Molecular screening of *Nilaparvata lugens* (Brown plant hopper) resistance genes in Hasanta rice variety using SSR markers

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Abstract

The menace of Brown Plant Hopper has been a problem mainly in the rice growing regions of India. Hasanta variety, reported to be resistant to *Nilaparvata lugens*, at the phenotypic level in the field has been chosen in this study to confirm the presence of resistance genes against BPH in it. This paper focuses on the molecular validation part of the experiment. Six flanking SSR markers were selected for one major resistant gene (*BPH31*) and three QTLs (*qBPH4.3, qBPH4.4* and *qBPH9*). The outcomes were compared with a resistant check; CRCPT 2 and a susceptible check; TN1. The banding pattern of gel electrophoresis studies for the genomic DNA has confirmed the presence of one major resistance gene *BPH31* and minor QTLs, *qBPH4.3, qBPH4.4* and *BPH9*. It was validated that the resistance displayed by Hasanta variety is due to the presence of these genes and further expression studies can be carried out to study their functional proteins.

Keywords: Hasanta, BPH31, *Nilaparvata lugens*, qBPH4.3, qBPH4.4, qBPH9

Introduction

Rice (*Oryza sativa* L.) is one of the oldest domesticated food crops in the world and provides two-third of calorie intake of more than 3 billion people in Asia and one-third of calorie intake of nearly 1.5 billion people in Africa and Latin America [1]. In India, the area under rice cultivation is 43.79 million hectares with an annual production of 112.91 million metric tonnes and an average yield of 3.87 metric tonnes per hectare (USDA, 207-18). It is cultivated globally under various agro-climatic conditions and hence faces major abiotic and biotic stresses due to adverse climatic changes, susceptible varieties and breakdown of resistance genes in elite cultivars.

About 52% of the global production of rice is lost annually by biotic factors, out of which 21% damage is due to insect pests [2]. As the crop is genetically very diverse it is attacked by more than 100 number of pests, 20 are considered as major ones [3]. Particularly, *Nilaparvata lugens* Stål. (Brown plant hopper), a typical phloem sap feeder, an erstwhile minor pest, has grown to be a major pest widely distributed in South, South East and East Asia, the South Pacific Island and Australia. It causes yield loss up to 60%. Both nymphs and adults suck sap from the leaves and leaf sheaths, which results in yellowing of leaves, reduced tillering, reduced plant height and increase in number of unfilled grains. Brown Plant Hopper (BPH) also causes the reduction in chlorophyll, protein content of leaves and photosynthetic rate, whereas severe attack of BPH causes ‘hopper burn’ symptoms [4-6]. It also transmits virus diseases like grassy stunt, ragged stunt and wilted stunt [7]. The losses to rice production caused by BPH in Asia have been estimated as more than US$300 million annually [8]. The control of this pest has predominantly relied on chemicals, over which it has already acquired resistance. Field population collected in 2016 in China reflected extremely high resistance to imidacloprid, thiamethoxam and buprofezin [9]. Four different biotypes of the insect have been identified in this regard that has developed virulence e.g., IR26 possessing *Bhp1* resistant gene became susceptible in 1976-77 due to biotype 2. The resistance conferred by *bph2* gene was considered durable as BPH resistance of IR36 lasted for 14 years until 1991 [10]. Several major genes and QTLs conferring resistance to BPH have been located on various chromosomes of rice [11, 12]. Varieties with various levels of resistance are deployed for insect control in combination with other components of pest management.
It also helps in conservation of natural enemies and minimizing the number of pesticide applications. So availability of BPH resistant rice varieties with desired agronomic traits should be ensured to the farmers for reducing the BPH menace in rice. One such promising variety, Hasanta (OR2328-5), released from OUAT, Odisha, India, has shown potential against BPH attack. During kharif 2017, there was a large scale BPH infestation in Odisha and caused major losses. Under field condition, while all the other varieties were completely damaged, Hasanta withstood the infestation with minimal crop loss. Observing the field level tolerance of Hasanta to BPH, it was thought imperative to take up the validation and confirmation studies about the presence of BPH resistance genes at the molecular level.

Materials and Methods
An experiment was carried out in OUAT, Bhubaneswar to study and confirm the presence of BPH resistance genes. Three varieties viz., TN1 (15120 days duration) as susceptible check, CRCPT 2 (135-140 days duration) as resistant check and Hasanta (143145 days duration) as test variety were taken. All the varieties were collected from National Rice Research Institute, Cuttack, Odisha and OUAT Research Farm for various experiments.

Genomic DNA extraction and quantification
The three rice genotypes were collected and germinated in trays under controlled condition of RGA-cum-Phytootron facility. Big trays were made ready for putting sterilized soil mixture and placing seeds for germination. Seeds were placed in rows for germination. The seedlings were harvested (15 days) for Deoxyribonucleic Acid (DNA) extraction. Healthy leaf sample of 2-4 gms was collected from 15 days old plant in microfuge tubes and stored in ice. The leaf tissue were cut into small pieces and crushed by liquid nitrogen to make fine powder form. 300µl CTAB buffer was added to the sample and tissue was homogenized. The sample was transferred to 2ml tubes and volume as adjusted to 600µl using CTAB mixture and placing seeds for germination. Seeds were placed in the tray under controlled condition of RGA.

Validation of Hasanta variety through SSR markers
DNA templates from all the three varieties were amplified using a set of 6 flanking Simple Sequence Repeat (SSR) markers mentioned in Table 1. The PCR cycle regime was carried out with following specifications for 35 cycles: Denaturation at 94 °C for 1 minute, Annealing at 55 °C for 1 minute, extension at 72 °C for 1 minute and Final extension at 72 °C for 10 minutes. For quantification of genomic DNA 2µl sample along with 2µl of loading dye was run in 0.8% agarose gel containing 0.8µg/ml Ethidium Bromide for electrophoresis in 1X TBE (pH 8.0). At least one lane was loaded with 50bp DNA ladder. The gel was run at 25V/cm for 4hrs and photographed using a Gel Documentation System (Syngene).

Results and Discussion
The molecular validation was taken up to confirm the presence of resistant genes in the variety. One resistant gene (BPH31) and three QTLs (qBPH4.3, qBPH4.4 and qBPH9) conferring resistance to the Indian biotypes of the insect are targeted in this validation work. For qBPH4.3 resistance gene, SSR markers RM551 and RM335 were deployed to know the presence of the gene in the variety. DNA banding pattern obtained using these two markers are shown in Fig 1 and Fig 2. The banding analysis using these two primers revealed the expected resistant band in Hasanta variety and the resistant check. Thus the variety is positive for qBPH4.3. The analysis for qBPH4.4 and qBPH9 also showed the presence expected resistance band size by amplifying the markers R5633 and RM242, respectively. Hence, the variety also shows the presence of qBPH4.4 or qBPH9 also. Genotyping for major gene BPH31 was performed using markers RM251 and RM335. Banding results confirmed the presence of BPH31. Thus the variety Hasanta was found to contain all the three genes/QTLs tested for their presence.

Conclusion
Genotyping results revealed the presence of one major resistance gene BPH31 and minor QTLs, qBPH4.3, qBPH4.4 and qBPH9. Apart from the genes (that has been used in this study) it is also possible that many other gene loci for BPH resistance might be present in the candidate variety. Earlier mapping results of [14] at ICAR-NRRI is validated in this experiment for presence of major resistance gene BPH31. Also two QTLs (qBPH4.3 and qBPH4.4) reported by [15], also qBPH9 are validated in this experiment to be present in Hasanta. Therefore with a major gene and QTLs in its background Hasanta variety can be expected to tolerate well the biotic stress posed by BPH with minimal economical damage to the crop. Further the crop variety can be subjected to expression studies using the mRNA and protein product to confirm the expression of the already present genes.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Chromosome And QTL</th>
<th>Expected Product size (bp)</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Repeat motif</th>
</tr>
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<tr>
<td>RM251</td>
<td>3, Bph31</td>
<td>147</td>
<td>GAATGCCAATGGGCTAG</td>
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<tr>
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<tr>
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<td>115</td>
<td>GTACACACCCACATCGAGA</td>
<td>GCTCTATTGCGATATCCATG</td>
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<tr>
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<td>RM242</td>
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<td>225</td>
<td>GGGCAACGTGTGATGTC</td>
<td>TATATGCGAAGCGGTGGG</td>
<td>(CT)26</td>
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</tbody>
</table>
Fig 1: Electrophoregram in 3 rice genotypes using SSR marker RM242, RM335, RM2334, RM251, RM551 and RM5663. In each Lane1: Molecular ladder (50bp); Lane2: Resistance check; Lane3: TN1 & Lane4: Hasanta

Fig 2: Electrophoregram in 3 rice genotypes using SSR marker RM242, RM335, RM2334, RM251 & RM551. In each Lane1: Molecular ladder (50bp); Lane2: Hasanta; Lane3: Resistant check & Lane4: TN1

References