The Rice Blast Resistance Gene *Pid* Family has been Strictly Diverged into Indica and Japonica Subspecies

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Abstract

Background: Rice blast (causative agent the fungus *Pyricularia oryzae*) represents a major constraint over the productivity of one of the world's most important staple foods. Genes encoding resistance have been identified in both the *indica* and *japonica* subspecies genepools, and combining these within new cultivars represents a rational means of combating the pathogen.

Results: In this research, a deeper allele mining was carried out on *Pid-2*, *Pid-3*, and *Pid-4* by their specific FNP markers in the three panels consisting of 70 *indica* and 58 *japonica* cultivars. Within *Pid-2*, three functional and one non-functional alleles were identified; the former were only identified in *indica* type entries. At *Pid-3*, four functional and one non-functional alleles were identified, and once again, all of the former were present in *indica* type entries. However, the pattern of variation at *Pid-4* was rather different: here, the five functional alleles uncovered were dispersed across the *japonica* type germplasm. Among all the 12 candidate functional alleles, both *Pid2-ZS* and *Pid3-ZS* were predominant.

Conclusions: Variation in both *Pid-2* and *Pid-3* appears to have evolved in response to pathogen pressure exerted on *indica* type cultivars, while that in *Pid-4* reflects the interaction between the fungus and the host in *japonica* type crops. Owing to the founder lineage, 'Zhenzhuai 11-ZS97', rather limited genotypes of the *Pid* family have been effective in both *indica* and *japonica* rice groups, of which *Pid2-ZS*+*Pid3-ZS* is present in a large proportion of Chinese *indica* cultivars released since the 1960s.

Background

Rice (*Oryza sativa*), a crop domesticated in Asia but now cultivated worldwide, is used as a staple food for half of mankind (Huang et al. 2012; Zhang et al. 2016; Du et al. 2017; Wang et al. 2018; Lv et al. 2020). As a result of two major and independent domestication events, two subspecies have been recognized, namely ssp. *indica* and ssp. *japonica* (Huang et al. 2012; Zhang et al. 2016; Choi & Purugganan 2018; Wang et al. 2018). The two genepools have differentiated over time through them been grown in distinct eco-geographical environments, and have diverged with respect to both the structure of the genome and their gene content (Huang et al. 2012; Ouyang & Zhang 2013; Zhang et al. 2016; Du et al. 2017; Wang et al. 2018). Introgression from one gene pool to the other is seen as a useful strategy for increasing the crop's genetic diversity.

One of the major pathogens of the crop is the fungus *Pyricularia oryzae* Cavara (syn. *Magnaporthe oryzae* Couch), the causative agent of the damaging disease rice blast (Dean et al. 2012; Liu and Wang 2016; Zhang et al. 2017, 2019; Zhao et al. 2018; Huang et al. 2021). A wealth of genes determining resistance to this pathogen has supported the success of using breeding to provide a sustainable means of mitigating the damage caused by blast (He et al. 2012; Zhai et al. 2014; Deng et al. 2017; Zhang et al. 2017, 2019). The genetics of resistance largely follows the gene-for-gene principle, involving an interaction between a host's resistance gene and a matching avirulence gene in the pathogen (Flor 1971; Ebbole 2007; Wu et al. 2014; Zhang et al. 2015). As a result, following the mutation of matching

avirulence genes, major gene-based resistances are prone to rapid breakdown. That in turn enable it to create a new genotype of resistance gene to overcome the emerged new race with its new resistance specificity (Zeigler et al. 1995; Wu et al. 2014; Zhang et al. 2015; Wang et al. 2017; Zhang et al. 2017, 2019; Zhao et al. 2018; Huang et al. 2021). New resistance specificities can be generated by mutations to a resistance gene's coding sequence, either in the form of a single nucleotide polymorphic (SNP) and even multiple nucleotide polymorphic mutations (so-called insertion/deletion, InDel) (Bryan et al. 2000; Lin et al. 2007; Liu et al. 2007; Fukuoka et al. 2009; Hua et al. 2012; Zhai et al. 2011, 2014; Zhao et al. 2018); where such changes result in an altered reaction to the pathogen, the mutation is referred to as a functional nucleotide polymorphism (FNP) (Hua et al. 2012; Zhai et al. 2011, 2014). As per the gene-for-gene principal, it is envisaged that the stronger arms race led to more FNPs emerged in any given resistance gene (Wu et al. 2014; Zhang et al. 2015; Wang et al. 2017; Zhang et al. 2017, 2019; Zhao et al. 2018; Huang et al. 2021). Searching for FNPs in established host cultivars is considered as an efficient way of identifying the novel resistances required for crop improvement (Li et al. 2019; Teerasan et al. 2019; Tian et al. 2020; Xiao et al. 2020; Zhou et al. 2020).

At least 100 major genes encoding resistance to *P. oryzae* are known, an increasing number of which have been isolated (Liu and Wang 2016; Zhao et al. 2018). Among the latter are the three genes *Pid-2*, *-3* and *-4*, present as a cluster on chromosome 6 (here after the *Pid* family) (Chen et al. 2006, 2018; Shang et al. 2009). The objective of the present study was to devise a set of reliable FNP markers based on variations in genomic sequences of the *Pid* family, and to use these to exploit the extent of allelic variation available in rice germplasm. A particular focus was to reveal the genetic basis underlying resistance gene divergence between *indica* and *japonica* subspecies.

Methods

Development of FNP markers

The DNA sequences of *Pid-2* (FJ915121.1), *Pid-3* (FJ745364.1), and *Pid-4* (MG839283.1) present in Digu as well as in a number of reference cultivars were retrieved from GenBank (<http://www.ncbi.nlm.nih.gov/>), and each set of alleles was aligned using Multalin (<http://multalin.toulouse.inra.fr/multalin/>). Two types of FNPs were targeted, the first represented either a functional or a nonfunctional haplotype, and the second an individual allele. An interval sequence of each candidate FNP was subjected to various marker designations including CAPS (cleaved amplified polymorphism sequences), and dCAPS (derived CAPS) using dCAPS Finder 2.0 (<http://helix.wustl.edu/dcaps/dcaps.html>). The necessary primer sequences were generated using Primer3 software (<https://primer3.ut.ee>). As for sequence largely diverged intervals, PCR was drove by triple and/or degenerate primers (Table S1; Zeng 2020; Zhang 2020).

Marker verification

The FNP assays were validated by testing a larger set of control cvs (so-called CKs), namely Digu (DIG), Tetep (TTP), CO39, Zhenshan97 (ZS97), Tadukan (TDK), Nipponbare (NPB), Koshihikari (KSH) and Shennong265 (SN265). Each 20 µL PCR contained 0.1 µL 5 U/µL rTaqase (TaKaRa, Dalian, China), 2.0 µL 10× rTaq Mg²⁺ plus buffer, 0.5 µL 10 mM dNTP (TaKaRa), 1.0 µL 2.5 µM primers (Sangon Biotech, Guangzhou, China), 1.0 µL 10-20 ng/µL template DNA and 14.4 µL ddH₂O. The PCR regime was initiated with a denaturing step (94°C/3 min), which was followed by 35 cycles of 94°C/30 s, 50-62°C/30 s, 72°C/25-30 s and completed with a 72°C/5 min final extension. The resulting amplicons were digested for 3 h with the appropriate restriction enzyme (NEB Inc., Ipswich, MA, USA) at the recommended temperature in a 10 µL reaction containing 1.5 µL PCR product, 0.2 µL 3 U/µL enzyme, 1.0 µL 10× digestion buffer, 8.3 µL ddH₂O. The digested amplicons were electrophoretically separated through 10-12% polyacrylamide gels in the presence of Tris-boric acid-EDTA buffer run at 250 V for 50-60 min.

Genotyping and data analysis

A smaller set of control cultivars, i.e., DIG, TTP, ZS97, NPB, SN265, and CO39, were involved in each genotyping experiment (Table S2). The functional and nonfunctional haplotypes of each member of the *Pid* family were firstly determined with a couple of the functional and nonfunctional FNP markers, and new alleles were then determined with a set of allelic FNP markers by testing a regular panel consisting of each 30 representative *indica* and *japonica* type cultivars. For confirming genetic divergence of alleles between *indica* and *japonica* rice groups (if any), allele mining was then extended to two additional germplasm panels, one consisting of 40 *indica* type cultivars used as parents in rice breeding programs based in the southern province of Guangdong and the other of 28 *japonica* type cultivars used -similarly in the north-eastern province of Heilongjiang (Table S2). A χ^2 test was used to determine whether the two genepools had or had not experienced divergence. The test was based on the formula

$$\chi^2 = \frac{N \left[|ad-bc| - \left(\frac{1}{2} \right) N \right]^2}{(a+b)(c+d)(a+c)(b+d)},$$

where *a* and *b* represent the number of *indica* type entries scored as, respectively, harbouring or not harbouring a given allele or genotype, while *c* and *d* represent the same for the *japonica* type entries. *N* denotes the total number of alleles or genotypes detected for each *Pid* gene or genotype (Fernando et al. 2018; Zhang et al. 2019; Huang et al. 2021). If all alleles derived from a given resistance gene, which was extremely diverged into *indica* group, then the resistance gene was defined as *indica* type one, and that in turn called as *japonica* type one.

Results

Pid-2 alleles

An alignment of *Pid-2* coding sequences (CDSs) of the 15 reference cultivars revealed the presence of seven single nucleotide polymorphisms (Fig. S1). A pair of FNPs, *Pid2-F/N*^{C1022T} and *Pid2-F/N*^{A1383G} effectively distinguished between the functional and the non-functional alleles. DIG, TTP, CO39, ZS97 and TDK each carried a functional allele, while NPB, KSH and SN265 carried a non-functional one (Fig. 1). *Pid2-DIG*^{T2058C} was informative for *Pid2-DIG* allele (DIG), and *Pid2-ZS*^{A555G} for *Pid2-ZS* allele (TTP, CO39, ZS97, and TDK). When the regular panel was screened, all 30 *indica* type entries were found to carry a functional *Pid-2* allele, but this was the case for only four of the 30 *japonica* types (Fig. 2; Table S2). Of the 34 *Pid-2* carriers, 14 belonged to *Pid2-DIG*, and 18 to *Pid2-ZS*, and two carried a distinct allele (hereafter referred to as *Pid2-New*). The screen of the additional 40 *indica* rice panel revealed that of the 39 carrying a functional copy of *Pid-2*, 32 had the *Pid2-ZS* allele, six the *Pid2-DIG* allele and one the *Pid2-New* allele. None of the additional *japonica* germplasm panel carried a functional copy of *Pid-2* (Fig. S2). A homogeneity test suggested that divergence of *Pid-2* was specific to the *indica* gene pool (Table 1). It was, therefore, defined as an *indica* type resistance gene.

Pid-3 alleles

The variation in the *Pid-3* CDSs identified in the 15 reference cultivars comprised 29 SNPs and one indel (Fig. S3); 18 of the SNPs and the indel were targeted for marker development (data not shown). The *Pid3-F/N*^{G2009A} and *Pid3-F/N*^{C2209T} were both effective for distinguishing between functional and non-functional alleles: the five cultivars DIG, TTP, CO39, ZS97 and TDK each carried a functional allele, while NPB, KSH and SN265 each carried a non-functional one (Fig. 3; Table S2). Three pairs, *Pid3-DIG*^{G775A} vs *Pid3-DIG*^{G2695A}, *Pid3-TTP*^{C1136T} vs *Pid3-TTP*^{C1623G}, and *Pid3-ZS*^{G477A} vs *Pid3-ZS*^{C525T}, were ensured as allele-specific FNP markers responsible for *Pid3-DIG*, *Pid3-TTP*, and *Pid3-ZS*, respectively (Fig. 3). As was the case for *Pid-2*, all 30 members of the *indica* panel carried a functional *Pid-3* haplotype, whereas only four of the *japonica* panel did so (Fig. 4; Table S2). The distribution of effective alleles was highly uneven: 29 of the *Pid-3* carriers harboured the *Pid3-ZS* allele, three the *Pid3-DIG* allele, one the *Pid3-TTP* and one a novel allele (*Pid3-New*). The distribution was similarly uneven in the additional *indica* panel, where 28 of the *Pid-3* positive entries carried the *Pid3-ZS* allele, three the *Pid3-DIG* allele, one the *Pid3-TTP* allele and one *Pid3-New*; none of the members of the additional *japonica* panel carried an effective allele (Fig. S4). A homogeneity test implied that divergence for *Pid-3* has only occurred in the *indica* gene pool (Table 1). It was, also, referred to as an *indica* type resistance gene.

Pid-4 alleles

Pid-4 was by far the most diverse of the three members, with 149 SNPs and six InDels identified in the CDSs plus one intron of the 13 reference cultivars (Fig. S5), a sample of these (17 SNPs and two InDels) were targeted for marker development (data not shown). Both *Pid4-F/N*^{C1217G} and *Pid4-F/N*^{A1452G} were informative with respect to functionality: five cultivars, DIG, NPB, KSH, CO39, and SN265, were recognized as carriers of functional alleles, while TDK, TTP and ZS97 harboured non-functional alleles (Fig. 5; Table

S2). Two pairs, *Pid4-DIG*^{A1149T} vs *Pid4-DIG*^{A1898G}, and *Pid4-NPB*^{G1362A} vs *Pid4-NPB*^{C1554A}, were confirmed as allele-specific FNP markers responsible for *Pid4-DIG* and *Pid4-NPB*, respectively; and *Pid4-SN/CO*^{T1841A} coupled with *Pid4-SN/CO*^{G2250C} responsible for both *Pid4-SN* and *Pid4-CO* (Fig. 5). Unlike the situation in both *Pid-2* and *Pid-3*, functional *Pid-4* alleles were present in many (28/30) of the *japonica* type entries, while the frequency of functional alleles was only moderate (12/30) in the *indica* germplasm (Fig. 6; Table S2). The distribution of the various alleles was more even than was the case for *Pid-2* and *Pid-3*, with 14 entries carrying the *Pid4-SN* allele, 11 the *Pid4-NPB* allele, eight *Pid4-New* and six the *Pid4-CO* allele (Fig. 6). Extending the screen to the two additional panels revealed that 28/40 *japonica* type cultivars harboured a functional allele, while only 7/40 *indica* type cultivars did so. Of the 35 functional haplotypes, 12 were present in entries carrying the *Pid4-CO* allele, 11 in those carrying the *Pid4-NPB* allele, seven in those carrying *Pid4-New*, five in those carrying the *Pid4-SN* allele, while just a single entry carried the *Pid4-DIG* allele (Fig. S6). A homogeneity test confirmed that significant divergence at *Pid-4* has occurred in the *japonica* gene pool (Table 1). Thus, it was termed '*japonica* type resistance gene'.

Discussion

The FNP markers for the *Pid* family have been comprehensively integrated

In the present study, a deeper allele mining was carried out on the *Pid* family in the three panels consisting of 70 *indica* and 58 *japonica* cultivars, which were selected from various regions across landrace and modern rice eras (Tables 1; S2). Three sets of FNP markers for allele mining of the *Pid* family were devised based on several criteria such as representative of FNPs over a given CDS, clear genotyping, and easy-accession to users (Zeng 2020; Zhang 2020). As almost resistance genes have been initially diverged into functional and nonfunctional haplotypes (Figs. S1, S3, S5; Bryan et al. 2000; Lin et al. 2007; Liu et al. 2007; Fukuoka et al. 2009; Hua et al. 2012; Yuan et al. 2011; Zhai et al. 2011, 2014; Zhao et al. 2018; Zeng 2020), allele mining of each member of the *Pid* family was, therefore, initiated from haplotype differentiation with haplotype specific FNPs, which enabled us to identify any new allele in a given cultivar belonging to the functional haplotype. That in turn, allele mining could be stopped when the panel where was not any functional haplotype (Figs. S2, S4). Whereas allele mining was pursued to individual alleles with a series of allele-specific FNPs, which helped us finding out more certain alleles within the functional haplotypes. Even so, there were still 3, 2, and 14 cultivars in *Pid-2*, *Pid-3*, and *Pid-4* categories, respectively, which were presumed to carry new types of alleles, compared to the defined alleles (Table 1). Collectively, the FNP markers for allele mining in the present study were largely improved from those used in the previous researches, as almost of those were focused on individual SNPs and/or InDels, which were unable to find out a series of new alleles as were the three cases shown in the current study (Shang et al. 2009; Lv et al. 2017; Promchuay and Nilthong 2017; Teerasan et al. 2019; Tian et al. 2020; Zhou et al. 2020).

The *Pid* family has been strictly diverged into *indica* and *japonica* subspecies

Four alleles were detected at *Pid-2*, of which the three functional ones were almost entirely restricted to *indica* type cultivars, while the null allele was only present in *japonica* type germplasm (Table 1). The distribution of alleles at *Pid-3* was very similar: the four functional ones were harboured for the most part by *indica* type entries and the null allele was common in the *japonica* genepool (Table 1). The latter result echoes a prior finding that the alleles of *Pid-3* present in *japonica* type cultivars are pseudogenes (Shang et al. 2009; Lv et al. 2017). In contrast, the distribution of alleles at *Pid-4* featured five functional alleles which were shared evenly among the *japonica* type entries, with the null allele found only in *indica* type ones (Table 1). It might be the first time to find out and define both *indica* and *japonica* type resistance genes within individual cultivars through allele mining with a set of comprehensive FNP markers (Table S2). The data revealed by the FNP screen suggest a plausible genetic basis for the stable and broad blast resistance exhibited by the modern cultivars, Digu, R207, Lu28S, TianfengB, R217, Zhonghua 11, Gumeizao 4, Moliruanzhan, Yuehesimiao and Yuejingsimiao 2, in that all of these cultivars harbour a functional allele at each of the three *Pid* genes (Table S2). It might be truly expected that integration of both *indica* and *japonica* resistance genes into upcoming cultivars would be one of the most promising ways to enlarge their genetic diversities of resistance genic resources thereby withstanding ever-growing pressure from the pathogen across *indica* and *japonica* rice areas (Shang et al. 2009; Zhang et al. 2016; Lv et al. 2017, 2020).

Rather limited genotypes of the *Pid* family have been effective in both *indica* and *japonica* rice groups

By owning three members of the *Pid* family, there would be seven all possible genotypes (combinations) ($2^3-1=7$), shortly as: *d2*, *d3*, *d4*; *d2-d3*, *d2-d4*, *d3-d4*; and *d2-d3-d4*, irrespective of specific alleles. However, only three genotypes, *d4*, *d2-d3*, and *d2-d3-d4*, were detected in the three panels consisting of 128 diversified rice germplasms (Tables 1, S2). The indication is therefore that rather limited genotypes of the *Pid* family have been integrated into both *indica* and *japonica* rice cultivars in China. Since all the three members have been strictly diverged into the two subspecies across landrace and modern rice eras, *d2-d3* was centralized in *indica* group and *d4* in *japonica* one both reached at overwhelming proportions; and *d2-d3-d4* also in *indica* group but with a rather moderate rate (Table 1). The genetic structure of the region harbouring the *Pid* family does not suggest any obvious barrier to local recombination (Fig. S7). That is, there were four types of such barriers in the target genomic region, the key subspecies hybrid sterile gene cluster *S5* (Chen et al. 2008), the heading date gene *Hd1* (Yano et al. 2000), the photonasty gene *Se5* (Izawa et al. 2000), and the centromere of rice chromosome 6 (Zhao et al. 2019), all of which were enough far from the genic positions of the *Pid* family. In addition, genomic intervals among the three members were also enough long for independently segregation each other in a given genetic cross (Fig. S7).

The most possible genetic determinants leading to establish such specific allelic and genotypic structures of the *Pid* family was, therefore, due to the specific lineage(s) of the Chinese rice population. That is, the Chinese rice population has, indeed, been derived from rather limited founder parents for an age. By reviewing the pedigrees of Top-10 of several cultivar types including the general cultivars and F₁ hybrid crosses, Liu (2021) pointed out that the specific lineage, 'Zhenzhuai 11-ZS97', both were recognized as *Pid2*-ZS_*Pid3*-ZS, has been central to Chinese *indica* type rice breeding programs since the 1960s (also see www.ricedata.cn/variety). The specific lineage perfectly addressed to both questions: why there were two *indica* type alleles with much higher rates in the respective allelic structures, *Pid2*-ZS with 71.4% (50/73), and *Pid3*-ZS with 86.3% (63/73); and why there was not any single gene genotype for *d2* or *d3*, but *d2-d3* for being predominant among the three effective genotypes for a long time (Tables 1, S2). That is, the unique allelic structures of the three members of the *Pid* family have been mainly constructed by the genotype, *d2-d3*, exactly, *Pid2*-ZS_*Pid3*-ZS, carried by the lineage in rice breeding programs in China since the 1960s. It was, again, concerned that updating the lineage would be the key to enlarge genetic diversities of rice cultivars in the next generation in China (Shang et al. 2009; Zhang et al. 2016; Lv et al. 2017, 2020; Liu 2021; www.ricedata.cn/variety).

A further priority will be to identify the functional specificities of the various *Pid* alleles by challenging near isogenic lines with a set of both *indica* and *japonica* races of the pathogen (Zhang et al. 2017, 2019; Huang et al. 2021).

Conclusions

The study has demonstrated that all the three members of the *Pid* family have been strictly diverged into *indica* and *japonica* subspecies: *Pid-2* and *Pid-3* were defined as *indica* type resistance genes, and *Pid-4* as *japonica* one. Owing to the specific lineage, 'Zhenzhuai 11-ZS97', rather limited genotypes of the *Pid* family have been effective in both *indica* and *japonica* rice groups, of which *Pid2*-ZS + *Pid3*-ZS has been central to the Chinese rice population since the 1960s.

Declarations

Author's contributions

Project conception (QHP), Marker development (JYW, JQW, XW, QHP), Material composition (YZ, YXY, LW, XW, JFZ, YHZ), Allele mining (RPC, JQW, XW, XMY), Data analysis (JYW, RPC, QHP), Manuscript preparation (QHP, JYW). All authors read and approved the final manuscript.

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Availability of Data and Materials

The data sets supporting the results of this article are included within the article and its supporting files.

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Table

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Figures

Figure 1

Development of a set of FNP markers able to distinguishing both haplotypes and alleles of Pid-2. (a-c) Discriminating between functional (F) and non-functional (N) haplotypes and between Pid2-DIG and Pid2-ZS alleles. CK1, Digu (Pid2-DIG); CK2, Tetep (Pid2-ZS); CK3, CO39 (Pid2-ZS); CK4, ZS97 (Pid2-ZS); CK5, Taluka (Pid2-ZS); CK6, Nippon bare (Pid2-Null); CK7, Kochukaru (Pid2-Null); CK8, Shennong265 (Pid2-Null). M, DL-500 size marker.

Figure 2

Alleles of Pid-2 represented in the regular panel consisting of both indica (CV1-30) and japonica (CV31-60) types. (a) Functional and non-functional haplotypes, (b) the Pid2-DIG allele, (c) the Pid2-ZS allele. CK1, Digu (Pid2-DIG; red); CK2, Tetep (Pid2-ZS; blue); CK3, ZS97 (Pid2-ZS; blue); CK4, Nipponbare (Pid2-Null; black); CK5, Shennong265 (Pid2-Null; black); CK6, CO39 (Pid2-ZS; blue); and the undefined alleles carried by CV6 and CV14 was marked in purple. The detailed information on each entry was shown in Table S2. M, DL-500 size marker.

Figure 3

Development of a set of FNP markers able to distinguishing both haplotypes and alleles of Pid-3. (a-d) Discriminating between functional (F) and non-functional (N) haplotypes and among Pid3-DIG, Pid3-TTP, and Pid3-ZS alleles. CK1, Digu (Pid3-DIG); CK2, Tetep (Pid3-TTP); CK3, Tadukan (Pid3-TTP); CK4, ZS97 (Pid3-ZS); CK5, CO39 (Pid3-ZS); CK6, Nipponbare (Pid3-Null); CK7, Shennong265 (Pid3-Null); CK8, Koshihikari (Pid3-Null). M, DL-500 size marker.

Figure 4

Alleles of Pid-3 represented in the regular panel consisting of both indica (CV1-30) and japonica (CV31-60) types. (a) Functional and non-functional haplotypes, (b) the Pid3-DIG allele, (c) the Pid3-TTP allele, (d) the Pid3-ZS allele. CK1, Digu (Pid3-DIG; red); CK2, Tetep (Pid3-TTP; blue); CK3, ZS97 (Pid3-ZS; green); CK4, Nipponbare (Pid3-Null; black); CK5, Shennong265 (Pid3-Null; black); CK6, CO39 (Pid3-ZS; green); and the undefined allele carried by CV14 was marked in purple. The detailed information on each entry was shown in Table S2. M, DL-500 size marker.

Figure 5

Development of a set of FNP markers able to distinguishing both haplotypes and alleles of Pid-4. (a-d) Discriminating between functional (F) and non-functional (N) haplotypes and among Pid4-DIG, Pid4-NPB, Pid4-CO, and Pid4-SN alleles. CK1, Digu (Pid4-DIG); CK2, Nipponbare (Pid4-NPB); CK3, Koshihikari (Pid4-NPB); CK4, CO39 (Pid4-CO); CK5, Shennong265 (Pid4-SN); CK6, Tadukan (Pid4-Null); CK7, Tetep (Pid4-Null); CK8, ZS97 (Pid4-Null). M, DL-500 size marker.

Figure 6

Alleles of Pid-4 represented in the regular panel consisting of both indica (CV1-30) and japonica (CV31-60) types. (a) Functional and non-functional haplotypes, (b) the Pid3-DIG allele, (c) the Pid3-NPB allele, (d) the Pid4-SN and Pid4-CO alleles. CK1, Digu (Pid4-DIG; red); CK2, Tetep (Pid4-Null; black); CK3, ZS97 (Pid4-Null; black); CK4, Nipponbare (Pid4-NPB; blue); CK5, Shennong265 (Pid4-SN; green); CK6, CO39 (Pid4-CO; rose-red); and the undefined alleles carried by CVs 10, 20, 21, 29, 30, 42, 50, and 55, were marked in purple. The detailed information on each entry was shown in Table S2. M, DL-500 size marker.

Supplementary Files

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- [Fig.S3.Pid3CDS.pptx](#)
- [Fig.S4.Pid3identitiesinGDHLJ.pptx](#)
- [Fig.S5.Pid4CDS.pptx](#)
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