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Molecular markers linked to the blast resistance gene *Pi-z* in rice for use in marker-assisted selection

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Abstract Rice blast, caused by the fungal pathogen *Pyricularia grisea*, is a serious disease affecting rice-growing regions around the world. Current methods for identification of blast-resistant germplasm and progeny typically utilize phenotypic screening. However, phenotypic screens are influenced by environmental conditions and the presence of one resistance gene can sometimes phenotypically mask other genes conferring resistance to the same blast race. *Pi-z* is a dominant gene located on the short arm of chromosome 6 that confers complete resistance to five races of blast. Using sequence data found in public databases and degenerate primer pairs based on the P-loop, nucleotide binding sites and kinase domain motifs of previously cloned resistance genes, we have developed PCR-based DNA markers that cosegregate with the gene. These markers are polymorphic in a wide range of germplasm, including the narrow crosses characteristic of applied rice-breeding programs. They can now be used as a low cost, high-throughput alternative to conventional phenotypic screening for direct detection of blast resistance genes, allowing rapid introgression of genes into susceptible varieties as well as the incorporation of multiple genes into individual lines for more-durable blast resistance.

Keywords Degenerate primers · Disease resistance · *Oryza sativa* L. · *Pyricularia grisea* · *Magnaporthe grisea* · Marker-assisted selection · Microsatellite markers · Rice blast

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Introduction

The potentially devastating economic impact resulting from blast infection has prompted worldwide efforts to produce blast-resistant rice varieties. A number of blast resistance genes have been mapped (Kiyosawa 1972; Yu et al. 1991; Chen et al. 1999; Ahn et al. 2000) and two of them, *Pi-b* and *Pi-ta*, have been cloned (Wang et al. 1999; Bryan et al. 2000). However, obtaining long-lasting durable resistance to blast from a single gene is not likely, as the fungus has the ability to quickly mutate and attack formerly resistant cultivars (Garces de Araujo et al. 2000). For example, when the variety Katy was released, its wide-spectrum blast resistance gene *Pi-ta*^{2} conferred resistance to all of the predominant U.S. races of blast (Moldenhauer et al. 1992). However, within a few years, a new race of blast, IE-1k, was identified that overcame resistance to *Pi-ta*^{2}.

One way to improve the durability of blast resistance is to "pyramid" resistance genes by crossing rice varieties with complementary genes to provide multigenic resistance against a wide spectrum of blast races. If the disease is able to overcome the resistance due to one gene, the cultivar will still have resistance from the remaining genes. Unfortunately, pyramiding genes is difficult using traditional greenhouse screens because plants that contain one resistance gene are generally fully resistant to the particular races of blast corresponding to that gene. As a result, plants with single gene resistance to a given race of blast often cannot be distinguished from plants with multigene resistance without a test cross. Greenhouse screens used to detect multiple genes can also be

influenced by environmental conditions and gene interactions, and are typically scored on a continuous scale based on the extent and severity of lesion formation. Therefore, the exact demarcation between resistant and susceptible classes is not always clear. The use of DNA markers linked to resistance genes is one way to overcome the ambiguity of greenhouse screens, and has already been used to successfully pyramid blast resistance genes (Hittalmani et al. 2000).

Pi-z was originally identified in the cultivar Zenith (Kiyosawa 1967) and is generally found in temperate *japonica* varieties. It was initially shown to confer resistance to four races of blast: IH-1, IG-1, IC-17 and IE-1. Subsequent work has shown that *Pi-z* also confers resistance to the race IE-1k (M. Marchetti, personal communication). *Pi-z* provides a very useful complement to the blast resistance gene *Pi-k*^{h}, which is predominantly found in tropical *japonica* varieties, as well as to *Pi-ta*^{2} which is found in the variety Katy and several *indica* varieties. It is also a useful complement to *Pi-b*, a resistance gene found in *indica* varieties such as Tohoku Il 9 and Teqing.

Here we report five new markers for the blast resistance gene *Pi-z* which were identified using both degenerate primers and microsatellites found in public databases. As expected, the new markers are on the short arm of rice chromosome 6 above the centromere. Unlike many DNA markers developed in wide crosses or mapping populations, these markers are polymorphic in many narrow crosses of practical importance in applied rice breeding. Thus, they can be directly used as tools to introduce *Pi-z* into blast susceptible varieties and to pyramid blast resistant genes to develop varieties with more durable blast resistance.

Materials and methods

Plant material and disease resistance evaluation

Crosses were made using the cultivars Lafitte, Jefferson and a closely related sibling of Jefferson (Jeff-sib) as sources of *Pi-z* (see Table 1). For evaluation of blast resistance, F3 or F4 individuals were randomly selected from the field and were screened in the greenhouse using race IE-1k in Beaumont, Tex., as described by Marchetti et al. (1987). Plants were classified as resistant, moderate or susceptible, based on the size and number of lesions produced. Individual plants that were clearly resistant (no lesions) and susceptible (large lesions) were selected for further DNA analysis.

To determine whether the markers developed in this project would be widely applicable in an applied breeding program, approximately 220 F8 lines from a variety of other crosses in the California public rice-breeding program were also screened for blast resistance in the same manner at the USDA-ARS Rice Experiment Station in Beaumont, Tex., using race IE-1k. The markers were also screened in a wide range of *indica* and *japonica* germplasm.

DNA extraction

Samples were extracted using a FastPrep 120 Instrument (Qbiogene, Carlsbad, Calif.). Approximately 2–3 cm of frozen leaf tissue or 20 embryos cut from seeds and 500 μl of extraction buffer [6.25 mM potassium ethyl xanthogenate (Fluka), 0.7 M Tris-HCl, pH 7.5, 0.7 M NaCl, 10 mM EDTA, pH 8.0] were placed in a 2-ml screw-cap tube. Samples were homogenized for 20 s using a 7-mm ceramic bead (Qbiogene, Carlsbad, Calif.) followed by incubation at 65 °C for 1 h. DNA was isolated using chloroform:isoamyl alcohol (24:1) and precipitated with 0.1 vol of 3 M sodium acetate, pH 5.2, and 2 vol of ethanol for 15 min at –80 °C. The DNA was pelleted by centrifugation at 13,000 rpm for 10 min, washed with 70% ethanol, air dried, and resuspended in 200 μl of TE buffer. DNA was quantitated on a Beckman Model LS50B fluorimeter (Fullerton, Calif.) and diluted to 10 ng/μl. For bulked segregant analysis, equal amounts of DNA from LFTE/93Y82 progeny classified as resistant or susceptible were pooled into groups of three.

Table 1 Crosses and markers used for mapping the blast resistance gene *Pi-z* with genetic distances (cM)

Resistant	Susceptible	Marker	Genetic distance	Generation	No. of progeny
Jefferson	Lemont/Katy	MRG5836	0.0	F4	30
Jeff-sib	Katy	KIN2	10.4	F4	251
Lafitte	93Y82	DP1	0.0	F3	49
Lafitte	93Y82	MRG5836	3.9	F3	49
Lafitte	93Y82	MRG2431	7.4	F3	49
Lafitte	93Y82	MRG4963	7.7	F3	49
Lafitte	M202	DP1	19.5	F3	206
Lafitte	M202	MRG5836	3.5	F3	206
Lafitte	M202	MRG2431	5.2	F3	206
Lafitte	M202	MRG4963	6.4	F3	206
Lafitte	M103	DP1	5.8	F3	54
Lafitte	M103	MRG5836	11.5	F3	54
Lafitte	M103	MRG2431	5.8	F3	54
Lafitte	M103	MRG4963	7.7	F3	54
Lafitte	Koshihikari	DP1	11.7	F3	98
Lafitte	Koshihikari	MRG5836	1.3	F3	98
Lafitte	Koshihikari	MRG2431	4.0	F3	98
Lafitte	Koshihikari	MRG4963	5.3	F3	98
VARIOUS	VARIOUS	DP1	1.4	F8	217
VARIOUS	VARIOUS	MRG5836	2.9	F8	217
VARIOUS	VARIOUS	MRG2431	3.1	F8	217
VARIOUS	VARIOUS	MRG4963	4.1	F8	217
VARIOUS	VARIOUS	KIN2	15.0	F8	217

Table 2 Primers used for *Pi-z* markers

Primer	Primer sequence (5′–3′)	Marker type
DP1F	ATATGCGGCCGCGGTGGGGTWGGKAARACNAC[a]	Degen. primer
DP1R	TATAGCGGCCGCIARIGCIARIGGIARNCC	(LRR-NBS)
KIN2F	TGATACTGGATGATGTCTGG	Degen. primer
KIN2R	GTGCTTCTTATGAACCCTTC	(Kinase)
MRG4963F	CGAAAAGTGGGAAGCAAATG	Microsatellite
MRG4963R	GCGTACCCCTAGTGGCTGTA	
MRG5836F	TATAAGCCGCAGCCAAATTC	Microsatellite
MRG5836R	AAAAACCTAGAAAATGGGAAAATG	
MRG2431F	ATCCAAATCCAATGGTGCAG	Microsatellite
MRG2431R	GTGGCGAAAGGGAACATTCT	

[a] W = A + T, K = G + T, R = A + G, N = A + C + G + T, I = Inosine

Selection of candidate markers and PCR amplification

The Monsanto microsatellite database (http:\\www.rice-research.org) contains the DNA sequence flanking approximately 7,000 microsatellites. It also contains the most-likely chromosomal location of the markers relative to previously mapped markers found in Gramene (http:\\www.gramene.org).

Starting with the location of *Pi-z* on the Japanese morphological map, microsatellites were identified on the corresponding region of chromosome 6 as discussed below. Primers for each candidate marker were designed using Primer3 (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi) and were synthesized by Genosys (The Woodlands, Tex.). Degenerate primers designed previously (Chen et al. 1998; Speulman et al. 1998) were also synthesized. PCR was performed in a total volume of 10 μl containing 10 ng of DNA template, 20 pmol of each primer, 1.5 mM of $MgCl_2$, 0.2 mM of dNTPs and 5 U of *Taq* polymerase (Invitrogen, Carlsbad, Calif.). Reaction conditions for degenerate primer pairs were initial denaturation for 4 min at 94 °C, followed by 40 cycles at 94 °C for 1 min, 45 °C for 1 min, and 72 °C extension for 2 min, and final extension at 72 °C for 7 min. Reaction conditions for microsatellites were initial denaturation for 2 min at 94 °C, followed by 35 cycles at 94 °C for 1 min, 55 °C for 1 min, and 72 °C extension for 1 min, and final extension at 72 °C for 5 min. Primer pairs used are listed in Table 2.

Six microliters of loading dye (98% formamide, 10 mM EDTA, 0.025% bromophenol blue, 0.025% xylene cyanol) were added to each reaction and samples were denatured at 80 °C for 3–4 min before gel analysis.

Electrophoresis and marker mapping

Four microliters of the reaction mix were loaded onto a 10% non-denaturing polyacrylamide gel (National Diagnostics, Atlanta, Ga.). Samples were electrophoresed at 55 V for 1.5 h or until the bromophenol blue reached the bottom of the gel. After electrophoresis, the gels were stained with SYBR gold (Molecular Probes, Eugene, Ore.). Five microliters of SYBR gold stain were mixed with 25 ml of 1× TBE (pH 7.5–8.0) and spread over the gel with a glass pipet. Gels were allowed to stain for 10 min. Bands were visualized on a Dark Reader optical system (Clare Chemical, Denver, Colo.), and images were recorded using an Olympus C-3030 digital camera.

To determine their position on chromosome 6, markers were mapped in 192 F8 Lemont/Teqing (LMNT/TQNG) recombinant inbred lines using Mapmaker 3.0 (Lander et al. 1987). Markers with a LOD > 2.0 were integrated into the map, while those mapping with LOD < 2.0 were assigned to the most-likely intervals.

Results

Identifying candidate markers in public databases

From the reported position of *Pi-z* on chromosome 6 relative to *Pgi-2, Amp-3* and *Est-2* (Kiyosawa 1967; Shinoda et al. 1971; Kiyosawa 1972), it is possible to estimate its position on the Cornell rice map (near RM136) and to identify the corresponding approximate location on the JRGP map (near R1679 and S2359) (Fig. 1). Using this information, 30 SSRs were identified from this region of chromosome 6 from the Gramene database and from the Monsanto rice microsatellite database as potential markers for *Pi-z*. These markers were first tested for linkage to *Pi-z* in pooled LFTE/93Y82 samples. Of the 30 microsatellite sequences tested, three were tightly linked to *Pi-z*, 18 were not polymorphic, and nine, all of which were AT repeats, did not amplify with the primers that were designed.

Linkage between the markers and blast resistance was confirmed by screening individual F3 and F4 plants from crosses segregating for *Pi-z* (Table 1). Genetic distances between the markers and the *Pi-z* locus ranged from 0.0 (no recombination between the marker and resistance factor) to 11.7 cM.

Based on the markers' chromosomal locations as reported in Gramene and the Monsanto database, the three microsatellite markers should be located between RM136 and RM150b. Neither RM136 nor RM150b was polymorphic in any of the crosses segregating for *Pi-z* that were tested. However, as expected, the three microsatellites mapped between these markers in the F8 LMNT/TQNG mapping population described by Tabien et al. (2000). Based on map positions, the markers were expected to span 3.5 cM, which is in reasonable agreement with the average distance of 5.9 cM we obtained between the markers in the F3 populations and 2.9 cM we observed in Lemont/Teqing.

Of the three linked microsatellite markers, MRG5836 was the most useful. It was polymorphic and linked to *Pi-z* in all of the F3 and F4 populations used for marker development. It was also polymorphic in most of the 220 F8 lines from the California public breeding program expected to be segregating for *Pi-z*, with an average genetic distance of 2.9 cM.

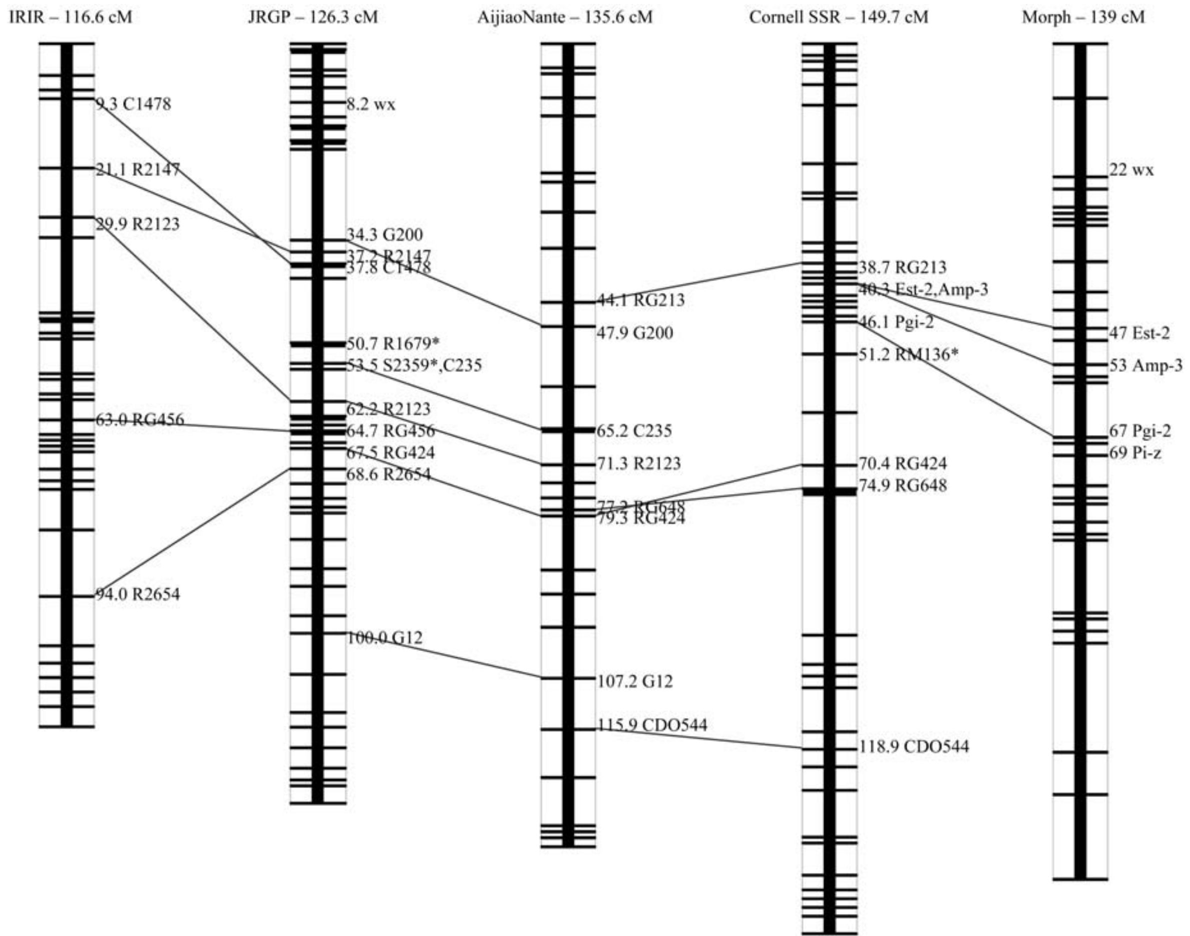

Fig. 1 Genetic maps of rice chromosome 6. As discussed in the text, there are slight differences in distances between markers, as well as some marker rearrangements between maps. These differences are similar to those in genetic distances seen in the both the mapping and breeding crosses for each of the *Pi-z* markers. The locations of S2539 (MRG5836), RM136 (MRG2431) and R1679 (MRG4963) are denoted by *asterisks*

To examine whether the markers would be of general utility in the wider range of *indica* and *japonica* germplasm as well as the narrow crosses characteristic of applied breeding programs, the alleles of each marker were examined in a cross-section of rice varieties and compared to the reported inheritance of *Pi-z* (Fig. 2). Zenith, the variety in which *Pi-z* was first identified (Kiyosawa 1967), has the (CTT)16 allele of MRG5836. As would be expected, this same allele is also present in all of the Zenith progeny that inherited *Pi-z* such as Gulf Rose, Mars and Bengal. The (CTT)16 repeat is relatively uncommon among cultivars that lack *Pi-z*. However there are some varieties that have inherited the (CTT)16 allele independent of the gene. For example, L202, and its progenies Cypress and Cocodrie have the CTT16 allele but are susceptible to IE-1k. An expanded pedigree showing the inheritance of *Pi-z* and MRG5836 alleles is shown in the electronic supplementary material that accompanies this paper.

Amplification of resistance gene analogs using degenerate primers

To find flanking markers for the gene, degenerate primers designed from known disease resistance genes were tested. Each primer combination produced 25–30 bands of which approximately 10–30% were polymorphic between *indica* and *japonica* cultivars.

One marker linked to *Pi-z* was polymorphic in bulked samples from the Lafitte/93Y82 cross. The primers DP1 F/R, which are based on the NBS and LRR motifs of the resistance genes *RPS2*, and *N*, produced a marker that was present in Lafitte and all resistant pools. The genetic distance between DP1 and *Pi-z* was substantially different in the pools and four F3 populations were examined, ranging from no recombination in Lafitte/93Y82 to 19.5 cM in the Lafitte/M202 population. Among the F8 lines examined where this marker was polymorphic between the IE-1k resistant and susceptible parents, there was only 1.4 cM between DP1 and *Pi-z*.

While DP1 is useful in crosses where it is polymorphic, there is also a common allele seen in both *indica* and

Variety	*Pi-z*	MRG5836	DP1	KIN2
Panda	yes	CTT16	A	A
Bengal	yes	CTT16	A	A
Jefferson	yes	CTT16	A	A
Gulf Rose	yes	CTT16	A	A
Mars	yes	CTT16	A	A
Saturn	yes	CTT16	A	A
Orion	yes	CTT16	A	A
Zenith	yes	CTT16	A	A
Mercury	yes	CTT16	A	A
Cadet	yes	CTT16	A	A
Vista	yes	CTT16	A	A
Lafitte	yes	CTT16	A	A
Fukunishiki	yes	CTT16	A	A
Teqing	no	CTT8	B	B
Tetep	no	CTT8	B	B
Zhe 733	no	CTT8	B	B
Zhong You Zao 3	no	CTT8	B	B
IR24	no	CTT8	A	B
IR48	no	CTT8	A	B
IR36	no	CTT8	A	B
Jasmine	no	CTT8	A	B
Taichung Native	no	CTT8	A	B
Leah	no	CTT20	A	A
Toro2	no	CTT20	A	B
Magnolia	no	CTT16	A	B
Rexmont	no	CTT16	A	B
L202	no	CTT16	A	B
Cypress	no	CTT16	A	B
Cocodrie	no	CTT16	A	B
Fortuna	no	CTT16	A	B
Bluebelle	no	CTT16	A	B
Bluebonnet	no	CTT16	A	B
Newrex	no	CTT16	A	B
Koshihikari	no	CTT15	B	B
M104	no	CTT15	B	B
Dawn	no	CTT15	A	B
Maybelle	no	CTT15	A	B
L201	no	CTT15	A	B
Rosemont	no	CTT15	A	B
Carolina Gold	no	CTT15	A	B
Lemont	no	CTT15	A	B
Colusa	no	CTT14	B	B
Calrose	no	CTT14	B	B
CS-M3	no	CTT14	B	B
Calrose 76	no	CTT14	B	B
Calmochi 101	no	CTT14	B	B
M204	no	CTT14	B	B
Caloro	no	CTT14	B	B
Early Wataribune	no	CTT14	B	B
Arkrose	no	CTT14	B	B
M202	no	CTT14	B	B
M103	no	CTT14	B	B
Brazos	no	CTT14	A	B
Pecos	no	CTT14	A	B
Rico	no	CTT14	A	B
Nipponbare	no	CTT14	A	B
Supreme Blue Rose	no	CTT14	B	B
Improved Blue Rose	no	CTT14	B	B
Blue Rose	?	CTT14	B	B
Nova	?	CTT16	A	B

Fig. 2 *Pi-z* and marker data for rice germplasm. Each column represents data for one marker and each row represents a cultivar. *Black boxes* represent cases in which marker data indicate the presence of *Pi-z*. *Gray boxes* represent cases in which marker data indicate that *Pi-z* is not present. As described in the text, there are some cultivars that have the resistant alleles of MRG5836 and DP1 although they lack *Pi-z*. In these instances, KIN2 can still be used to accurately predict the presence of *Pi-z*

japonica cultivars that is not linked to *Pi-z*. This unlinked allele gives a DNA fragment of exactly the same size and sequence, and is particularly common among long-grain varieties. Thus, DP1 will not be generally useful for rapid introgression of *Pi-z* into some material.

Eight more degenerate primers pairs, designed from additional conserved disease resistance gene motifs (Collins et al. 1998), were used to search for additional markers linked to *Pi-z*. This was done in progeny from a cross between the variety Katy and a sibling of the variety Jefferson that was segregating for IE-1k resistance, but where none of the markers discussed thus far were polymorphic. Using this method one marker, KIN2, was identified which has an apparent recombination rate of 10.4% with IE-1k susceptibility in Jeff-sib/Katy. As expected, KIN2 was inherited in a fashion very similar to *Pi-z* and can distinguish between the resistant and susceptible *japonica* cultivars.

Discussion

Even with the availability of several high-density rice maps, linking markers to genes using the information in the public domain is not a simple matter. The problem lies in consolidating individual marker maps. As shown in Fig. 1, there are few markers present on multiple maps, their order is sometimes inverted, and distances between them can differ substantially between crosses.

The location of *Pi-z* at position 69 on chromosome 6 was reported (Kiyosawa 1967; Shinoda et al. 1971; Kiyosawa 1972) relative to a number of morphological and isozyme markers. Using information in the public domain, these markers cannot be directly linked with the microsatellites in Gramene or in the Monsanto microsatellite database. However, based on the position of *Pi-z* relative to *Pgi-2, Amp-3* and *Est-2*, it was possible to estimate its likely position on the Cornell SSR map (near RM136) and to identify the corresponding approximate location on the JRGP map (near R1679 and S2359). This approximation proved to be accurate since the polymorphic markers MRG4963, MRG2431 and MRG5836 are linked to *Pi-z*. Based on this information as well as calculated genetic distances, we can tentatively assign positions for *Pi-z* on both the JRGP and Cornell SSR maps. As expected, this position is in general agreement with the position of *Pi-z* on the morphological map.

MRG5836 is the closest linked microsatellite marker, located 3.8 cM from *Pi-z* in the F3 and F4 progeny, and 2.9 cM in the F8 lines tested. It is very useful since the (CTT)16 allele that is linked to *Pi-z* in all of the Zenith progeny examined is relatively uncommon in germplasm lacking *Pi-z* (see Fig. 2). This indicates that MRG5836 will be informative in most crosses between varieties having *Pi-z* and those that lack this blast resistance gene.

It should be noted that there are at least two lineages where the (CTT)16 allele of MRG5836 is not linked to *Pi-z*. A (CTT)16 allele of MRG5836 that is not linked to *Pi-z* can be traced from Fortuna into Bluebonnet,

Bluebelle, Newrex and Rexmont. A (CTT)16 allele that is not linked to *Pi-z* can also be traced from L202 into Cypress and Cocodrie. In both cases the origin of this allele is unclear, as the original parent of Fortuna is no longer available, and L202 has an unknown parent. Since the linkage between MRG5836 and *Pi-z* has been broken in these lineages, MRG5836 will not be informative in progeny of these varieties.

The first marker found for *Pi-z* using degenerate primers was DP1. The distance between DP1 and *Pi-z* ranged from 5.8 cM in LFTE/M103 to 19.5 cM in the LFTE/M202 population. This large variation is not particularly surprising since, as shown in Fig. 1, recombination rates in this region of chromosome 6 have previously been shown to differ substantially between crosses.

As would be expected for flanking markers, DP1 and MRG5836 are very accurate when used together. They failed to correctly predict the presence of *Pi-z* in less than 1% of the F3 and F8 progeny where both were polymorphic. Unfortunately, the utility of DP1 for introgression of *Pi-z* into some varieties will be limited because many varieties contain an allele of DP1 that is not linked to *Pi-z* but which gives a band of exactly the same size and sequence.

To use marker-assisted selection for *Pi-z* as a routine tool in applied breeding, it was necessary to develop additional markers for *Pi-z* that have a wider range of polymorphism than DP1. In particular, a marker was needed to complement MRG5836 in cases where linkage between *Pi-z* and the 'resistant' allele of the marker had been broken. These include prominent commercial varieties such as Cypress as well as other key breeding material. We identified one marker, KIN2, that was polymorphic in this material and was located approximately 10 cM from *Pi-z*. KIN2 was also polymorphic in most of the F8 lines where we had previously mapped MRG5836 and DP1 relative to *Pi-z*, and was approximately 15.0 cM from *Pi-z* on the far side of DP1. As expected, KIN2 works very well in conjunction with the flanking marker MRG5836 (Fig. 2 and electronic supplementary material).

It should be noted that KIN2 is a dominant marker. Thus, heterozygous samples cannot be distinguished from those homozygous for *Pi-z*. Also, samples that lack *Pi-z* can not be distinguished from failed reactions. DP1 has similar limitations, but it has tighter linkage to *Pi-z* and its primers produce several other bands, providing a positive control for amplification. In contrast, MRG5836 is a codominant marker and therefore more informative in terms of heterozygous samples.

Pi-z5, previously identified as *Pi2*(*t*), has been mapped to chromosome 6 and is thought to be allelic to *Pi-z* (Inukai et al. 1992; Jiang and Wang 2002). It is possible that these new markers will be useful for this gene, but further studies will be necessary to confirm this.

Pi-z was originally identified based on work done using the cultivar Zenith (Kiyosawa 1967). However, the origin of this gene is unclear. Since Zenith, Improved Blue Rose and Supreme Blue Rose are all claimed to be selections from the variety Blue Rose, one would expect them all to be resistant to the blast race IE-1k and to have the same allele of MRG5836. This is not the case. Zenith alone has the (CTT)16 allele linked to resistance, while Blue Rose, Improved Blue Rose and Supreme Blue Rose all have the (CTT)14 allele. Also, in a recent greenhouse test conducted at the USDA-ARS facility in Beaumont, Improved Blue Rose and Supreme Blue Rose were found to be susceptible to IE-1k and Blue Rose gave a mixed response (A. McClung, personal communication). Another indication that Zenith is not simply a selection from Blue Rose comes from analysis with other DNA markers. In a preliminary screen, there were 11 markers on six chromosomes for which Zenith differs from Blue Rose, indicating that Blue Rose is not the sole parent of Zenith (our unpublished data). Questions regarding parentage are seen frequently in studies such as this, and are often caused by mixtures of seed lots, pollen contamination and/or sample mislabeling.

The very limited polymorphism in this region of chromosome 6 between varieties that have *Pi-z* and those that do not, suggests that the gene now recognized as *Pi-z* may have arisen from a very small genetic change. It should be noted in this respect that for the *Pi-ta* blast resistance gene, a single amino-acid substitution differentiates resistant and susceptible alleles (Bryan et al. 2000). Lack of polymorphism between cultivars in this region of the genome could be explained if the function of *Pi-z* arose in a similar manner.

In summary, we have developed DNA markers that can be used to accurately predict IE-1k resistance and thus the presence of *Pi-z*, even in narrow crosses characteristic of applied breeding programs. The benefits of such markers include a reduction in time and space requirements, as well as a lack of interference from environmental conditions or the presence of additional blast resistance genes. These markers are now being utilized in public breeding programs to rapidly develop cultivars with enhanced blast resistance.

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