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Biochemical responses in transgenic rice plants expressing a defence gene deployed against the sheath blight pathogen, *Rhizoctonia solani*

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Diverse defence responses were studied in transgenic Pusa Basmati1 (PB1) rice lines engineered with rice chitinase gene (*chi11*) for resistance against the sheath blight pathogen, *Rhizoctonia solani*. Enhancement of phenylalanine ammonia lyase, peroxidase, and polyphenoloxidase enzyme activities in response to the pathogen challenge under controlled conditions resulted in reduced symptom development and containment of disease in transgenic rice lines compared to non-transgenic control plants. Loss of chlorophyll resulting from *R. solani* infection was comparatively less in transgenic plants. Our results provide new information on the biochemical basis of chitinase-based fungal resistance in transgenic plants.

Keywords: Defence gene, resistance, *Rhizoctonia solani*, transgenic rice.

PLANTS live in a milieu of potential pathogens under natural conditions. Though plants have no antibody-mediated resistance mechanisms, they defend themselves against pathogens with an arsenal of defence mechanisms. These include both passive and induced defence responses, wherein the latter plays a vital role in the active defence mechanism and it requires the host metabolism to function[1,2]. Further, these factors appear to play an important role in transgenic plants engineered with defence genes. This study reports the role of the introduced chitinase gene in triggering the defence pathway in response to sheath blight pathogen.

Rice (*Oryza sativa* L.) is an important food crop, providing a major source of sustenance to over half of the world's population. Rice production is severely affected by several pests and diseases. Among the several devastating diseases, rice sheath blight (ShB) caused by *Rhizoctonia solani* Kühn, is a major limiting factor hampering the rice production[3]. Through genetic engineering approaches it is now possible to engineer ShB resistance in cultivated rice by introducing genes encoding chitinases[4] and thaumatin-like proteins[5,6]. These pathogenesis-related

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(PR) proteins are often associated with the plant's defence against pathogens[7]. Chitinase releases water-soluble chitin fragments from the cell walls of invading fungal pathogens[8]. This in turn may act as an elicitor in triggering a *de novo* synthesis of enzymes of shikimate and phenyl propanoid pathways leading to the synthesis of phenols and phytoalexin[9–11].

Disease-resistant transgenic crops are generally analysed for the expression of the introduced gene. The complete role played by these foreign genes in the host plant is often not studied. In the case of transgenic plants expressing defence-related resistance genes, a greater understanding of the defence pathways triggered in response to pathogen attack will be more useful.

Recently, we reported the development of transgenic rice lines with rice chitinase gene[12]. These plants exhibited ShB resistance. In this context, it is imperative to study the active defence components triggered by the transgene *chi11* in response to the ShB pathogen *R. solani*. Hence the objective of the present study was to analyse the response of the major defence components, viz. phenylalanine ammonia lyase (PAL), peroxidase (PO) and polyphenoloxidase (PPO) in the transgenic rice lines when challenge-inoculated with *R. solani*.

Three transgenic homozygous lines (KK-PB-1, KK-PB-3 and KK-PB-5) of Pusa Basmati1 (PB1), an indica rice cultivar expressing chitinase[12] were used in the present study. The transgenic and non-transgenic control PB1 plants were maintained in the transgenic greenhouse. *R. solani* isolate[13] *Rs7* maintained on potato dextrose agar was used throughout the present study. Forty-five days after transplanting, both transgenic and non-transgenic control plants were challenge-inoculated. After the lapse of each incubation period (24, 48, 72, 96, 120, 144 and 168 h after inoculation; HAI) leaf sheath samples were collected and used for biochemical analyses.

Figure 1. Sheath blight development in transgenic Pusa Basamati 1 rice lines 168 HAI of *Rhizoctonia solani*.

The leaf sheath tissue (1 g fr wt) of healthy and infected transgenic and non-transgenic rice plants was collected and protein content estimated following Lowry *et al.*[14]. Assays of PAL[15], PO[16] and PPO[17] were performed as reported. The data were statistically analysed and treatment means were compared by Duncan's multiple range test[18]. The package used for analysis was IRRISTAT version 92, developed by the Biometrics Unit, International Rice Research Institute, Philippines.

The disease progression occurred at a slower pace in the transgenic plants compared to the non-transgenic counterparts. This was visualized from the observations recorded at 24, 48, 72, 96, 120, 144 and 168 h after pathogen challenge (data not shown). In the case of non-transgenic plants, lesions were found throughout the leaf sheath followed by yellowing and drying up of the lamina. Transgenic plants exhibited slower progression, featured localized lesions and browning of tissue arrested further spread of the disease (Figure 1). Earlier reports on *chi11* over-expression have explained its plausible role in enhanced resistance on the basis of increased browning around lesions and reduction in the size of lesions[12,19].

Transgenic and non-transgenic rice lines challenge-inoculated with *R. solani* showed increased defence related enzyme activity. Elevation in PAL activity is always favourable, as it is associated with the production of fungi toxic phenolics and some phytoalexins[20]. The transgenic rice lines showed higher PAL activity in response to *R. solani* infection. PAL is the key enzyme that controls the interface between shikimate pathway and the secondary phenyl propanoid pathway. PAL activity was measured in healthy and infected transgenic and non-transgenic rice plants. In control plants, four-fold increase in PAL activity was observed in infected leaf sheaths 120 h following inoculation peaked at 120 hpi (Figure 2). PAL activity was 50 to 80% higher in transgenic plants infected with *R. solani* compared to control inoculated plants (Figure 2).

Plant peroxidases are involved in a broad range of physiological processes all along the plant life cycle. Peroxidases can generate reactive oxygen species, polymerize cell wall compounds, and regulate H_2O_2 levels[21]. In control plants, a three-fold increase in PO activity was observed 120 hpi. Transgenic plants exhibited 80 to 100% higher PO activity than the non-transgenic counterparts (Figure 3). In the case of PPO, control plants showed a three-fold activity 120 h after *R. solani* infection. Increase in PPO activity was three-fold higher in transgenic plants compared to control (Figure 4).

Based on the present study an overview of various defence components being triggered in the transgenic rice plants in response to the pathogen challenge is depicted schematically (Figure 5). In conclusion, biochemical analyses of various disease-responsive components have thrown

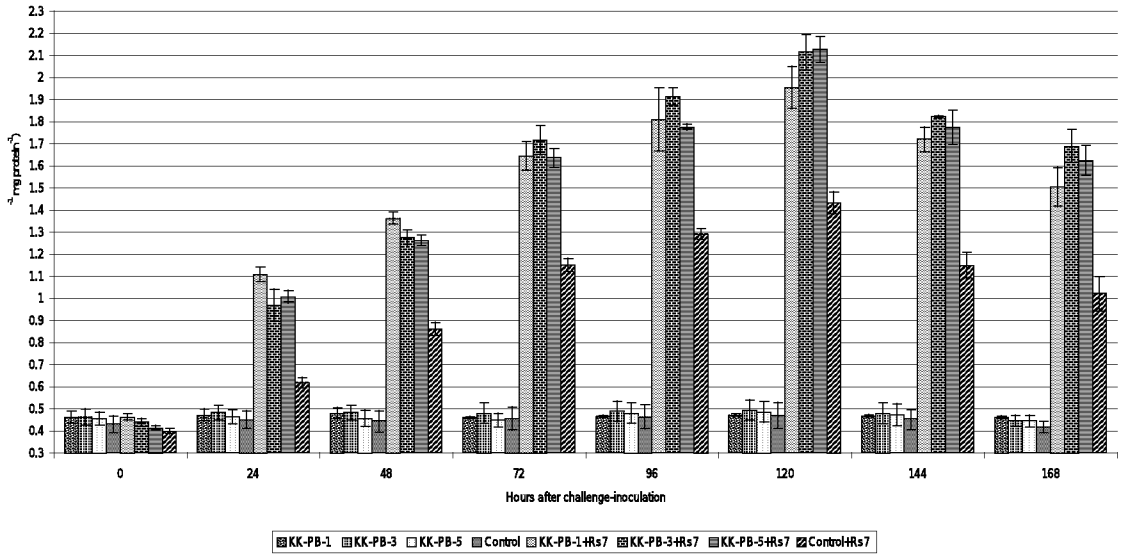

Figure 2. Induction of PAL activity in transgenic plants challenge-inoculated with *R. solani*.

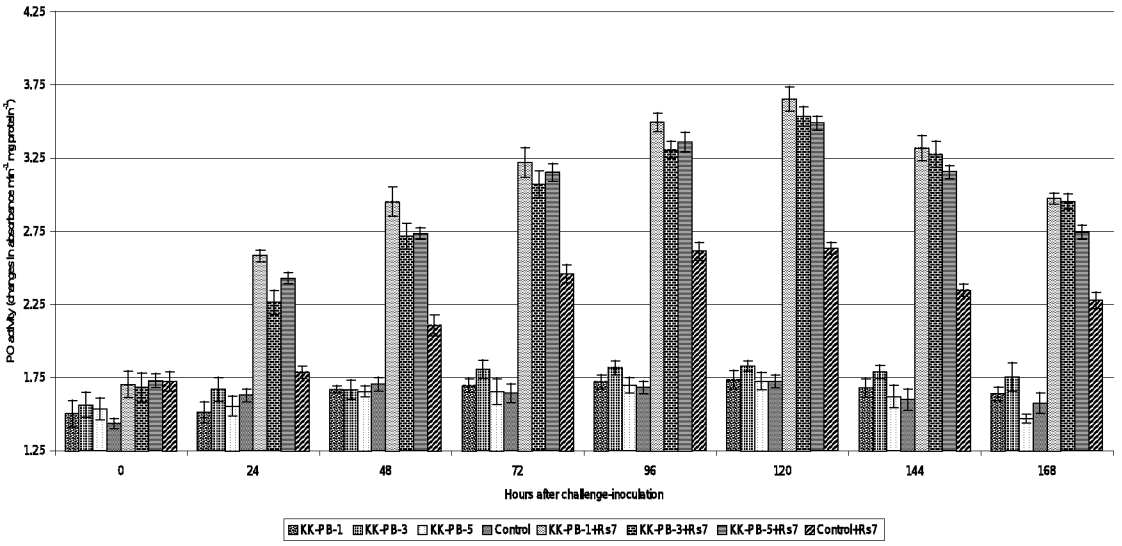

Figure 3. Induction of PO activity in transgenic plants challenge-inoculated with *R. solani*.

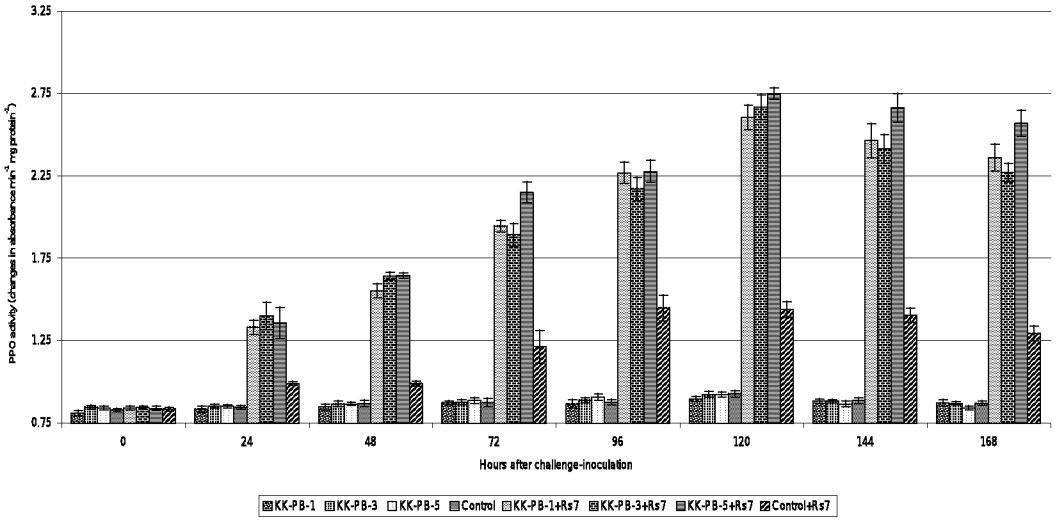

Figure 4. Induction of PPO activity in transgenic plants challenge-inoculated with *R. solani*.

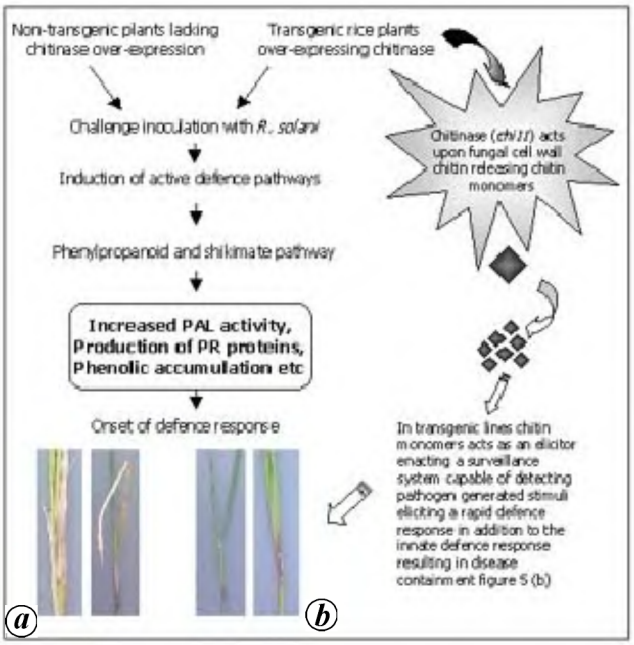

Figure 5. Schematic representation of various defence components induced in response to pathogen challenge in transgenic and non-transgenic plants. Drying of control PB1 leaf and leaf sheath in response to *R. solani* inoculation (***a***), while such drying was absent in transgenic lines expressing chitinase containing the disease spread (***b***).

more light on the pivotal role of defence gene over-expression. Transgenic plants are found to exhibit superior defence response tactics compared with the non-transgenic counterparts. The existing void in our knowledge on the active defence response of transgenic plants could be made up through this study. Further, focus should be directed towards individual components of the defence pathway for a broad spectrum resistance. Thus, the present study promises a new beginning in the efficient confrontation of plant diseases by the introduction of defence genes.

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