Tagging *Pi-1(t)* gene for blast resistance in rice via linkage to microsatellite markers

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Abstract

The present work was conducted to identify microsatellite markers linked to the rice blast resistance gene *Pi-1(t)* for a marker-assisted selection program. Twenty-four primer pairs corresponding to nineteen microsatellite loci were selected from the Gramene database (www. gramene.org) considering their relative proximity to *Pi-1(t)* gene in the current rice genetic map. Progenitors and DNA bulks of resistant and susceptible families from F_3 segregating populations of a cross between the near-isogenic lines C101LAC (resistant) and C101A51 (susceptible) were used to identify polymorphic microsatellite markers associated to this gene through bulked segregant analysis. Putative molecular markers linked to the blast resistance gene *Pi-1(t)* were then used on the whole progeny for linkage analysis. Additionally, the diagnostic potential of the microsatellite markers associated to the resistance gene was also evaluated on seventeen rice varieties planted in Latin America by amplification of the specific resistant alleles for the gene in each genotype. Comparing with greenhouse phenotypic evaluations for blast resistance, the efficiency of the microsatellite assay was corroborated. As expected, the phenotypic segregation in the F_3 generation agreed to the expected segregation ratio for a single gene model. Of the twenty-four microsatellite sequences tested, six resulted polymorphic and linked to the gene. Two markers (RM1233*I and RM224) mapped in the same position (0.0 cM) with the *Pi-1(t)* gene. Other three markers corresponding to the same genetic locus were located at 18.5 cM above the resistance gene, while another marker was positioned at 23.8 cM below the gene. Microsatellite analysis on elite rice varieties with different genetic background showed that all known sources of blast resistance included in this study carry the specific *Pi-1(t)* allele. Results are discussed considering the potential utility of the microsatellite markers found, for marker-assisted selection in rice breeding programs aiming at developing rice varieties with durable blast resistance based on a combination of resistance genes.

Introduction

Rice blast caused by *Pyricularia grisea* (Cooke) Sacc., the anamorphous state of *Magnaporthe grisea* (T.T. Hebert) Barr (Rossman et al., 1990), is the most limiting biotic factor for rice production in the world. The use of resistant cultivars is the most effective and economical way of controlling blast disease, therefore, breeding efforts for developing resistant cultivars continue to be a priority of rice breeding programs.

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. Combining these resistance genes broadens the number of races that a variety can resist, and there is evidence that multiple resistance genes make it more difficult for virulent races to evolve (Correa-Victoria et al., 2002). Unfortunately, pyramiding genes is difficult using conventional greenhouse screening procedures because blast races carrying individual avirulence genes to be used in inoculations for the identification of the corresponding resistance gene are normally not present in nature. As a result, accumulation of several resistance genes in a common background cannot be easily distinguished without a test cross. Greenhouse tests 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.

Recent advances in molecular marker technology, such as development of tightly linked molecular markers, has made it possible to pyramid major genes and QTL's into one genotype and to simultaneously select several complex characters. The benefits of marker-assisted selection (MAS) in applied breeding programs include a reduction in time and space requirements, as well as a lack of interference from environmental conditions or the presence of additional resistance genes (Mohan et al., 1997; Witcombe & Hash, 2000). In rice, molecular markers have been used to pyramid several bacterial blight resistance genes (*Xa4, xa5, xa13* and *Xa21*) into isogenic lines (Huang et al., 1997); some of which were later transferred to breeding lines possessing desirable agronomic characteristics (Sanchez et al., 2000) and to commercial hybrids (Chen et al., 2000). Hittalmani et al. (2000) used MAS to combine three blast resistance genes (*Pi-1(t), Piz-5* and *Pita*) in a single genotype. The authors confirmed that the markers were efficient in developing gene pyramids and that the lines containing all three resistance genes had a broader spectrum of resistance than lines with individual genes. More recently, Jiang et al. (2004) showed the utility of MAS to pyramiding insect and disease resistance genes in commercial rice genotypes.

The blast resistance gene *Pi-1(t)*, originally identified in the cultivar LAC23 (Mackill & Bonman, 1992), an upland cultivar from Liberia within the subspecific isozyme group IV according to Glaszmann's classification (Glaszmann, 1987), confers complete resistance to several blast populations from Latin

America when combined with the blast resistance genes *Pi-2(t)* and *Pi-33(t)* (Correa-Victoria et al., 2002). The Pi-1 gene confers resistance to all races present in one of the most predominant genetic lineages (SRL-4) from Colombia, while the other two genes confer resistance to all races within two other predominant lineages (SRL-5 and SRL-6), respectively. Allelism tests conducted by Inukai et al., (1994) using near isogenic lines and the Japanese differential cultivars indicated that *Pi-1(t)* was closely linked but nonallelic to the *Pi-k* locus, a blast resistance gene present in the japonica cultivar, Kusabue. Mapping studies showed that the *Pi-1(t)* gene is located near the end of chromosome 11, linked to the Npb181 and RZ536 RFLP markers at a distance of 3.5 and 14.0 cM, respectively (Yu et al., 1996; Hittalmani et al., 2000).

However, RFLP approaches are expensive and laborious limiting their use in applied breeding programs, where a considerably high number of samples need to be analyzed. Convenient and cost-effective microsatellite markers, particularly those that can be scored on agarose gels (Chauhan et al., 2002), seem to be promising for the identification of blast resistance genes and for pyramiding or introgression of these genes into rice commercial varieties and elite lines (Mackill & Ni, 2001). Microsatellite markers are hypervariable, abundant and well distributed throughout the rice genome and they are now available through the published high-density linkage map (Temnykh et al., 2000; 2001; McCouch et al., 2001, 2002) or in the public database (www. gramene.org).

We have designed a molecular marker-assisted breeding program in rice aiming at developing durable blast resistance in elite rice lines and cultivars by pyramiding the resistance genes *Pi-1(t)*, *Pi-2(t)* and *Pi-33(t)*; which are potentially useful to control blast pathogen populations in the Latin American region (Correa-Victoria et al., 2002). Closely linked microsatellite markers are available for *Pi-2(t)* and *Pi-33(t)* (Jiang & Wang, 2002; Berruyer et al., 2003; Deng et al., 2006), but not for *Pi-1(t)* gene. Here we report new microsatellite markers that cosegregate with the blast resistance gene *Pi-1(t)*, using sequences available in a public database. These markers can be potentially used in MAS to introduce this gene into blast susceptible varieties, and provide the basis for map based cloning of this blast resistance gene.

Material and Methods

Plant material and disease evaluation

The near-isogenic lines C101LAC (resistant line to isolates carrying avr *Pi-1(t)*) and C101A51 (susceptible line) developed at IRRI (Mackill & Bonman, 1992) were crossed (cross CT 13432) and F_1 seeds generated. The F_2 progeny, resulting from self-pollination of F_1 individuals, were self-pollinated to generate 283 CT13432 F_3 lines. Rice varieties from Latin America were obtained from CIAT's rice germplasm bank.

Ten rice seedlings 21 days old per pot were sprayed with 2.0 ml of blast inoculum suspension ($5x10^5$

spores/ml of isolate Oryzica Yacu 9-19-1 carrying avr *Pi-1(t)*) and incubated in the greenhouse at a temperature of 24-28°C and relative humidity above 85 %. Plants were evaluated 15 days (two life cycles of the pathogen) after inoculation and scored for resistance and susceptibility in two replications as described by Correa-Victoria and Zeigler (1993), and considering the susceptible/resistance criteria indicated in the Standard Evaluation System for Rice (INGER-IRRI, 1996). Thus, resistant genotypes exhibit complete resistance with no lesions or few non-sporulating lesions type 1 or 2, and susceptible genotypes exhibit typical sporulating blast lesions type 3 or 4 covering more than 1 % of leaf area.

DNA extraction and PCR assay

The DNA extraction was conducted as described by Dellaporta et al. (1983). DNA concentrations were determined in a TKO 100 minifluorometer (Hoeffer, San Francisco, CA) with the DNA-specific fluorescent dye Hoechst 33258 according to manufacturer's directions. DNA bulks were prepared from 13 resistant and 13 susceptible lines within the CT13432 F_3 families evaluated for their blast reaction using the blast isolate Oryzica Yacu 9-19-1. Polymerase chain reaction (PCR) was conducted in a final volume of 20 µl containing between 25-50 ng of template DNA, 0.5 µM of each primer, 200 µM of each dNTP, 3.1 mM $MgCl_2$ and 1 unit of Taq DNA polymerase. For the majority of microsatellite markers studied the reaction was processed as follow: 94°C for 1 min, followed by 40 cycles consisting of 94°C for 30 sec, 50 and/or 55°C for 30 sec and 72°C for 30 sec and a final extension step of 72°C for 10 minutes. After the PCR reaction, 5 µl of blue juice (30 % glycerol, 0.25% bromophenol blue) was added to the amplification product and 20 µl per sample were loaded on high-resolution agarose gels prepared mixing 1.5 % Sinergel (Diversified Biotech) and 0.7 % molecular grade (Invitrogen Life Technologies) products and containing 0.5mg/mL of ethidium bromide.

Microsatellite polymorphism analysis

Twenty-four primer pairs corresponding to nineteen microsatellite loci (Figure 1B) were selected from the Gramene database (www. gramene.org) considering their relative proximity to the *Pi-1(t)* gene in the current rice genetic map (Figure 1A). The isogenic lines C101LAC and C101A51 and their common genetic background, the susceptible recurrent parent CO39, were used to identify microsatellite polymorphisms associated to the blast resistance genes. Polymorphic markers identified above were assayed by bulked segregant analysis (BSA) as indicated by Michelmore et al. (1991).

(Include Figure 1)

Genetics and Linkage analyses

Genetic analysis of the resistance was conducted measuring the goodness-of-fit to the expected ratio for a single gene model using a chi-square test. For this purpose, we used 283 F_3 near-isogenic lines derived from 283 F_2 plants with no selection. Molecular markers that resulted positive in BSA for the *Pi-1(t)* gene were used in linkage progeny analysis using 158 F_3 near-isogenic lines. The determination of sample size for this analysis was carried out using a Genetic Power Calculator software (Purcell et al., 2003) controlling type-I and type-II errors [type-I error = probability that the test rejects H_0 (no linkage) although H_0 is true; type-II error = probability that the test fails to reject H_0 although H_0 is false] as indicated for co-dominant molecular marker-assisted linkage detection for a monogenic qualitative trait (Hühn & Piepho, 2003). Associations between markers and the resistance gene were demonstrated using a chi-square test. Linkage analysis was performed using the software MAPMAKER/EXP V 3.0 (Lander et al., 1987) on the segregation data obtained from markers and blast resistance scoring of the CT13432 F_3 population. Conversion of the recombination fraction into centiMorgans (cM) units was obtained with the Kosambi's mapping function. The final map was drawn using the software QGene V 3.04 (Nelson, 1997).

Amplification of Pi-1(t) resistant allele in commercial rice varieties

The diagnostic potential of the markers associated with the *Pi-1(t)* gene was also evaluated on DNA obtained from nineteen rice genotypes including seventeen elite cultivars grown in Latin America. For this purpose, the criteria followed for determining the presence or absence of the resistance gene was the amplification of the specific *Pi-1(t)* microsatellite allele in each rice genotype. Comparing with phenotypic evaluation obtained as indicated above, the veracity of the assay was corroborated.

Results

Genetic analysis of blast resistance

Genetic analysis of the resistance was conducted using 283 F_3 near-isogenic lines of the cross CT 13432. Expected and observed segregation ratios for this population are shown in Table 1. The population analysis showed a good fit to the expected segregation ratio (1:2:1) for a single gene model confirming the hypothesis of a single dominant gene for *Pi-1(t)* locus, which is in agreement with genetic studies conducted by Mackill & Bonman (1992).

(Include Table 1)

Microsatellite polymorphism and linkage analyses

From the reported position of *Pi-1(t)* on chromosome 11 relative to RZ536 RFLP marker, it was possible to estimate its approximate position on the Rice-Cornell microsatellite genetic map (Figure 1A). Using this information, twenty-four microsatellite sequences were selected from this region of chromosome 11 from the Gramene database (www.gramene.org) as potential markers for *Pi-1(t)*. These markers were first tested for polymorphism in the susceptible and resistant parent and later for linkage to *Pi-1(t)* in pooled C101LAC/C101A51 samples. Of the twenty-four primer pairs tested six (corresponding to four microsatellite loci) were polymorphic in agarose gel electrophoresis, all of them showing positive results in bulked segregant analysis; five markers were not polymorphic, and thirteen principally repeats with TA sequences did not show consistent amplification with the different annealing temperatures assayed.

Linkage between these six markers and blast resistance was confirmed by screening 158 F_3 near-isogenic lines from the cross C101LAC/C101A51 segregating for *Pi-1(t)*. Chi-square test indicated that these markers were linked to *Pi-1(t)* (all χ^2 values were greater or equal to 128.99, $p < 0.0001$). Occurrence probability of the type-I error here expressed as false linkage (α) was zero for co-dominant markers (RM1233*I and RM224) and the type-II error (β) was always lower than 1.7 %. In this analysis, these two markers were highly powerful, sensible and specific showing a predictive capacity of the resistance events (PVP value) that reached 100 %. RM1233*I and RM224 markers also showed PVN values higher than 95% indicating that they are statistically robust markers. The occurrence probability of the type-I (α) and type-II (β) errors using dominant markers as RM7654*A and RM7654-2 were also low, while the markers RM7654*H and RM6094 showed α and β values higher than 5 %. In all these cases, although these were similarly powerful and sensible, they showed specificity and PVN values lower than 95 % (Table 2).

(Include Table 2)

The genetic distance between the markers and the *Pi-1(t)* locus ranged from 0.0 (no recombination between the markers and the resistance factor) to 23.8 cM (Figure 1C). Among the six makers linked to *Pi-1(t)* gene, two (RM1233*I and RM224) mapped in the same position (0.0 cM) with the *Pi-1(t)* gene. Other three dominant markers corresponding to the same genetic locus (RM7654) were located at 18.5 cM above the *Pi-1(t)* gene, while marker RM6094 was identified at 23.8 cM below the gene. This last microsatellite locus was characterized by the presence of a band in the susceptible genotypes and by the absence of the band in the resistant lines being not potentially useful for MAS. Therefore, this marker was no used in the next analysis.

Using Pi-1(t) associated microsatellite markers to detect the resistance gene in rice cultivars

To examine whether the markers identified would be of general utility on a wider range of rice germplasm used in applied breeding programs in Latin America, the presence of resistant bands for five markers were examined in elite rice cultivars and compared to the reported inheritance of *Pi-1(t)* (Table 3). For this purpose, we used known sources of blast resistance as positive controls and considered as predictive criteria of the resistance event the amplification in each variety of the resistant microsatellite band (Figure 2) and therefore the presence of the resistant allele for the *Pi-1 (t)* gene.

(Include Figure 2)

Comparing with phenotypic data on blast resistance our results showed that our known sources of resistance (C101LAC, Cica 8, Oryzica 2, BR IRGA 409, CR 1113, El Paso 144 and Panama 1048) carry the resistance *Pi-1(t)* allele; on the other hand, the susceptible cultivars (Colombia XXI, Epagri 108, Capirona and Oryzica 1 and CO-39) had not the resistant allele. In addition, other seven varieties (Jucarito-104, Fedearroz 2000, CR 1821, Primavera, Cimarrón, Bonanza and Fedearroz 50), which were resistant in the pathogenicity assay, did not show the allele characteristic of the *Pi-1(t)* gene.

(Include Table 3)

Discussion

This study demonstrates that approaches combining near isogenic progeny analysis and rice genome information available in a public database constitute a very useful tool for identifying molecular markers closely linked to blast resistance genes. The reported marker most closely linked to blast resistance gene *Pi-1(t)* was the cDNA Npb181, identified at 3.5 cM from the gene (Hittalmani et al., 2000). Here, using a segregating population with identical genetic background (CO39) but with a higher number of segregant lines than the one used by these authors, we have identified two new microsatellite markers (RM1233*I and RM224) highly linked to gene *Pi-1(t)* (at 0 cM of the gene). Working with several rice populations, Fjellstrom et al. (2004) found RM1233*I and RM224 markers highly linked to the resistance genes *Pi-k*^{h} and *Pi-k*^{s}. However, it has been shown more recently that *Pi-k*^{h} mapped at 101.9 cM on Chromosome 11 closely linked to RM206 marker (Sharma et al., 2005).

The fact that markers previously reported in the neighborhood of *Pi-1(t)* in the Rice-Cornell microsatellite map, did not amplify or resulted in being not polymorphic in this study limits our intent to reassign the position of this gene on rice chromosome 11; however some advances can be obtained to this aim. The *Pi-1(t)* gene was previously mapped between markers RG303 and RZ536 (Yu et al., 1996), which are about 24.7 cM apart on chromosome 11 (Chen et al., 1997). Hittalmani et al. (2000) further localized the gene

within an interval of about 11.4 cM region flanked by RZ536 and the cDNA marker Npb181, an expressed sequence derived from the Japanese rice program. From the reported position of the *Pi-1(t)* gene relative to the RZ536 marker, it was possible to estimate its putative position on the Rice-Cornell microsatellite genetic map flanked by the microsatellite RM254 and the RFLP RZ536 markers between the 110.0 and 125.6 cM at the end of chromosome 11. Here we have reported two microsatellite markers very closely linked to gene *Pi-1(t)*, which is in agreement with the information included in the Rice-Cornell microsatellite genetic map, positioning these markers between 112.9 and 120.1 cM at the end of chromosome 11. However, the two remaining microsatellite loci RM7654 and RM6094 were outside of the mentioned 7.2 cM chromosomal region, mapping to 18.5 and 23.8 cM from the gene *Pi-1(t)*, respectively. This situation could be explained by the dominant nature of such markers as suggested by the quality indicators (Table 2), where we can compare the limitations of a dominant marker with respect to a co-dominant marker.

The potential use of microsatellite markers as a screening tool for detecting the presence of resistance genes in different cultivars was also addressed as recommended by other authors (Yi et al., 2004). We have shown that the known *Pi-1(t)* resistance sources such as C101LAC, Cica-8, Oryzica 2, BRIRGA409, El Paso 144, Panamá 1048 and CR1113 (Correa-Victoria et al., 2002) exhibited microsatellite alleles associated with this gene of resistance, while susceptible varieties don't. Interestingly, six varieties (Fedearroz 2000, Fedearroz 50, Primavera, Bonanza, Cimarrón and Jucarito-104) that were resistant to the rice blast isolate Oryzica Yacu 9-19-1 did not show the resistant *Pi-1(t)* alleles. One possibility for this resistance reaction in these cultivars could be the presence of different resistance genes interacting with corresponding avirulence genes different from the avr *Pi-1(t)* in the pathogen. It has been previously indicated (Conaway-Bormans et al. 2003) that the presence of one resistance gene can sometimes phenotypically mask other genes conferring resistance to the same blast race.

This study is part of a molecular marker-assisted rice breeding program aiming at developing durable blast resistance in rice cultivars by pyramiding the resistance genes *Pi-1(t)*, *Pi-2(t)*, and *Pi-33(t)*, which are potentially useful for controlling blast pathogen populations in the Latin American region (Correa-Victoria et al., 2002). Disease assays to evaluate resistance to rice blast are time-consuming and laborious procedures that also require specialized facilities. PCR analysis can greatly reduce the amount of labor needed for evaluating phenotypes by prescreening with MAS. Cost-effective microsatellite markers linked to the blast resistance *Pi-1(t)* gene and suitable for agarose gel electrophoresis (Figure 2) facilitating the introgression and pyramiding of the gene into rice commercial cultivars, were developed here.

Compared with previously reported RFLP markers linked to the *Pi-1(t)* gene (Yu et al., 1996; Hittalmani et al., 2000), these microsatellite markers are potentially useful for assisting rice breeding programs in developing countries where financial support is the principal limiting factor to establish a marker assisted

selection breeding program. However, the practical usefulness of the identified markers in assisting an applied rice-breeding program should be tested in segregating populations with different genetic backgrounds. This is not uncommon that the order of markers on a chromosome be inverted in rice cultivars with different genetic backgrounds. Thus, the distance between the markers and tagged genes can differ substantially for different genetic crosses as it has been previously indicated by Conaway-Bormans et al. (2003).

In addition to the gene *Pi-1(t)*, other four blast resistance genes, *Pi-k, Pi-sh, Pi-f* and *Pi-18(t)*, have been mapped at the end of chromosome 11 (Ahn et al., 2000; Sallaud et al., 2003), and several major genes for resistance to rice bacterial blight (*Xa3, Xa4, Xa10, Xa21* and *Xa22(t)*) are clustered near the *Pi-1(t)* locus (Causse et al. 1994; Mackill & Ni, 2001). Expressed sequence tags (EST) and resistance gene analogs (RGR) markers for resistance involved in elicitor molecule recognition and defense response triggered by the recognition events have been also mapped closely linked to the *Pi-1(t)* and *Pi-k*^{m} genes (Mago et al., 1999; Wang et al., 2001; Ramalingam et al., 2003; Sallaud et al., 2003). All these independent studies support the importance of this chromosomal region in studying broad-spectrum resistance, i.e., resistance to multiple races or even resistance to multiple pathogens. The microsatellites reported in this study seem to be suitable for assisting rice breeders in the introduction of the *Pi-1(t)* resistance gene in different rice cultivars, and serve as an indicator for the presence of others. Thus, the *Pi-1(t)* gene markers may serve as indicators for the presence of resistance gene clusters in the indicated chromosome region and for the selection of breeding parents for developing rice cultivars with a broader-resistance spectrum to blast as it has been indicated for other clusters of resistance genes (Jia et al., 2002; Narayanan et al., 2002; Wen et al., 2003; Deng et al., 2006). Additionally, these microsatellite markers could provide a starting point for efforts eventually aimed at cloning and isolating this gene.

Concluding remarks

The present work evidenced the usefulness of combining near-isogenic progeny analysis with rice genome information available in public databases to identify molecular markers highly linked to blast resistance genes in rice. Although a limited number of polymorphic markers can be expected when near-isogenic lines are used as progenitors, here we found six polymorphic markers in a region of only 13 cM surrounding the blast resistance gene *Pi-1(t)* (Figure 1). Additionally, two of these markers (RM1233*I and RM224) were closely linked to the gene. This finding supports the hypothesis that when polymorphisms are found in near-isogenic derived populations, differing only in the presence or absence of a gene, the probability that these markers be closely linked to the gene is very high. Besides, polymorphic markers linked to resistance genes in near-isogenic populations, can also be expected to detect polymorphism and presence of the linked genes in commercial rice varieties with certain level of

inbreeding as it has been previously suggested by other authors (Conaway-Bormans et al. 2003). Our results support the utility of these DNA markers in MAS and gene pyramiding rice breeding programs addressing the improvement of blast resistance in rice cultivars; and eventually to map based cloning of the gene. However, the use of these markers as a diagnostic tool for determining the presence of the resistance gene *Pi-1(t)* in a wider range of rice germplasm require additional studies for further confirmation of the results reported here. The speed, simplicity and reliability of PCR based approaches make microsatellite analysis on agarose gels an attractive tool for marker-assisted selection in rice breeding programs aiming at developing durable rice blast resistant cultivars.

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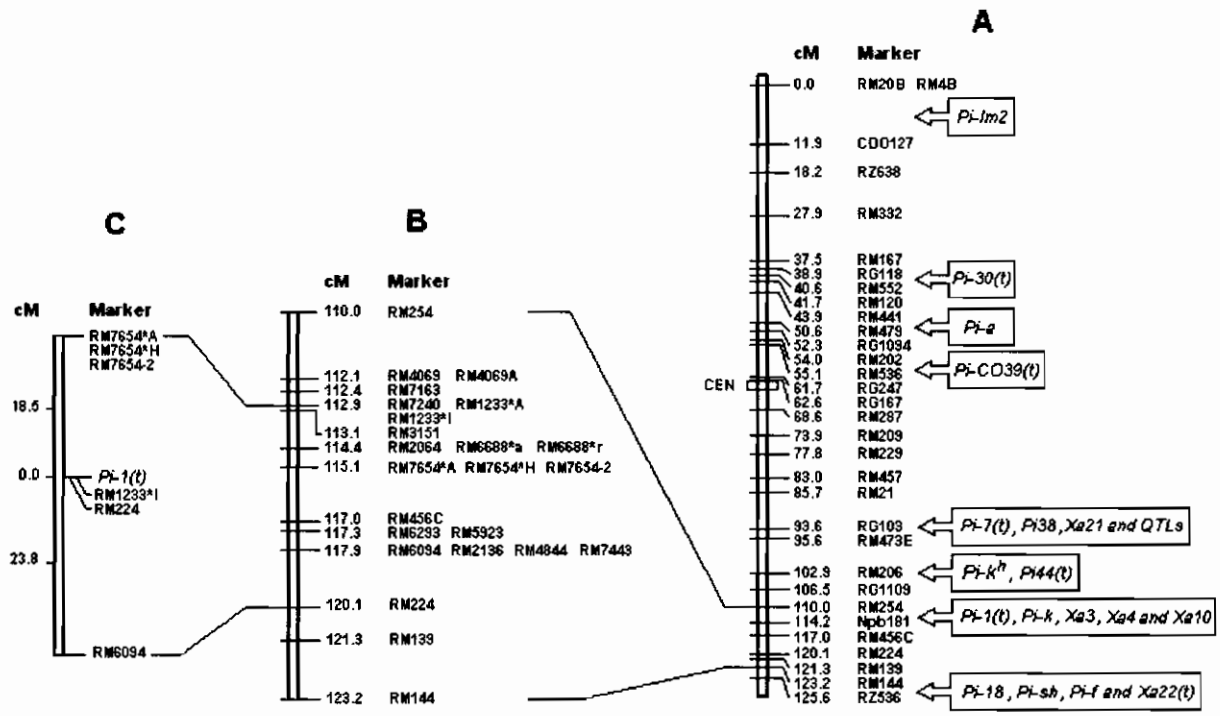

Figure 1. Genetic map of rice chromosome 11 (A) as indicated by Temnykh et al. (2001) and by McCouch et al. (2001). Region between the 110.0 and 123.2 cM (B) was complemented with public information available at Gramene database (www.gramene.org). Information about position of the resistance genes on chromosome 11 was obtained as follow: *Pi-1(t)* (Yu et al. 1996; Hittalmani et al. 2000), *Pi-7(t)* and quantitative trait locus (QTL) to partial resistance to blast (Wang et al. 2001; Zenbayashi et al. 2002), *Pi-CO39(t)* (Chauhan et al. 2002), *Pi-18(t)* (Ahn et al. 2000), *Pi38* (Gowda et al., 2006), *Pi-44(t)* (Chen et al. 1999), *Pi-a, Pi-k, Pi-sh, Pi-f, Pi-lm2 and Pi-30(t)* (Sallaud et al. 2003), *Pi-k^h* (Sharma et al., 2005), *Xa3, Xa4, Xa10, Xa21* and *Xa22(t)* (Causse et al. 1994; Mackill and Ni, 2001). Chromosome 11 generated through linkage analysis (C).

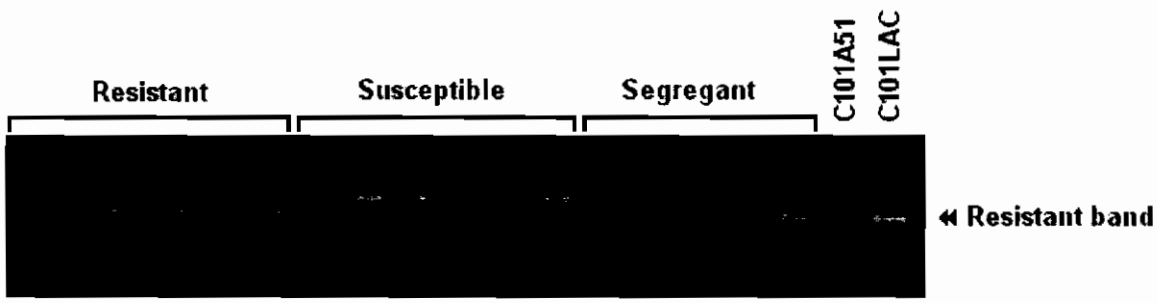

Figure 2. Microsatellite agarose patterns of selected susceptible, segregant and resistant lines analyzed with the RM1233*I marker. Susceptible (C101A51) and Resistant (C101LAC) checks.

Table 1. Segregation of F_3 near-isogenic lines of the genetic cross between C101LAC (*Pi-1(t)*)/C101A51 inoculated with the blast isolate Oryzica Yacu 9-19-1 of *Pyricularia grisea*.

Population	Expected ratio[1]	No. of lines expected			No. of lines observed		
		S	SG	R	S	SG	R
F_3 near-isogenic lines	1:2:1 ($\chi^2 = 1.0$, $p < 0.05$)	71	141	71	76	133	74

(1) According to a model based on a single dominant gene as indicated in materials and methods; (S): Susceptible, (SG): Segregant; (R): Resistant

Table 2. Quality indicators for six microsatellite markers computed as indicated by Hühn and Piepho (2003).

Probability qualification (%)	Marker analyzed					
	RM1233*I	RM7654*A	RM7654*H	RM7654-2	RM6094	RM224
False linkage (α)	0.0	2.9	5.7	2.9	8.0	0.0
False no linkage (β)	0.8	1.6	2.5	2.4	9.9	0.8
Power ($1-\beta$)	99.2	98.4	97.5	97.6	90.1	99.2
Sensitivity	100.0	99.2	98.3	99.2	91.9	100.0
Specificity	97.2	94.4	91.7	91.8	90.1	97.2
Predictive-value positive (PVP)	100.0	99.2	98.3	99.2	91.9	100.0
Predictive-value negative (PVN)	97.2	94.4	91.7	91.9	90.1	97.2

(α) = type-I error or probability that the test rejects H_0 (no linkage) although H_0 is true, (β) = type-II error or probability that the test fails to reject H_0 although H_0 is false. The power ($1-\beta$) is the probability of detecting linkage when it exists. Sensitivity is the probability that the test will be positive if a line has the disease. Specificity is the probability that a line, which does not have the disease will test negative. (PVP) = probability that a line were resistant given that the test was positive. (PVN) = probability that a line were susceptible given that the test was negative.

Table 3. Analysis of the predictive capacity of six microsatellite markers for blast resistance gene *Pi-1(t)* in 19 commercial rice cultivars.

Variety	Origin	PA	Marker analyzed				
			RM1233*1	RM7654*A	RM7654*H	RM7654-2	RM224
CO-39 [1]	Philippines	S	-	-	-	-	-
C101LAC [2]	Philippines	R	+	+	+	+	+
Cica-8	Colombia	R	+	+	+	+	+
Fedearroz 2000	Colombia	R	-	-	-	-	-
Colombia XX1	Colombia	S	-	-	-	-	-
Oryzica 1	Colombia	S	-	-	-	-	-
Oryzica 2	Colombia	R	+	+	+	+	+
Fedearroz 50	Colombia	R	-	-	-	-	-
Epagri 108	Brazil (irrigated)	S	-	-	-	-	-
BRIRGA409	Brazil (irrigated)	R	+	+	+	+	+
Primavera	Brazil (upland)	R	-	-	-	-	-
Bonanza	Brazil (upland)	R	-	-	-	-	-
El Paso 144	Uruguay, Argentina	R	+	+	+	+	+
Cimarron	Venezuela	R	-	-	-	-	-
Capirona	Peru	S	-	-	-	-	-
Panamá 1048	Panama	R	+	+	+	-	+
CR 1113	Costa Rica	R	+	+	+	+	+
CR 1821	Costa Rica	R	-	-	-	-	-
Jucarito-104	Cuba	R	-	-	-	-	-

(1): Susceptible control; (2): Resistant control; PA: Results of the pathogenicity assay using blast isolate Yacu 9-19-1, R: resistant genotype, S: susceptible genotype, (+) presence of resistant allele, (-) absence of resistant allele.