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Phenotypic screening and molecular analysis of blast resistance in fragrant rice for marker assisted selection

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ABSTRACT

Experiments were conducted to identify blast-resistant fragrant genotypes for the development of a durable blast-resistant rice variety during years 2012–2013. The results indicate that out of 140 test materials including 114 fragrant germplasms, 25 differential varieties (DVs) harbouring 23 blast-resistant genes, only 16 fragrant rice germplasms showed comparatively better performance against a virulent isolate of blast disease. The reaction pattern of single-spore isolate of *Magnaporthe oryzae* to differential varieties showed that *Pish*, *Pi9*, *Pita-2* and *Pita* are the effective blast-resistant genes against the tested blast isolates in Bangladesh. The DNA markers profiles of selected 16 rice germplasms indicated that genotype Chinigura contained *Pish*, *Pi9* and *Pita* genes; on the other hand, both BRRI dhan50 and Bawaibhog contained *Pish* and *Pita* genes in their genetic background. Genotypes Jirakatari, BR5, and Gopalbhog possessed *Pish* gene, while Uknimodhu, Deshikatari, Radhunipagol, Kalijira (3), Chinikanai each contained the *Pita* gene only. There are some materials that did not contain any target gene(s) in their genetic background, but proved resistant in pathogenicity tests. This information provided valuable genetic information for breeders to develop durable blast-resistant fragrant or aromatic rice varieties in Bangladesh.

1. Introduction

Rice (*Oryza sativa* L.) confronts several major and minor diseases, among which blast caused by *Magnaporthe oryzae* [1] is a serious constraint causing moderate to huge economic losses worldwide [2–4]. Outbreaks of this disease are a serious and recurrent problem, and are extremely difficult to control in all rice-growing regions of the world, including Bangladesh [5,6]. Bangladesh experienced several epidemic outbreaks of blast disease since 1980 [7], presumably because of the popularity of certain susceptible cultivars and/or generation of new pathogenic races. Most of the fragrant varieties are highly susceptible to this disease under favourable environment.

There are some chemicals available on the market to control this disease, but they are costly for resource-poor farmers, and sometimes effective chemical control is difficult in case of outbreaks, especially during neck blast.

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In addition, reduction of chemical application is also desirable for environmental protection in such a heavily farmed country as Bangladesh [8]. Cultivars resistance to blast is therefore the most eco-friendly and economic approach for managing blast diseases in rice. However, cultivars released as resistant often show high levels of susceptibility within a few years, even shortly after cultivar release, due to inadequate information of genotypes used as resistant sources and also due to a lack of information about the pathogen population structure. So, knowledge on population genetics of rice genotypes is important for breeding for blast disease resistance. Though cultivation of resistant varieties is the most promising method to control blast, the breeding-for-resistance programme is still at an early developmental stage in Bangladesh [9].

Fortunately, the Bangladesh Rice Research Institute (BRRI) has already collected more than 8000 rice germplasms. We believe that there are some resistant aromatic or fragrant sources in those materials that may contain blast-resistant gene(s) suitable for developing durable blast-resistant fragrant rice varieties. Recently, in collaboration with the Japan International Research Centre for Agricultural Sciences (JIRCAS), BRRI has developed good research facilities and started an international collaborative study under the "Blast Research Network for Stable Rice Production" project. In connection with this work, the present studies were undertaken with two major objectives: (1) to screen out the aromatic germplasms that are resistant against blast isolate and (2) to confirm the major blast-resistant genes in selected aromatic germplasms using tightly linked molecular markers for the development of durable blast-resistant rice varieties.

2. Materials and methods

2.1. Collection, isolation, purification, and preservation of blast isolate

Rice panicles of high yielding fragrant variety (BRRI dhan34) showing typical blast symptoms were collected from the rain-fed lowland ecosystem of Bangladesh. To obtain a single conidial isolate, an individual conidium was identified with a compound microscope (Olympus BX41) and aseptically transferred into a water agar medium. Finally, the fungus was grown on sterile filter paper and stored at −20 °C after aseptic drying, necessary for repeated access to original isolates [10,11].

2.2. Inoculum preparation and inoculation

To produce inoculum, the stock isolate (paper disc) of single-spore culture was re-cultured in an oatmeal agar medium. The inoculated plates were incubated at temperatures from 25 to 28 °C for 12 to 14 days. The culture was scraped with a sterilized tooth brush and the plates were exposed to continuous light for 4–5 days to induce heavy sporulation. Conidia were dislodged by gentle rubbing with a paintbrush from the incubated plates to sterilized distilled water with 0.01% Tween 20. Spore suspensions were filtered through four layers of gauze mesh and concentration was adjusted to 10^5 conidia per millilitre using a haemocytometer.

Inoculation of 140 test materials, including 114 fragrant germplasms, 25 differential varieties (DVs) harbouring 23 blast-resistant genes [12–14] and LTH (universal susceptible check) was carried out following the standard methods [10,11]. The seeds of DVs were collected from JIRCAS, Tsukuba, Japan. Inoculated seedlings were incubated in a dew chamber at 25 °C for 20 h, then transferred into a greenhouse maintained at 25 ± 1 °C with 70 to 80% of relative humidity.

2.3. Disease assessment and data collection

Disease reactions of the inoculated plants were evaluated seven days after inoculation using a 0 to 5 scale, where 0 = no evidence of infection, 1 = brown specks smaller than 0.5 mm in diameter, no sporulation, 2 = brown specks about 0.5 to 1 mm in diameter, no sporulation, 3 = roundish to elliptical lesions about 1 to 3 mm in diameter with a grey centre surrounded by brown margins, lesions capable of sporulation, 4 = typical spindle-shaped blast lesions capable of sporulation, 3 mm or longer with necrotic grey centres and water-soaked or reddish brown margins, little or no coalescence of lesions, and 5 = lesions as in 4 but about half of one or two leaf blades killed by coalescence of lesions. The reactions of plant were further categorized as 0 to 2 as resistant (R), whereas 3 to 5 as susceptible (S) in most cases. The scores 2 to 5 were only categorized as susceptible for the differential variety of IRBL5-M and 4 to 5 for IRBLsh-B and IRBLta2-Pi [11,15]. The disease scoring data were then converted into the percent disease index (PDI) by using the following formula [16]:

$$\text{Percent disease index (PDI)} = \frac{\text{Sum of the scores}}{\text{Number of observation} \times \text{highest number in rating scale}} \times 100$$

Cluster analysis was done using Ward's hierarchical method using JMP 7.0.2 (JMP Statistics and Graphic Guide, Version 7.0.2: SAS Institute, Inc., Cary, NC, USA) based on a PDI of 140 tested materials. Canonical variant analysis was done using GenStat 5.3 software.

2.4. Confirmation of target genes in selected germplasms by molecular assay

2.4.1. Plant materials

Sixteen selected germplasms, four differential varieties (IRBLsh-B, IRBLta, IRBL9-W and IRBLta-2) that showed low PDI with blast isolate and one susceptible check (LTH) were used for the PCR assays. The plants were grown in a seedling tray for DNA isolation.

2.4.2. DNA extraction and PCR assay

DNA was isolated from leaves collected from 30-day-old seedlings as per the method of Murray and Thomson [17], with modifications. Polymerase chain reaction (PCR) was performed using gene-specific primers (Table 1). The