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Differential expression of proteins in resistant and susceptible rice genotypes against blast infection

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ABSTRACT

Blast is one of the most important diseases affecting rice cultivation worldwide. The current study employed Two-Dimensional Difference Gel Electrophoresis (2D-DIGE) plus Matrix-assisted laser desorption/ionization time-of-flight-mass spectroscopy (MALDI-TOF MS) and TOF/TOF tandem MS/MS to investigate the differentially regulated proteins in the highly resistant (HR) genotype, FL 127 and highly susceptible (HS) genotype, Rustic against blast pathogen in rice. The differential expression of seventy eight protein spots were observed in 2D-gel analysis of HR and HS genotypes. The comparision of protein expression ratio between HR and HS genotypes showed that twenty protein spots were predominant in differential expression against blast pathogen. Of which, seventeen were upregulated and three were down regulated. Characterization of these proteins using MALDI-TOF (MS) and TOF/TOF tandem MS/MS analysis with protein databases revealed that most of their functions either directly or indirectly related to plant defense and stress response, transcription and photosynthesis. The knowledge on various proteins characterized in this study suggested their possible role in expressing resistance by gentotype FL 127 against *P. oryzae*. Further, the knowledge generated in this study would be useful in the development of molecular markers for screening blast resistance using molecular biology approaches.

1. Introduction

The plants have greater capacity to offer resistance against biotic and abiotic stresses through the changes in biochemical reactions. Such changes at molecular levels comprehensively understood by the use of advanced molecular techniques. In recent years, proteomic based approaches have been employed to investigate the changes in expression levels of proteins in plants exposed to various biotic and abiotic stresses [1–3]. Among these, a good number of researches have been conducted to study the differential expression of proteins in plants against biotic stresses caused by fungal [4–7], bacterial [8–10] and viral [3,11–13] pathogens. Apart from the study on general expression of protein levels in plants upon pathogen infection, nowadays the proteomic based approaches have gained more attention among the researchers, to compare the resistant and susceptible cultivars against pathogen infection [3,13]. This will allow for better understanding of mechanisms associated with resistance in resistant cultivars over susceptible.

In case of rice cultivation in Guyana, the occurrence of blast disease caused by *Pyricularia oryzae* has resulted in severe yield loss every year. The genetic nature and unique feature of adoption to weather conditions have made the pathogen difficult to control. The experiments conducted at multiple locations from 2015 to 2017 have resulted in identification of FL-127 and Rustic as highly resistant (HR) and highly susceptible (HS) to blast disease respectively [14]. Several studies have been reported so far on the reaction of rice genotypes against blast infection. Few studies further demonstrated the differential expression of proteins in resistant genotypes against blast infection under inoculated and uninoculated conditions [5–7]. Kim et al. [5] studied the pathogen responsive proteins in rice leaves inoculated with blast pathogen using proteomic approach. Similar approach was employed to study the resistance offered by *Pi5* gene in rice plants against blast infection [6]. However, there is no study directly targeted the comparison of differential expression of proteins between resistant and susceptible genotypes against blast infection under inoculated and uninoculated conditions. Thus, the current research is carried out (i) to study the differential expression of proteins in HR and HS genotypes challenged with and without inoculation of *P. oryzae* and (ii) to understand the possible disease resistant mechanisms in HR, FL 127 genotype compared to susceptible genotype, Rustic.

The complete genome sequencing and characterization of proteins make the rice as ideal crop to conduct various molecular and genetic level studies [15]. When the susceptible and resistant plants are

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challenged by artificial inoculation of various pathogens, it reacted differently by triggering an array of defense responses [16–18]. These host defense responses often triggered by an activation of host resistance upon infection. Thus, the understanding of differential response of the host to infection provide a great scope for the proteomic analysis. Of several techniques employed in proteomics, the use of 2D-DIGE (Two-Dimensional Difference Gel Electrophoresis) clubbed with Matrix-assisted laser desorption/ionization time-of-flight-mass spectroscopy (MALDI-TOF MS) and TOF/TOF tandem MS/MS have been demonstrated for its high efficacy in quantification and characterization of proteins. The use of 2D-DIGE facilitates the direct labeling of extracted proteins with fluorescent dyes prior to isoelectric focusing (IEF) [19]. Further, 2D-DIGE allows the separation of more than two samples labelled with different dyes in the same gel. This also avoids the variability from gel to gel making the quantification and matching of spots easier and simpler compared to 2D-GEL (Two Dimensional Gel Electrophoresis) run with silver staining methods [20]. Therefore, 2D-DIGE plus MALDI-TOF (MS) and TOF/TOF tandem MS/MS was adopted in this study to identify the differentially expressed proteins in HR and HS genotypes associated with *P. oryzae*.

2. Material and methods

2.1. Selection of resistant and susceptible genotypes

The genotypes FL-127 and Rustic identified as highly resistant (HR) and highly susceptible (HS) (Fig. 1) through blast resistant screening experiments from 2015 to 2017 [14] were used in this study.

2.2. Isolation and preparation of inoculum of P. oryzae

The leaves expressing typical symptoms of blast disease were collected from the disease nursery of GRDB, RRS, Burma for isolation of the pathogen.

The isolation was done as described by Ghazanfar et al. [21], where infected leaves were cut into small pieces (0.5–1.0 cm) and surface sterilized with 2% sodium hypochlorite for 2 min. The cut leaf pieces were washed three times with sterilized water and plated on Petri plates containing PDA. These PDA plates were incubated at 26 ± 2 °C for 5 days. The pathogen was identified by studying the colony characteristics of the isolates on PDA plates by following the method described in a technical bulletin on seed borne disease and seed health testing of rice [22]. The agar slants and petri plates of pure culture of the virulent isolate were prepared and stored at 4 °C for further use. The virulent isolate was mass multiplied in PDA prepared plates in the Plant Pathology laboratory at RRS, Burma. The conidia was harvested from 9 days old culture of *P. oryzae* plates by adding 5 ml water and 0.05% Tween-20. After adding the water and tween the plates were agitated with the help of a sterile scalpel by gently scraping the fungal spores. The suspension was then filtered through two layers of cotton cloth. Then distilled water was added to the suspension and conidial suspension was adjusted to 1×10^6 conidia per ml with the help of a hemocytometer.

2.3. Inoculation with P. oryzae

The seeds of genotypes FL-127 and Rustic sowed separately in pots (45 cm × 30 cm) filled with 4 kg of field soil, which classified as Litchfield clay- (Description: humic gley, very poorly drained, strongly acidic surface soil to neutral, thick very dark grey surface soil, Low in P, Ca and K). Each genotype was sowed in six pots. The plants were grown under semi-controlled screen house conditions with an average day temperature of 25 to 28 °C and night temperature of 18 to 22 °C; average humidity of 90–95%; long period of free moisture or extension

Fig. 1. Highly resistant (FL 127) and highly susceptible (rustic) rice genotypes against blast disease under field conditions.