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Screening and validation of known DNA markers for BPH resistance in Germplasm of rice (*Oryza sativa* L.)

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Abstract
Rice productivity is adversely impacted by numerous biotic and abiotic factors. Diseases and insect pests are the major biotic agents causing significant yield losses. DNA marker based technology is being increasingly used to overcome difficulties of plant breeding based on phenotypic characters like insect resistance. The experimental material consisted of 24 elite rice genotypes was involved, where T- 12 and ARC 10550 were used as resistant check and Swarna used as susceptible check, respectively. Total rice genomic DNA was extracted from young succulent, disease and insect free seedlings by mini prep method. The DNA was extracted from 24 lines out of which 13 were resistant and 11 were susceptible.PIC value ranges from B (monomorphic) to A (very high discriminative with many alleles in equal frequencies). The average PIC value for all 10 microsatellite loci in the present study was 0.60, with a range from 0.329- 0.818. PIC was highest for the SSR primer RM5479 (0.818), and was lowest for the primer RM589, (0.329). Hence, primer RM 5479 is highly informative in the present study. The association between trait and markers were estimated using single marker analysis (SMA) in Microsoft Excel program. The significant marker trait associations were indicated by a P-value (< 0.05) along with corresponding R^2 for each marker is the total phenotypic variation for a trait that is accounted by markers. We detected a significant resistance marker-trait association (P<0.05). All of the significant SSR loci were indentified for the agronomic traits, with the R^2, percentage of the total variation explained ranging from 9.73% to 35.5%. In the analysis of susceptibility/resistance index marker RM6869 show the resistance, it shows significant (at 5%) P-value 0.041% and %R^2 is highest 35.5% that indicates the Marker RM6869 is associated with gene *Bph2* and *Bph18* genes present in BPH resistance germplasm.

Keywords: Validation, DNA, BPH, Germplasm, *Oryza sativa* L.

Introduction
Globally, more than 3 billion people from Asia and other countries depend on rice (*Oryza sativa* L.) as their staple food, and by 2025 about 60% more rice must be produced to meet the needs of the growing population (Khush, 1979) [5]. Rice productivity is adversely impacted by numerous biotic and abiotic factors. Diseases and insect pests are the major biotic agents causing significant yield losses.

Among the biotic stresses, the brown plant hopper (BPH) *Nilaparvata lugens* (Stal.) is one of the most destructive monophagous insect pest, one of the main bioticconstraint of rice productivity causing huge yield losses every year in rice grown throughout tropical, subtropical and temperate areas in Asia (Park *et al.*, 2008) [7]. The plant would suffer 40% to 70% yield loss if attacked by 100–200 first instar nymphs of BPH at 25 day after rice seedling transplanting (Bae and Pathak, 1970) [1].

The primary methods of control are chemical insecticides and host plant resistance (HPR) as part of an integrated pest management (IPM) strategy. Cultivation of resistant varieties is the better and environmentally safe alternative. Such varieties will also help in conservation of natural enemies, increasing their effectiveness and minimizing the pesticide applications (Panda and Khush, 1995) [6]. Hence, breeding programme for development of BPH resistant varieties with different mode of host plant resistance is extremely important. DNA marker based technology is being increasingly used to overcome difficulties of plant breeding based on phenotypic characters like insect resistance. It is most appropriate for inter-sub specific and intra-specific transfer of insect resistance that has been difficult to improve using conventional methods.

It also paves the way for selecting the target gene based on DNA marker with a predictable rate of accuracy. As such, this study has used these tools to make the study an exhaustive one and to provide the base material for the rice breeders for exploitation of landraces possessing one or more desirable characters. Keeping these points in view, the present study was carried out.

Material and Methods

Generation and maintenance of rice brown plant hopper breeding materials along with molecular work were done at the research farm and molecular biology laboratory of Department of Genetics and Plant Breeding. The studies extended over a period of one cropping wet seasons (*Kharif*) 2016.

The experimental material consisted of 24 elite rice genotypes are IC75889, IC75844, IC75767, IC217492, IC75964, IC76046, IC216609, IC75832, IC217509, IC216606, IC75845, IC75829, IC 540584, IC 216579, IC75795, IC75839, IC218650, IC75874, IC216612, T-12, IC216563, Swarna, ARC-10550 and IC218607, where T- 12 and ARC 10550 were used as resistant check and Swarna used as susceptible check, respectively.

Screening for BPH resistance

Initial population of BPH was collected from Maharajpet farm, Barwale Foundation and mass rearing of BPH and screening was done by following three methods, (i) standard seed box screening technique (SSST) developed at IRRI by Heinrichs *et al.* (1985) [3], (ii) Honeydew test and (iii) Nymphal survival method. Observations were recorded 7-10 days after releasing insects, when 90% of the plants in the susceptible check line Swarna were killed. The observations were recorded on the basis of 0-9 scale as per the International Standard Procedure (IRRI, 1996).

Molecular work was carried out in Plant Molecular Biology Laboratory, Department of Genetics and Plant Breeding, IGKV, Raipur (C.G.).

DNA Extraction

Total rice genomic DNA was extracted from young succulent, disease and insect free seedlings by mini prep method. The DNA was extracted from 24 lines out of which 13 were resistant and 11 were susceptible. The DNA was isolated from the leaves of 3 weeks old plants. Mini prep method was followed for the isolation of DNA.

PCR amplification using SSR primers

PCR analysis was done using a set of ten random SSR (simple sequence repeat) markers (Table 1) to identify the parental polymorphism between 24 elite lines.

Visualization of amplified products in Polyacrylamide gel electrophoresis

Five per cent polyacrylamide gels (vertical) were used for better separation and visualization of PCR amplified microsatellite products, since polyacrylamide gels have better resolution for amplified products.

Microsatellite marker analysis

A total of 24 rice genotypes were used in the SSR analysis. The primer sequences and chromosomal positions for primer pairs were downloaded from genome database, Rice Genome Microsatellite Markers (http:/www.gramene.org/db/markers.html). Ten primers were chosen randomly covering three the chromosomes or genomic regions. Four primers exhibited monomorphic fragments and were therefore excluded from further analysis.

Scoring and Data Analysis

All the genotypes were scored for the presence and absence of the SSR bands. Ethidium bromide staining of gels generally showed several bands. The size of the most intensively amplified band for each microsatellite marker was determined based on its electrophoretic mobility relative to molecular weight markers (increments of 50). Clearly resolved unambiguous bands were scored visually for their presence or absence with each primer. The score were obtained in the form of matrix with "1" and "0", which indicate the presence and absence of bands in each variety respectively.

Results and Discussion

Out of 24 rice germplasm lines and varieties, 13 germplasm lines *viz*., IC75889, IC75767, IC217492, IC216609, IC217509, IC216606, IC75829, IC216579, IC216612, T-12, IC216563, IC218607 and ARC 10550 showed the 70 average plant damage score of (0-5) 1.5, 3, 0.41, 0.71, 0.45, 1.36, 1.68, 0.63, 1.22, 0, 1, 1 and 0 respectively i.e. resistant.

Polymorphism Information Content (PIC)

PIC value ranges from B (monomorphic) to A (very high discriminative with many alleles in equal frequencies). The average PIC value for all 10 microsatellite loci in the present study was 0.60, with a range from 0.329- 0.818. PIC was highest for the SSR primer RM5479 (0.818), and was lowest for the primer RM589, (0.329). Hence, primer RM 5479 is highly informative in the present study. The above markers were found to be highly informative in revealing the genetic diversity among the varieties and will be useful in future genetic diversity analysis. The high PIC value obtained in the present investigation might be due to high genetic diversity among the rice germplasm lines and varieties. The PIC values are usually dependent on the genetic diversity of the accessions chosen (Garland *et. al.*, 1999) [2] for the specific study.

Table 1: List of SSR markers showing polymorphism among 24 rice germplasm lines

S. No.	Markers	Forward/ Reverse Primer Sequence	Chromosome No.	Product Size(bp)	PIC
1	RM119	CATCCCCCTGCTGCTGCTGCTG/ CGCCGGATGTGTGGGACTAGCG	4	166	0.594
2	RM261	CTACTTCTCCCCTTGTGTCG/ TGTACCATCGCCAAATCTCC	4	125	0.795
3	RM313	TGCTACAAGTGTTCTTCAGGAC/ GCTCACCTTTTGTGTTCCAC	12	111	0.708
4	RM463	TTCCCCTCCTTTTATGGTGC/ TGTTCTCCTCAGTCACTGCG	12	192	0.696
5	RM589	ATCATGGTCGGTGGCTTAAC/ CAGGTTCCAACCAGACACTG	6	186	0.329
6	RM5479	AACTCCTGATGCCTCCTAAG/ TCCATAGAAACAATTTGTGC	12	197	0.818
7	RM6217	CGCAGATGGAGATTCTTGAAGG/ ACAGCAGCAAGAGCAAGAAATCC	12	159	0.519
8	RM260	ACTCCACTATGACCCAGAG/ GAACAATCCCTTCTACGATCG	12	111	0.6782
9	RM3331	CCTCCTCCATGAGCTAATGC/ AGGAGGAGCGGATTTCTCTC	12	129	0.378
10	RM6869	GAGCTCCTTGTAGTGACCCG/ ATCAGCCTCGCCAGCTTC	12	126	0.52

Graphical genotyping of rice germplasm lines using SSR maker data

A graphical representation of molecular marker data can be an important tool in the process of selection and evaluation of plant material. A computer program was developed that enables representation of molecular marker data by simple chromosome drawings in several ways. Commonly used marker file types that contain marker information serve as input for this program, which was named "GGT". (An acronym of Graphical Genotypes) (GGT user manual 2007). Graphical outputs of genotyping data in this study were generated using GGT version 2.0 tool. The study showed genomic constitution analysis (marker allele contribution) of rice germplasm lines and varieties are based on chromosome-wise distribution of polymorphic SSR loci. The chromosome wise allelic contribution of marker alleles A and B carried out for 24 rice germplasm lines selected in the study. Marker alleles for each locus were marked in different colours and incorporated in ascending order of position of markers (in cM.) on the chromosomes. The analysis revealed that the two alleles more or less contributed equally in case of markers located on chromosome 6 rice germplasm lines whereas in case of chromosome 12 showed maximum contribution of allele A and minimum contribution of allele B.

Identification of DNA markers associated to BPH resistance in rice germplasm

The association between trait and markers were estimated using single marker analysis (SMA) in Microsoft Excel program. The significant marker trait associations were indicated by a P-value (< 0.05) along with corresponding R^2 for each marker is the total phenotypic variation for a trait that is accounted by markers. We detected a significant resistance marker-trait association (P<0.05). All of the significant SSR loci were indentified for the agronomic traits, with the R^2, percentage of the total variation explained ranging from 9.73% to 35.5%.

Table 2: Association between SSR markers and BPH resistance P<0.05.

Trait	Chromosome No.	Position	Marker	Gene	P-Value	%R ^{2}
Susceptibility/resistance index	12	105.5	RM6869	*Bph2, Bph18*	0.041*	35.5

*=Significant at 5% and **=Significant at 1%

Out of 10 markers 5 was polymorphic and 5 was monomorphic. Five polymorphic markers were RM261, RM589, RM3331, RM260 and RM6869 and remaining five monomorphic markers are RM119, RM313, RM5479, RM463 and RM6217. Marker RM261 and RM119 found in chromosome no.4, RM589 found in chromosome no.6 and marker RM6217, RM3331, RM3331, RM260, RM313, RM463, RM5479 and RM6869 found in chromosome no.12. The P-value of 0.041 for marker RM 6869 present on chromosome no.12 (at 105.5 cM) was found to be significantly associated with BPH resistance (p-value=0.041). This marker was reported to associated with BPH resistance gene *Bph2* and *Bph18* by Jena *et al.* (2006) [4]. Thus the association of the marker RM6869 found this study also and that indicates the presence of gene *Bph2* and *Bph18* on the chromosome no.12 in the genotyping of germplasm of rice. In the analysis of susceptibility/resistance index marker RM6869 show the resistance, it shows significant (at 5%) P-value 0.041% and %R ^{2} is highest 35.5% that indicates the Marker RM 6869 is associated with gene *Bph2* and *Bph18*genes present in BPH resistance germplasm.

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