



BIOCHEMICAL ANALYSIS OF KALANAMAK, TETEP AND ITS CROSSES AFTER *P. ORYZAE* INFECTION

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Abstract

Rice blast is not only one of the earliest known plant diseases but also one of the most widely distributed ones occurring in every region of the world where rice is grown. Kalanamak rice is susceptible to blast disease due to which its yield reduces significantly depending upon the infestation. To develop the blast resistant plant crosses were made in Kalanamak with blast resistant variety *i.e.* Tetep and biochemical analysis of their crosses (F_2 population : randomly five crosses were selected) as well as parents were done to know the biochemical process underlying the expression of resistance to *P. oryzae*. All the inoculated plants were found to had increased changes in the activities of peroxidase, catalase. 2-3 fold increase in activity of catalase, peroxidase found in *P. oryzae* inoculated plants of Tetep as well as in F_2 population of cross between Kalanamak and Tetep (KT) *i.e.* KT_5 , KT_1 , and KT_2 . Maximum enzymes activities were recorded in between 20 to 48 hrs. After infection with *P. oryzae* new protein bands appeared ranging from ~35 kDa to ~85 kDa in all inoculated parents and crosses. The greater enzymes activity and more protein induction in KT_5 , KT_1 , KT_2 may be manifestation of resistance transferred against *P. oryzae* in Kalanamak from Tetep.

Key words : *P. oryzae*, rice, catalase, peroxidase.

Abbreviations : CAT : Catalase, H_2O_2 : Hydrogen peroxide, PO : Peroxidase, ROS : Reactive oxygen species, KT : F_2 population of cross between Kalanamak & Tetep

Introduction

Blast disease is caused by *Magnaporthe grisea* (Hebert) Barr., anamorph *Pyricularia oryzae* (Rossmann *et al.*, 1990) is an important fungal disease of rice known to occur in most rice producing areas of the world (Ou, 1985). The disease results in yield loss as high as 70-80% (Ou, 1985). Kalanamak is one of the finest quality scented rice of India, also affected by *P. oryzae* causes reduction in yield however, there are several other reasons for decrease in production too (Singh *et al.*, 2005). Therefore to increase yield by developing blast resistant cultivar crosses were made between Kalanamak and Tetep. Selection of transferred trait was done with the help of molecular markers as well as screening under field and green house condition after *P. oryzae* inoculation (unpublished). In present study, biochemical analysis of parent and crosses were done because plants under stress conditions, such as pathogen attack, they exhibit increase in the production of active oxygen species such as

superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH) (Magbanua *et al.*, 2007 and Salam *et al.*, 2006). These active oxygen species (AOS) is one of the earliest responses of the plants to infection by pathogens (Tzeng and De Vay, 1993; Grant and Loake, 2000). The active oxygen species may act directly as toxins against the pathogens (Mehdy, 1994) as well as may act as second messengers for the activation of a variety of defence genes (Baker and Orlandi, 1995; Lamb and Dixon, 1997). There are several studies showing that protein pattern changes are accompanied by the biological changes, which makes the organism more fit to the altered environment (Singh *et al.*, 1985 and Hurkman *et al.*, 1988). The antioxidative systems of plants during pathogen infection have been well documented (Agrawal *et al.*, 2002; Diaz-Vivancos *et al.*, 2008; Kuzniak and Sklodowska, 2005). Therefore, biochemical analysis of Kalanamak, Tetep and their crosses were done to know the enhanced level of different enzyme and changes in protein pattern after *P. oryzae* inoculation.

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Materials and Methods

Plant materials

Seeds of Kalanamak were obtained from Department of Genetics and Plant Breeding, N.D.U.A. & T, Kumarganj, Faizabad and seeds of Tetep were obtained from Division of Genetics, IARI, New Delhi.

Experimental condition, fungal isolates and inoculation

Fungal isolates were collected from Division of Pathology, IARI, New Delhi, were maintained on potato dextrose agar (PDA) as well as on the Oat meal agar (OMA) media at 25°C. Highly pathogenic isolate was used for inoculation. Inoculation of Kalanamak (recurrent parent), Tetep (donor plant) and their crosses (F₂ population) was done as described by Chen (2001).

Sample preparation for different enzymatic analysis and protein analysis

Plants samples were collected from Kalanamak, Tetep and their crosses [five plants were randomly selected from F₂ population:KT] on four different time interval (16hr, 20hr, 24hr, 48hr after inoculation) for the biochemical analysis after *P. oryzae* inoculation.

Assay of catalase (CAT): CAT activity was determined as described by Chance and Maehly (1955) and units were defined as mmol oxygen released min⁻¹g fresh weight⁻¹.

Assay of peroxidase (PO): PO activity was determined as described by Srivastava (1987) and enzyme activity was expressed as kkat.g⁻¹ tissue.

Protein isolation and electrophoresis of proteins

Different plant samples were crushed in extraction buffer [100 mM Tris-HCl pH 6.8, 5 mM PMSF, 4% SDS, 30% glycerol, 200 mM dithiothreitol (DTT)]. 50µg protein of each samples were separated on 12% SDS-PAGE (Laemmli, 1970) and their relative changes in bands no. and intensity were studied.

Experimental design and statistical analysis

For the enzyme analysis, all experiments were performed in triplicate. The data obtained for each enzyme were analysed separately. Paired t test was performed in all the cases using Graph Prism software (Motulsky, 1999). Pearson correlation coefficients and P values were used to show correlations and their significance. Differences of P<0.05 were considered significantly.

Results

Catalase activity

The content of catalase in infected rice, Tetep and

crosses KT₅, KT₁, KT₂ increased after *P. oryzae* infection after inoculation and was significantly higher (up to two fold) in infected plants than the susceptible line and control plants. Although catalase content in infected Kalanamak & KT₃, KT₄, increased from 16 to 20 hrs after inoculation and were significantly lower than those of control plants. (Fig-a)

Peroxidase activity

Peroxidase activities >2-3 fold increased in Tetep, KT₅, KT₁, KT₂ crosses after inoculation period of 16 to 48hr. The peroxidase activity was found maximum in Tetep after 24 hr after inoculation followed by KT₅ (after 48 hr). Furthermore, peroxidase activity in infected susceptible rice cv. Kalanamak was highest from 24 to 48 hr after inoculation periods which was similar to control condition (fig. 1).

Induction and comparison of induced protein in Kalanamak, Tetep and their crosses

The relative protein bands induced or diffused from rice cv. Tetep, Kalanamak, KT crossed plant's leaves samples control and infected with *P. oryzae* are recorded. The total number of protein bands in the control and infected genotypes ranged from 10 to 13 bands. Generally, infection with *P. oryzae* induced the appearance of new protein bands in the all the plants. After comparing the protein profiling among the control and different inoculated samples of Kalanamak, Tetep and KT crossed plants, SDS-PAGE showed that infection by *P. oryzae* induced only few proteins in susceptible rice Kalanamak. It was found that intensity and induction of new protein bands were more prominent in Tetep (~75 kDa, ~70 kDa, ~66 kDa, ~35kDa) followed by KT₅ plant (~80kDa, ~75 kDa and 35kDa) in which intensity of 75kDa protein band increases after infection. In KT₄ induction of 80kDa, 75kDa, 70kDa, In susceptible parent *i.e.* Kalanamak intensity of band was low as well only two protein bands of 60 kDa and 35kDa was induced after infection similarly low intensity of protein also observed in KT₁ plant but four protein band was induced after infection *i.e.* 70kDa, 50kDa, 45kDa after 20 to 24hr after inoculation (fig. 2).

Discussion

During infection by pathogens, several reactive oxygen species are produced in plants (Sutherland, 1991). These active oxygen species have been shown to be associated with the hypersensitive response in plants (Grant and Loake, 2000). The results of the present study indicated that catalase and peroxidase activity significantly increased in Tetep & KT crosses, after inoculation with *P. oryzae*. Catalase activity was changed after infection

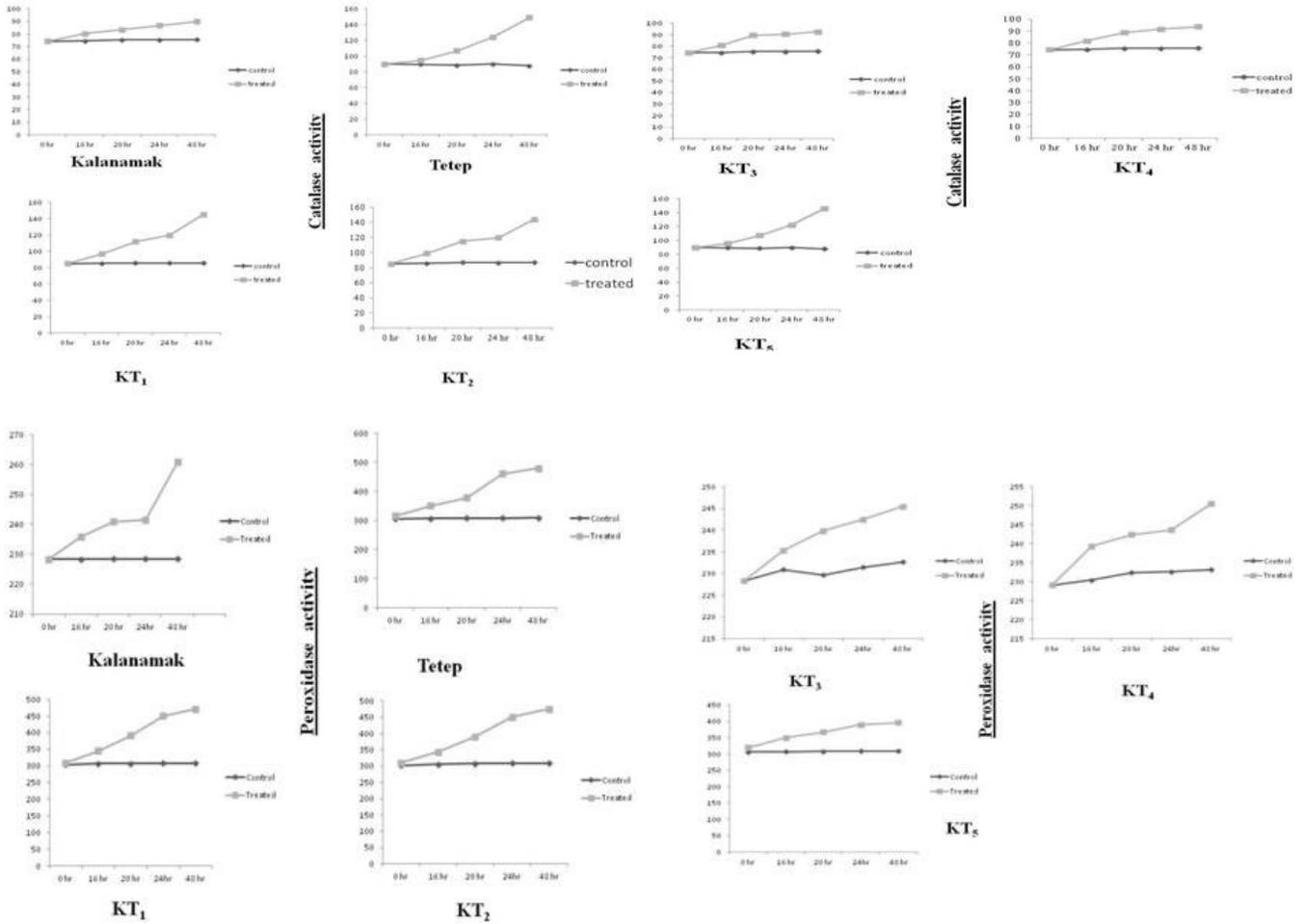


Fig. 1 : Catalase and peroxidase activity at different time intervals (16 hr, 20 hr, 24 hr, 48 hr) in Parents (Kalanamak, Tetep and their crosses (F2 population) after infection with *P. oryzae*. Statistical differences at $P \leq 0.05$ have been calculated for each test in all the assays.

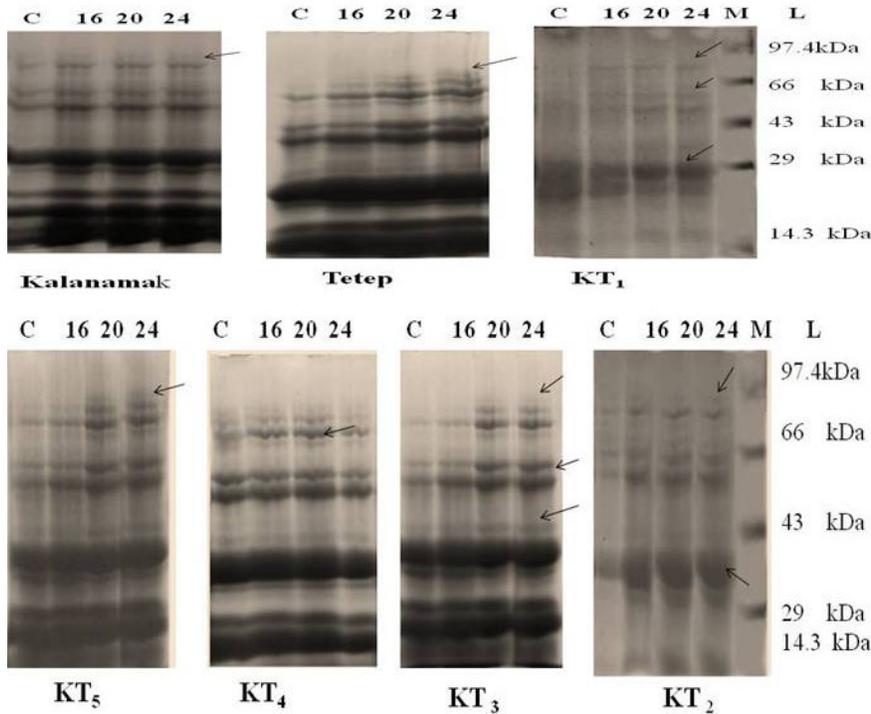


Fig. 2 : Comparative protein profiling of the on 12 % SDS-PAGE at different time intervals (16 hr, 20 hr, 24 hr, 48 hr) in Parents (Kalanamak, Tetep and their crosses (F2 population) after infection with *P. oryzae*. [M denotes marker, C control].

with *P. oryzae* from susceptible rice cv. Kalanamak however maximum induction of catalase activity were shown by Tetep. Catalase has been found in all aerobic cells containing cytochrome (Percy, 1984). The time course change in peroxidase activities showed that in the infected rice Tetep and crosses (KT₁, KT₂, KT₅) increase in the activity by > 2-3 fold between 16 hr to 24 hr. Sasaki *et al.* (2004) analyzed ten rice peroxidase responded to multiple stress including infection with rice blast fungus. Seven of the 10 *POX* genes were expressed at higher levels in the incompatible host than in the compatible host 6–24 h after inoculation by which time no fungus-induced lesions have appeared. In rice, *POX* activity increased after inoculation with rice blast fungus (Matsuyama and Kozaka, 1981) and the expression of *POX8.1* and *POX22.3* genes was induced by infection with *Xanthomonas oryzae* pv. *oryzae*, a bacterial leaf blight disease, especially in a resistant rice cultivar (Chittoor *et al.*, 1997). Wounding also induced *POX* gene expression in horseradish (Kawaoka *et al.*, 1994), rice (Hiraga *et al.*, 2000a, Ito *et al.*, 2000), tobacco (Sasaki *et al.*, 2002a) and tomato plants (Mohan *et al.*, 1993). The relative protein bands induced or diffused from rice cv. Tetep, Kalanamak, KT crossed plant's leaves samples control and infected with *P. oryzae* are recorded. Generally, infection with *P. oryzae* induced the appearance of new protein bands in the all the plants. Pathogenesis-related (PR) and similar proteins have been found to be inducible by infection with various types of pathogens in many plant families and have been classified into 17 families. In present study, intensity and induction of new protein bands were more prominent in Tetep followed by KT₅ plant. Proteins showing differential expression between treatments may have important roles in plant-stress responses (Van Loon, 2006). Induction of defence proteins makes the plant resistant to pathogen invasion. Matsubayashi *et al.* (2006) reported five proteins were included after Xoo infection in rice related to signal transduction: phyto-sulfokine receptor precursor, G protein, phosphatidylinositol 3-kinase-related protein kinase, CHP-rich zinc finger protein and auxin-regulated protein (U46). In the other proteomic analysis, Mahmood *et al.* (2006) also identified proteins related to signal transduction at 72 h after Xoo inoculation. Several other works also reported that after pathogen infection protein patterns were changed in rice (Katso *et al.*, 2001; Korfhage *et al.*, 1994; Liscum and Reed, 2002). Susceptible and resistant parent may induce a similar resistant response and activate different signal transduction pathways with different defence responses when *P. oryzae* pathogen was infected. As a general conclusion, we can also suggest

that protein changes in resistant parent Tetep and crosses KT₁, KT₂, KT₅'s leaves are somehow related to increased resistance level against *P. oryzae* and can be used as blast resistant cultivar in future after successive crossing.

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