 8 (2014).
17. Project, R. G. The 3,000 rice genomes project. Gigascience 3, 7 (2014).
18. Alexandrov, N. et al. SNP-Seek database of SNPs derived from 3000 rice genomes. Nucleic Acids Res 43, D1023–7 (2015).
19. Guo, L., Gao, Z. & Qian, Q. Application of resequencing to rice genomics, functional genomics and evolutionary analysis. Rice (N Y) 7, 4 (2014).
20. Huang, X. et al. Genome-wide association studies of 14 agronomic traits in rice landraces. Nat Genet 42, 961–7 (2010).
21. Huang, X. et al. Genome-wide association study of flowering time and grain yield traits in a worldwide collection of rice germplasm. Nat Genet 44, 32–9 (2011).
22. Chen, W. et al. Genome-wide association analyses provide genetic and biochemical insights into natural variation in rice metabolism. Nat Genet 46, 714–21 (2014).
23. Begum, H. et al. Genome-wide association mapping for yield and other agronomic traits in an elite breeding population of tropical rice (Oryza sativa). PLoS One 10, e0119873 (2015).
24. Yano, K. et al. Genome-wide association study using whole-genome sequencing rapidly identifies new genes influencing agronomic traits in rice. *Nat Genet* **48**, 927–34 (2016).
25. Wang, X., Jia, M. H., Ghai, P., Lee, F. N. & Jia, Y. Genome-Wide Association of Rice Blast Disease Resistance and Yield-Related Components of Rice. *Mol Plant Microbe Interact* **28**, 1383–92 (2015).
26. Skamniotis, P. & Gurr, S. J. Against the grain: safeguarding rice from rice blast disease. *Trends Biotechnol* **27**, 141–50 (2009).
27. Devanna, N. B., Vijayan, J. & Sharma, T. R. The blast resistance gene *Pi54* of cloned from *Oryza officinalis* interacts with Avr-Pi54 through its novel non-LRR domain(s). *PloS One* **9**, e104840 (2014).
28. Ma, J. et al. P64, Encoding a Novel CC-NBS-LRR Protein, Confers Resistance to Leaf and Neck Blast in Rice. *Mol Plant Microbe Interact* **28**, 558–68 (2015).
29. Xu, X. et al. Excavation of *Pi3* ortholog with differential expression spectra to *Magnaporthe oryzae* in rice resource. *PloS One* **9**, e93275 (2014).
30. Zhang, X. et al. A genome-wide survey reveals abundant rice blast *R* genes in resistant cultivars. *Plant J* **84**, 20–8 (2015).
31. Chen, X. et al. A B-lectin receptor kinase gene conferring rice blast resistance. *Plant J* **46**, 794–804 (2006).
32. Fujikake, S. et al. Loss of function of a proline-containing protein confers durable disease resistance in rice. *Science* **325**, 998–1001 (2009).
33. Wang, D. et al. Allele-mining of rice blast resistance genes at *AgCl134922* locus. *Biochem Biophys Res Commun* **446**, 1085–90 (2014).
34. Yang, S. et al. Rapidly evolving *R* genes in diverse grass species confer resistance to rice blast disease. *Proc Natl Acad Sci USA* **110**, 18572–7 (2013).
35. Vasudevan, K., Gruijissem, W. & Bhullar, N. K. Identification of novel alleles of the rice blast resistance gene *Pib* in *P*54. *Sci Rep* **5**, 15678 (2015).
36. Liu, X., Lin, F., Wang, L. & Pan, Q. The in silico map-based cloning of *Pib*36, a rice coiled-coil nucleotide-binding site leucine-rich repeat gene that confers race-specific resistance to the blast fungus. *Genetics* **176**, 2541–9 (2007).
37. Qu, S. et al. The broad-spectrum blast resistance gene *Pib9* encodes a nucleotide-binding site-leucine-rich repeat protein and is a member of a multigene family in rice. *Genetics* **172**, 1901–14 (2006).
38. Lee, S. K. K. et al. Rice Pe5-mediated resistance to *Magnaporthe oryzae* requires the presence of two coiled-coil-nucleotide-binding-leucine-rich repeat genes. *Genetics* **181**, 1627–38 (2009).
39. Zhai, C. et al. The isolation and characterization of *Pib*, a rice blast resistance gene which emerged after rice domestication. *New Phytol* **189**, 321–34 (2011).
40. Okuyama, Y. et al. A multifaceted genomics approach allows the isolation of the rice *Pia*-blast resistance gene consisting of two adjacent NBS-LRR protein genes. *Plant Cell* **46**, 667–79 (2011).
41. Lin, F. et al. The blast resistance gene *Pib57* encodes a nucleotide binding site leucine-rich repeat protein and is a member of a resistance gene cluster on rice chromosome 1. *Genetics* **177**, 1781–80 (2007).
42. Hayashi, N. et al. Durable panicle blast-resistance gene *Pib1* encodes an atypical CC-NBS-LRR protein and was generated by acquiring a promoter through local genome duplication. *Plant J* **64**, 498–510 (2010).
43. Hayashi, K. & Yoshida, H. Refunctionalization of the ancient rice blast disease resistance gene *Pit* by the recruitment of a retrotransposon as a promoter. *Plant J* **57**, 413–25 (2009).
44. Xia, J. et al. Identification of a new rice blast resistance gene, *Pi3*, by genewide comparison of paired nucleotide-binding site–leucine-rich repeat genes and their pseudogene alleles between the two sequenced rice genomes. *Genetics* **182**, 1303–11 (2009).
45. Chen, J. et al. A *Pib* allele from rice cultivar Gumei2 confers resistance to *Magnaporthe oryzae*. *J Genet Genomics* **38**, 209–16 (2011).
46. Lv, Q. et al. Functional analysis of *Pib3-4A*, an ortholog of rice blast resistance gene *Pib3* revealed by allele mining in common wild rice. *Phytopathology* **103**, 594–9 (2013).
47. Yang, S. et al. Genetic variation of NBS-LRR class resistance genes in rice lines. *Theor Appl Genet* **116**, 165–77 (2008).
48. Ellur, R. K. et al. Improvement of Basmati rice varieties for resistance to blast and bacterial blight diseases using marker assisted backcross breeding. *Plant Sci* **242**, 330–41 (2016).
49. Tanweer, F. A. et al. Current advance methods for the identification of blast resistance genes in rice. *C R Biol* **338**, 321–34 (2015).
50. Ni, D. et al. Marker-assisted selection of two-line hybrid rice for disease resistance to rice blast and bacterial blight. *Field Crops Research* **184**, 1–8 (2015).
51. Ashkani, S. et al. Molecular breeding strategy and challenges towards improvement of blast disease resistance in rice crop. *Front Plant Sci* **6**, 886 (2015).
52. Das, A. A. et al. A novel blast resistance gene, *Pib54rh* cloned from wild species of rice, *Oryza rhizomatis* confers broad spectrum resistance to *Magnaporthe oryzae*. *Funct Integr Genomics* **12**, 215–28 (2012).
53. Bryan, G. T. et al. A single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene *Pv-ta*. *Plant Cell* **12**, 2033–46 (2000).
54. Yang, Y. et al. Sequence variation of *Pib* for blast resistance in Yunnan rice landrace. *Chin J Rice Sci* **30**, 17–26 (2016).
55. Ronald, P. C. et al. Genetic and physical analysis of the rice bacterial blight disease resistance locus, *Xa21*. *Mol Gen Genet* **236**, 113–20 (1992).
56. Xie, H. G. et al. Development of hybrid rice variety FY7206 with blast resistance gene *Pib3* and cold tolerance gene *Ctb1*. *Rice Science* **23**, 266–73 (2016).
57. Wang, H. M. et al. Development and validation of CAPS markers for Marker-Assisted selection of rice blast resistance gene. *Planta* **238**, 1906–60 (2012).
58. Zheng, T. Q. et al. Rice functional genomics and breeding database (RFGB): 3K-rice SNP and InDel sub-database (in Chinese). *Chin Sci Bull* **60**, 367–71 (2015).
59. Bai, Y. L. Virulence to hybrid rice in *Magnaporthe oryzae* from Sichuan Province. Master dissertation, Sichuan Agricultural University, Chengdu City, Sichuan Province, China (2011).
60. Larkin, M. A. et al. Clustal W and Clustal X version 2.0. *Bioinformatics* **23**, 2947–8 (2007).
61. Rozas, J., Sánchez-DelBarrio, J. C., Meseguer, X. & Rozas, R. *DnaSP, DNA polymorphism analyses* by the coalescent and other methods. *Bioinformatics* **19**, 2496–7 (2003).
62. Lu, H. J., Zhou, X. R., Gong, Z. X. & Upadhyaya, N. M. Generation of selectable marker-free transgenic rice using double right-border (DRB) binary vectors. *Functional Plant Biology* **28**, 241–48 (2001).
63. Hei, Y., Ohta, S., Komari, T. & Kumashiro, T. Efficient transformation of rice (*Oryza sativa L.*) mediated by Agrobacterium and sequence analysis of the boundaries of the T-DNA. *Plant J* **16**, 271–82 (1994).

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Author Contributions
Lihuang Zhu and Qiming Lv conceived and designed the experiment, Qiming Lv, Xiao Xu, Zhiyuan Huang, Hai Liu, Li Tang, Junjie Xing, Zhirong Peng carried out the experiments, Yeyun Xin, Xiaobing Li, Tianqing Zheng collected the data, Qiming Lv, Lihuang Zhu, Tianqing Zheng, Zhuangshi Zhou and Chunchao Wang analyzed the data, Lihuang Zhu and Qiming Lv wrote the manuscript. All authors have read the manuscript and agree with its content.

Additional Information

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