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Development and validation of gene-derived Cleaved Amplified Polymorphic Sequences (CAPS) marker for blast resistance gene *Pi54*

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Abstract

Rice blast is one of the world's most damaging diseases attacking the rice crop. Functional or gene-based markers derived from the polymorphic sites within the nucleotide sequences of cloned R-genes are the potent tools for precise and speedy selection of resistance genes in marker-assisted breeding programmes. The *Pi54* gene identified from a broad spectrum genotype Tetep is known to exhibit resistance to predominant races of pathogen in India thus making it a potential resistance source for breeding blast resistant varieties. The *Pi54* gene has been cloned thus offering a scope for the development of gene-derived markers for this useful gene by using the sequence polymorphisms between the resistant and susceptible haplotypes/alleles of the gene. The development of a new gene-based Cleaved Amplified Polymorphic Sequences (CAPS) marker for *Pi54* gene and its utility in the marker-assisted selection of this broad-spectrum resistance gene has been reported. The developed marker has been shown to be perfectly linked with *Pi54* and works well for crosses where the previously known gene-based marker *Pi54MAS* fails to reveal polymorphism between the resistant and susceptible genotypes.

Key words: Blast resistance; CAPS; gene-derived markers; *Pi54;* rice.

Rice (*Oryza sativa* L.) is a queen of cereals which indeed is a life for millions of global population. The crop plays a key role in achieving global nutritional security too, as it is a plenary source of carbohydrate, protein, specific oils, dietary fibre, vitamins, many minerals and other disease-fighting phyto-compounds. These all qualities collectively make it a 'golden cereal' (Pradhan *et al.* 2019). Unfortunately, the crop suffers from several biotic and abiotic stresses that detrimentally affect its production and quality. Rice blast caused by a hemibiotropic fungal pathogen *Pyricularia oryzae* is a major production constraint for rice world-wide. The pathogen causes a serious damage to the crop throughout its growth stages, beginning from seedling to adult plant stages impacting leaves, nodes, collar, panicles and roots (Sharma *et al.* 2012). Its severe infestation may lead to complete harvest loss (Wang *et al.* 2014).

To curb paddy blast, number of effectual means like chemical, biological and development of transgenics have been suggested. However, development and deployment of disease resistant varieties is the most cost-effective and ecologically safe strategy to manage the disease. Approximately 120 blast resistance genes have been identified and molecularly mapped in rice; 25 of these genes have also been cloned and characterized (Kalia and Rathour, 2019).

The identification and utilization of broad-spectrum resistance genes has been advocated for achieving a durable resistance against the blast disease. The utilization of some of such genes like, *Pi-1, Pi-2, Pi-9, Pi-ta, Pi-ta2* and *Pi54* has prevented large scale outbreaks of the blast disease in many parts of the world (Jeon *et al*. 2003; Sharma *et al.* 2012). The *Pi54* gene identified from a broad spectrum genotype

Tetep has been reported to exhibit resistance to predominant races of pathogen in India (Sharma *et al.* 2012) thus making it a potential resistance source for breeding blast resistant varieties. Simple Sequence Repeat (SSR) markers linked to *Pi54* gene have been identified and effectively exploited to specifically transfer this gene to vulnerable rice varieties through marker-assisted selection (Ellur *et al.* 2016; Khanna *et al.* 2015). Yet, these linked SSR markers are not 100 % predictive of the existence/ non-existence of the trait allele due to genomic recombination between the marker and the trait allele, and can occasionally result in false positives and in many instances these markers are not polymorphic between *Pi54* donors and susceptible recipient genotypes. Functional markers (FMs) or gene-derived markers (GDMs) created from the polymorphic sites within the nucleotide sequences of cloned R- genes, however, can overcome the shortcomings of linked markers due to their perfect linkage with the trait allele and underlying phenotype. The *Pi54* gene has been cloned from 'Tetep' (Sharma *et al.* 2005) thus offering a scope for the development of gene derived markers for this useful gene by using the sequence polymorphisms between the resistant and susceptible haplotypes/alleles of the gene. In past, a functional marker *Pi54MAS* has been developed by targeting a 144 bp insertion/deletion (Indel) polymorphism in the coding region of *Pi54* (Ramkumar *et al.* 2011). Though *Pi54MAS* has been validated in a range of susceptible genotypes that do not have 144 bp deletion in the coding region of *Pi54* locus, it does not work in those crosses where the resistant and susceptible alleles differ only in single nucleotide polymorphisms (SNPs).

In present study, we report the development of a new Cleaved Amplified Polymorphic Sequences (CAPS) marker for *Pi54* gene and its utility in the marker-assisted selection of this broad-spectrum resistant gene. The developed marker has been shown to be perfectly linked with *Pi54* and works well for crosses where the *Pi54MAS* fails to reveal polymorphism between the resistant and susceptible genotypes.

Materials and Methods

Traditional Basmati rice variety Ranbir Basmati (IET-11348, a spontaneous early maturing selection of Basmati-370), two *indica* varieties, PR114 and HPR2795 and a *japonica* line Liziangxintuanheigu (LTH) were used as susceptible genotypes. A Vietnamese *indica* rice variety 'Tetep' from which *Pi54* was originally cloned and two *indica* genotypes DHMAS164 and HPR2880 that derive *Pi54* gene from Tetep were used as resistant lines (Table 1). A BC_1F_1 progeny derived from the cross Ranbir Basmati x DHMAS164 was used to validate the linkage of the gene–based CAPS marker developed during the study. The genomic DNA of different genotypes and BC_1F_1 progenies was isolated by standard CTAB method (Murray and Thompson, 1980).

For the development of co-dominant gene-based CAPS marker, the DNA sequence of cloned *Pi54* gene (AC.No. AY914077) was retrieved from the GenBank and used for designing a gene-specific sequence tagged site (STS) marker *Pi54STS-1* (forward: 5'TTCTCTGCTTCTGATCACCAAA3'; reverse: 5'GAGACATTGATGTTGAGGTGGA3') using primer 3.0 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). PCR amplification was performed in a 25 µl reaction volume containing 20 ng template DNA, 0.2 mM of each dNTP, 0.2 µM of each primer, 1.5 mM $MgCl_2$, 1X PCR buffer (10 mM Tris-HCl, 50 mM KCl, pH 8.3) and 1 unit *Taq* polymerase (MBI, Fermentas). PCR amplification was carried out in a thermocycler (ABI 9700, Applied Biosystems, USA) using the following temperature profile: Initial denaturation at 94°C for 5 min, followed by 39 cycles at 94°C for 1 min, 58°C for 1 min, 72°C for 1.5 min and a final extension at 72°C for 5 min, followed by rapid cooling to 4°C. Amplified PCR products were electrophoresed in a 2.0 % agarose gel in 1X Tris acetate-EDTA (TAE) buffer at 120 V and visualized with ethidium bromide (0.5µg/ml) staining. The *Pi54*STS-1 amplicons from Ranbir basmati and *Pi54* donor DHMAS164 were digested with a panel of restriction enzymes chosen from *in silico* restriction map of *Pi54* generated through restriction analysis tool of TAIR (http://www.arabidopsis.org/tools/). The cleaved products were separated on 2.0 % agarose gel using 1X TBE to detect restriction fragment length polymorphisms between the parental genotypes. The primer sequences and PCR amplification conditions for previously known gene-derived Indel marker

Pi54MAS for *Pi54* gene were adopted from Ramkumar *et al.* (2011).

Blast resistance screening of different rice genotypes and BC_1F_1 progeny of cross Ranbir Basmati x DHMAS164 was done by using a culture of blast isolate *Mo-nwi-114* (race = U01-i2–k133–z00–ta001) being maintained in our laboratory. The 21 days old rice seedlings of each genotype were spray inoculated with blast isolate *Mo-nwi-114* using the standard procedure as described in Rathour *et al.* (2004). The genotypes were rated for their reaction to blast after 7 days of inoculation using 0-5 scale given by Mackill and Bonman (1992).

The linkage of gene-based CAPS marker *Pi54STS-1(Dra*I*)* to *Pi54* was validated by testing the marker on 34 BC_1F_1 progeny plants of cross Ranbir Basmati x DHMAS164 that had been phenotyped for their reaction to blast. The zygosity of each BC_1F_1 plant at resistance locus was inferred from the segregation analysis of F_2 progenies of each plant.

Results and Discussion

Rice genotypes Ranbir Basmati, HPR2795, PR114 and LTH exhibited susceptibility to blast isolate *Mo-nwi-114*, whereas the *Pi54* gene carrying genotype Tetep and two other genotypes DHMAS164 and HPR2880 showed complete resistance to blast. The gene-based Indel marker *Pi54MAS* for *Pi54* gene amplified a 216 bp allele in all the resistant genotypes as well as in one of the susceptible genotypes Ranbir Basmati. The marker amplified a 359 bp allele characteristic of susceptible haplotype of *Pi54* in other blast susceptible genotypes, HPR2795, PR114 and LTH (Fig 1). Previously, Ramkumar *et al.* (2011) developed a functional gene-based marker *Pi54MAS* by targeting a 144 bp deletion in the resistance alleles of *Pi54* gene. However, the amplification of 216 bp allele in susceptible genotype Ranbir Basmati clearly suggests that 144 bp deletion does not have any relevance in the functionality of *Pi54* gene and instead suggest that SNP polymorphisms possibly determine the resistance specificity at the *Pi54* locus. Consistent with our view, Sharma *et al.* (2005) have also observed a single SNP polymorphism in the promoter region of *Pi54* gene that differentiates the resistance and susceptible alleles of *Pi54* gene. Structural comparisons of the cloned members of multi-allelic resistance loci like *Pi2* and *Pik* have also provided the evidence that SNP polymorphisms not only differentiate the resistance and susceptible alleles but also account for distinct resistance specificities of different alleles of same locus (Zhou *et al.* 2006; Zhai *et al.* 2011; Hua *et al.* 2012).

Since the *Pi54* gene-derived Indel marker *Pi54MAS* previously developed by Ramkumar *et al*. (2011) was not polymorphic between Ranbir Basmati and *Pi54* donor lines, we attempted to develop a co-dominant gene-derived CAPS marker for *Pi54* that could differentiate Ranbir Basmati and *Pi54* donor lines. The gene-derived STS marker *Pi54STS-1* targeting the coding region of *Pi54* generated a monomorphic amplicon of 1214 bp in Ranbir Basmati and *Pi54* donor line DHMAS164. The *Pi54STS-1* amplicons of two genotypes were digested with a panel 8 restriction enzymes *viz.*, *Alu* I, *Dpn* I, *Dra* I, *Hae* III, *Hinf* I, *Rsa* I, *Sca* I, and *Xho* I for generating cleaved amplified polymorphisms between the genotypes. Of the eight enzymes tested, three namely, *Hinf* I, *Rsa*I and *Dra* I generated cleaved amplified polymorphisms between the Ranbir Basmati and DHMAS164 (Fig 2). These results suggested that the resistance and susceptible genotypes have several SNP polymorphisms in the coding region of *Pi54* that resulted in the differential restriction patterns of their alleles. These polymorphisms can be utilized to develop gene-based markers for efficient selection of resistance allele of *Pi54* during resistance breeding.

To test efficacy of one of these CAPS polymorphisms in the marker-assisted selection of *Pi54* gene, a total of 33 BC_1F_1 plants of cross Ranbir Basmati x DHMAS164 that had been phenotyped for their reaction to blast isolate *Mo-nwi-114* were analysed with CAPS marker *Pi54STS-1*(*Dra*I). The *Pi54STS-1* derived amplicons of parental genotypes and BC_1F_1 plants were digested with enzyme *Dra*I before their analysis on 4% agarose gel. The CAPS marker *Pi54STS-1*(*Dra*I) produced a 400 bp polymorphic restriction fragment in susceptible parent Ranbir Basmati and 350 bp fragment in *Pi54* donor genotype DHMAS164. The marker produced 400 bp fragment characteristic of Ranbir Basmati in all the 10 susceptible BC_1F_1 plants and a discernible heterozygous pattern in all the 23 *Pi54* heterozygous

Table 1. List of rice varieties used for validating the gene-derived markers for *Pi54 gene*

Sr. No.	Genotype	Varietal type	Known resistance gene(s)	Reaction to blast isolate *Mo-nwi-114*
1	Ranbir Basmati	*Basmati*	-	S
2	DHMAS164	*Indica*	*Pi1, Pita, Pi54*	R
3	Tetep	*Indica*	*Pi1, Pita, Pi54*	R
4	HPR2880	*Indica*	*Pi54, Pita*	R
5	HPR2795	*Indica*	-	S
6	PR114	*Indica*	-	S
7	Liziangxintuanheigu (LTH)	*Japonica*	*Pik*^{l}	S

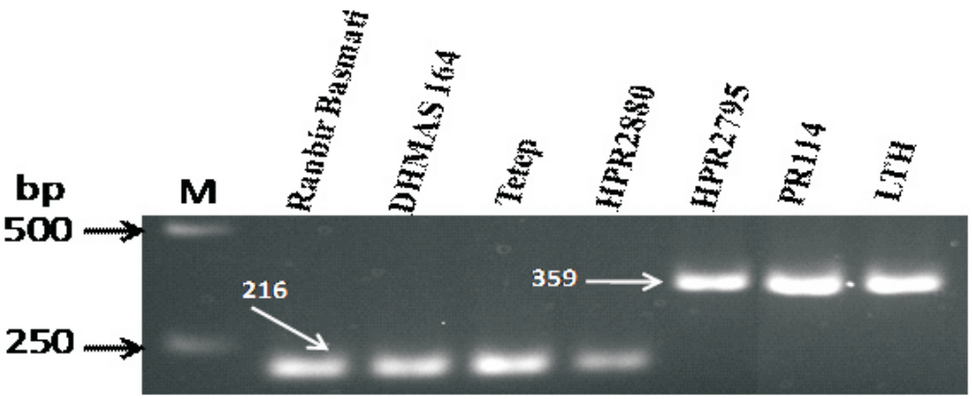

Fig 1. Amplification patterns of *Pi54MAS* Indel marker in resistant and susceptible rice genotypes. PCR products were resolved on 4% agarose and visualized by ethidium bromide staining. M= 1 Kb DNA ladder

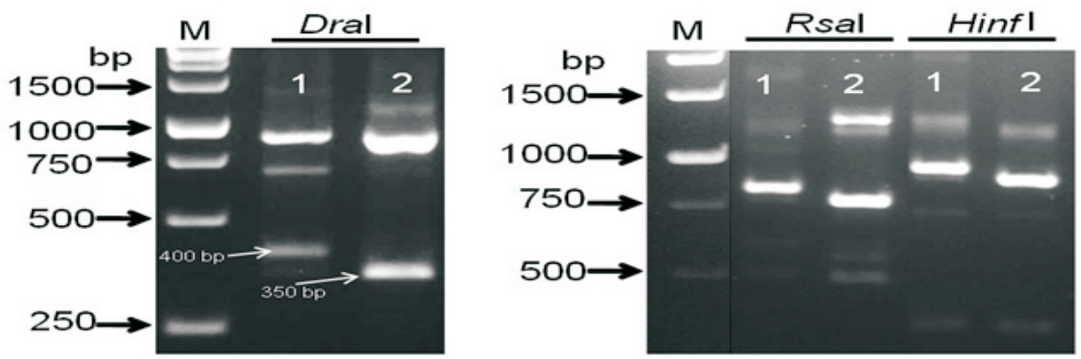

Fig 2. Electrophoretic patterns of restriction digested *Pi54STS-1* amplicons of parental genotypes with different restriction enzymes. 1= DHMAS164; 2= Ranbir Basmati. M= 1 Kb molecular weight ladder

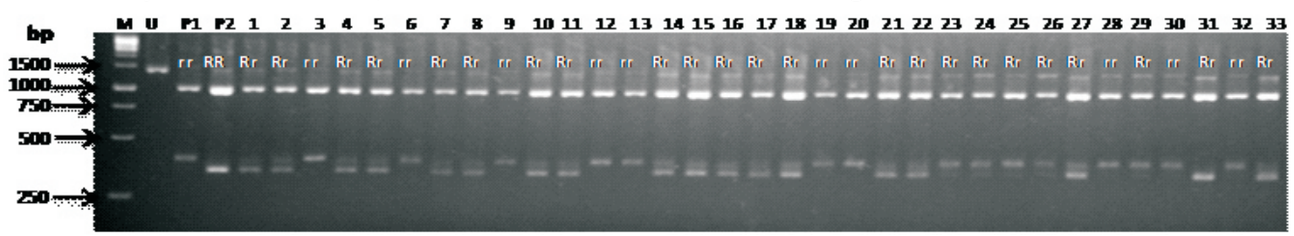

Fig 3. Genotyping of parental genotypes and BC_1F_1 progeny of cross Ranbir Bamsati x DHMAS164 for the validation of linkage of CAPS marker *Pi54STS-1*(*Dra*I) with blast resistance gene *Pi54*. The BC_1F_1 progeny was tested against a blast isolate Mo-nwi-114 that exhibits compatibility with Ranbir Basmati and incompatibility with DHMAS164. For each BC_1F_1 plant the zygosity at *Pi54* locus was inferred by testing the F_2 progeny for blast resistance. rr = susceptible; Rr = heterozygous resistant; RR= homozygous resistant. U= unrestricted amplicon of resistant parent

BC_1F_1 plants, thereby confirming the co-dominant inheritance and perfect linkage of the marker to *Pi54* gene (Fig 3). The CAPS marker *Pi54STS-1*(*Dra*I) and two other SNP based polymorphisms detected through restriction analysis with *Hinf* I and *Rsa*I can be exploited as reliable gene-based markers for efficient selection of *Pi54* gene in marker-assisted breeding programmes.

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References

Ellur RK, Khanna A, Yadav A, Pathania S, Rajashekara H, Singh VK, Krishnan SG, Bhowmick PK, Nagarajan M, Vinod KK and Prakash G. 2016. Improvement of Basmati rice varieties for resistance to blast and bacterial blight diseases using marker assisted backcross breeding. Plant Science **242**: 330-341.

Hua L, Wu J, Chen C, Wu W, He X, Lin F, Wang L, Ashikawa I, Matsumoto T, Wang L and Pan Q. 2012. The isolation of *Pi1*, an allele at the *Pik* locus which confers broad spectrum resistance to rice blast. Theoretical and Applied Genetics **125** (5): 1047-1055.

Jeon JS, Chen D, Yi GH, Wang GL and Ronald PC. 2003. Genetic and physical mapping of *Pi5(t)*, a locus associated with broad-spectrum resistance to rice blast. Molecular Genetics and Genomics **269** (2): 280-289.

Kalia S and Rathour R. 2019. Current status on mapping of genes for resistance to leaf and neck blast disease in rice. 3 Biotech **9**: 209.

Khanna A, Sharma V, Ellur RK, Shikari AB, Krishnan SG, Singh UD, Prakash G, Sharma TR, Rathour R, Variar M and Prashanthi SK. 2015. Development and evaluation of near-isogenic lines for major blast resistance gene(s) in Basmati rice. Theoretical and Applied Genetics **128** (7): 1243-1259.

Mackill DJ and Bonman JM. 1992. Inheritance of blast resistance in near-isogenic lines of rice. Phytopathology **82** (7): 746-749.

Murray MG and Thompson WF. 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Research **8** (19): 4321-4325.

Pradhan SK, Pandit E, Pawar S, Baksh SY, Mukherjee AK and Mohanty SP. 2019. Development of flash-flood tolerant and durable bacterial blight resistant versions of mega rice variety 'Swarna' through marker-assisted backcross breeding. Scientific Reports **9**: 12810.

Ramkumar G, Srinivasarao K, Mohan KM, Sudarshan I, Sivaranjani AK, Gopalakrishna K, Neeraja CN, Balachandran SM, Sundaram RM, Prasad MS and Rani NS. 2011. Development and validation of functional marker targeting an InDel in the major rice blast disease resistance gene *Pi54* (*Pik h*). Molecular breeding **27** (1):129-135.

Rathour R, Singh BM and Sharma TR. 2004. Population structure of *Magnaporthe grisea* from north western Himalayas and its implications for blast resistance breeding of rice. Journal of Phytopathology **152**: 304-312.

Sharma TR, Madhav MS, Singh BK, Shanker P, Jana TK, Dalal V, Pandit A, Singh A, Gaikwad, K, Upreti HC and Singh NK. 2005. High-resolution mapping, cloning and molecular characterization of the *Pi-k*^{h} gene of rice, which confers resistance to *Magnaporthe grisea*. Molecular Genetics and Genomics **274**: 569-578.

Sharma TR, Rai AK, Gupta SK, Vijayan J, Devanna BN and Ray S. 2012. Rice blast management through host-plant resistance: retrospect and prospects. Agricultural Research **1** (1): 37-52.

Wang X, Lee S, Wang J, Ma J, Bianco T and Jia Y. 2014. Current advances on genetic resistance to rice blast disease. In: *Rice - Germplasm, Genetics and Improvement,* eds Bao J and Yan W. IntechOpen, London, UK, pp 195-217.

Zhai C, Lin F, Dong Z, He X, Yuan B, Zeng X, Wang L and Pan Q. 2011. The isolation and characterization of *Pik*, a rice blast resistance gene which emerged after rice domestication. New Phytologist **189**: 321-334.

Zhou B, Qu S, Liu G, Dolan M, Sakai H, Lu G, Bellizzi M and Wang GL. 2006. The eight amino-acid differences within three leucine-rich repeats between *Pi2* and *Piz-t* resistance proteins determine the resistance specificity to *Magnaporthe grisea*. Molecular Plant-Microbe Interactions **19**: 1216-1228.