Activity profile of defence-related enzymes in rice genotypes (*Oryza sativa* L.) against rice blast (*Magnaporthe oryzae*)

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**Abstract**

The control and infected leaf samples of blast resistant and susceptible rice genotypes were evaluated for activities of defence-related enzymes viz., total phenol content, chitinase, phenylalanine ammonia lyase (PAL), β-glycosidase, antioxidative enzymes, superoxide dismutase, peroxidase and ascorbate peroxidase. The level of total phenol content and the activity profile of chitinase, PAL and β-glycosidase significantly increased in both blast-resistant and susceptible rice genotypes with comparatively higher level induction in Tetep, NLR-20104 and Swarnadhan the blast-resistant genotypes. The antioxidative enzymes were comparatively higher in the leaf samples of blast-resistant genotypes recording highest increase in NLR-20104 and KJT-5. The constitutive levels of total phenols and activity of defence-related and antioxidative enzymes in the control leaf samples differed among the genotypes and were even higher in the two blast susceptible genotypes (EK-70 and Chimansal). However, the level of induction as evident from the activity profile differences between control and infected leaf samples suggests higher level of induction was more which is indicative of the induced defence response. The genotype recording maximum induction of defence-related and antioxidative enzymes activity could be useful criteria in screening for blast resistant genotype in rice.

**Introduction**

Rice crop is often subjected to different biotic stresses caused by fungi, bacteria and viruses. Rice blast disease is caused by the filamentous ascomycete fungus *Magnaporthe oryzae*, is the most devastating fungal disease in the rice-growing world thus resulting in huge yield losses (Samalova et al. 2014). Most infection occurs on the leaves during vegetative phase, on panicle and neck during...
reproductive phase of crop. Generally, rice blast is favoured by moderate temperatures (24 °C) and periods of high moisture.

In India, management of Rice blast diseases is managed using chemical fungicides, due low levels of host plant resistance in the cultivated rice varieties. The use of chemical is neither practical nor environment-friendly for disease control and hence utilisation of host resistance has been the best way to manage the disease, for which identification of sources of resistance is necessary. The need for a better understanding of this disease becomes clear if we consider the poor durability of many blast-resistant cultivars of rice, which have a typical field life of only 2–3 growing seasons before disease resistance is overcome.

In plant defence, the systemic resistance induction process activates defence response pathways by increasing enzymatic activity of peroxidase (POX) and poly phenol oxidase which is responsible for catalysing lignin formation and phenylalanine ammonia lyase (PAL) which is involved in the biosynthesis of phytoalexins and phenols. Induced systemic resistance (ISR) induction also increases liposaccharides, a constituent of cell wall membranes (Radjacommare et al. 2004). The pathogenesis-related proteins β-1,3-glucanase and chitinase, enzymes that belong to PR-2 and PR-3 families, respectively, (Van Loon & Pieterse 2006) have been related more often to SAR and sometimes to ISR. All these enzymes have been shown to be involved in plant defence against pathogens in several pathosystem (Kini et al. 2000).

Plants have also developed complex antioxidant defence systems that respond to biotic and abiotic stresses and mitigate the deleterious effects of reactive oxygen species (Panda 2007). The levels of reactive oxygen species (ROS) and the extent of oxidative damage depend largely upon the level of coordination among ROS-scavenging enzymes (Liang et al. 2003). In transgenic rice plant, phenols and activity profile of some enzymes have shown active role in resistant mechanism. Biochemical studies on resistant and susceptible genotypes have often helped in understanding the nature and mechanisms of resistance which could aid in screening for disease-resistant genotypes. The constitutive and induced biochemical defence of rice genotypes against rice blast was therefore undertaken at Lonavala region of Pune district in India which is a hot spot of rice blast disease (Krishnaveni et al. 2012).

**Materials and methods**

The control and *M. oryzae* infected leaf samples of 35 days grown rice seedlings of same plant of different blast-resistant (KJT-2, Tetep, NLR 20104, KJT-5, Rp-Biopatho-3, Swarnadhan, RAU 631-9-10, CN-1447-9-4-2, CB-06-555) and susceptible (EK-70, and Chimansal) genotypes of rice were collected from the Rice Pathologist, Rice Research Station, Lonavala during the year 2014–2015 and were immediately frozen in liquid nitrogen for the biochemical analysis. The rice genotypes were screened by rice pathologist at location which is a hot spot for rice blast pathogen under glass house condition. The fungal inoculum load used to inoculate was 10⁶ spores per ml.
The estimation of total phenol was carried out with Folin–Ciocalteu reagent (Bray & Thorpe 1954). Exactly, 0.2 g of oven dried sample was macerated in mortar and pestle with 5 times volume of 80% ethanol. The homogenate centrifuged at 10,000 rpm for 20 min and the supernatant was collected. The residue re-extracted two more times with 80% ethanol, supernatant was collected and evaporated to dryness. Further the residue was dissolved in a known volume of distilled water (5 ml) from that 0.1 ml aliquots pipetted into test tubes. The volume in each tube made up to 3 ml with distilled water and 0.5 ml of Folin–Ciocalteu reagent was added. After 3 min, 2 ml of 20% Na₂CO₃ solution was added to each tube. Mixed thoroughly and placed tubes in boiling water bath for exactly 2 min and cool and absorbance was measured at 650 nm against a reagent blank. The activity measures from prepared standard curve using different concentration of catechol.

The chitinase (EC.3.2.1.14) activity was assayed by the method of Giri et al. (1998) Enzyme extraction 0.5 g of infected and control seedlings was weighed separately and macerated with 2 ml of 0.1 M sodium citrate buffer in precooled mortar and pestle. The homogenate was centrifuged at 10,000 rpm for 10 min at 10 °C and the supernatant was used as crude source of chitinase. 0.5 ml of supernatant was added to 2 ml of chitin suspension containing 7.5 mg of BSA and was incubated in water bath at 37 °C for 3 h. From that an aliquot of 0.1 ml was taken for the estimation of N-acetyl glucosamine as per the method of Nelson (1944). The chitinase activity was expressed in terms of \(\mu g\) N-acetyl glucosamine released min\(^{-1}\) mg\(^{-1}\) protein.

The PAL activity (E.C.4.1.3.5) was assayed by the method of Campos et al. (2004). Infected and control seedlings were separately weighed and 0.5 g were macerated with 2 ml of 50 mM borate buffer (pH 8.5) containing 5 mM of 2-mercaptoethanol and 0.4 g polyvinylpyrrolidone. The homogenate was centrifuged at 20,000 rpm at 4 °C for 20 min. The collected supernatant was used as an enzyme source. The assay mixture containing 1 ml aliquots of supernatant and 110 \(\mu l\) of 100 mM L-phenylalanine were incubated at 40 °C for 30 min. Then added 1 ml of 4% trichloro acetic acid (TCA) in it to terminate the reaction. Similarly, the TCA was added in one of the test tubes at zero min to serve as blank. The assay mixture was incubated with TCA for 5 min at room temperature and the absorbance was read at 290 nm. PAL activity was calculated as \(\mu\) moles of trans-cinnamic acid released min\(^{-1}\) mg\(^{-1}\) protein under the specific condition.

The β-glucosidase activity was assayed by the modified method of Agrawal and Bahl (1969). Enzyme extraction was done using 0.5 g of infected and control seedlings and samples were macerated with 0.05 M of sodium acetate buffer (pH 4.6). The homogenate was centrifuged at 23,000 rpm at 41 °C for 20 min. The supernatant was used as an enzyme source. A reaction mixture was prepared by adding 100 \(\mu l\) of a solution of p-nitro phenyl-β-D-glycopyranoside to 350 \(\mu l\) of 0.05 m sodium buffer (pH 4.6), followed by initial incubation at 30 °C for 5 min. After the addition of 50 \(\mu l\) of enzyme extract, the mixture was further incubated
at 30 °C for 15 min. The reaction was stopped by adding 700 μl of 0.2 M sodium carbonate. The yellow colour formed was measured at 420 nm by spectrophotometer. Enzyme activity was calculated as μ moles of p-nitro phenol released min⁻¹ mg⁻¹ protein. The activity was calculated based on molar extinction coefficient \(U = 1.12 \times 104\) M⁻¹ cm⁻¹.

Ascorbate POX (EC.1.11.1.11) activity was assayed as per the method described by Nakana and Asada (1981). Enzyme extract for ascorbate peroxidase (APX) was prepared by grinding 0.5 g of controlled and infected leaf samples separately with 2 ml of 100 mM Potassium phosphate buffer (pH = 7.5). The homogenate was centrifuged at 15,000 rpm for 15 min at 4 °C and the supernatant was used as the enzyme source. The reaction mixture contained 2.3 ml phosphate buffer, 0.2 ml ascorbic acid, 0.2 ml ethylenediaminetetraacetic acid (EDTA), 50 μl enzyme extract, 50 μl \(\text{H}_2\text{O}_2\) and 0.3 ml distilled water. The reaction was started with addition of 0.2 ml of hydrogen peroxide. Decrease in absorbance after 30 s was measured at 290 nm in UV–visible spectrophotometer. The activity was determined using molar extinction coefficient \(U = 2.8\) mM⁻¹ cm⁻¹

Superoxide dismutase (SOD) (EC.1.15.1.1) activity was measured immediately in fresh extract as per the method described by Dhindsa et al. (1981). Enzyme extract for SOD was prepared by grinding 0.5 g of controlled and infected leaf samples separately with 2 ml of 100 mM Potassium phosphate buffer (pH = 7.5). The homogenate was centrifuged at 15,000 rpm for 15 min at 4 °C and the supernatant was used as the enzyme source. The reaction mixture contained, 1.5 ml phosphate buffer, 0.2 ml methionine, 0.1 ml EDTA, 0.1 ml sodium carbonate, 0.1 ml enzyme extract, 0.1 ml NBT, 0.9 ml distilled water and 0.1 ml riboflavin. The reaction was started by adding 0.1 ml of riboflavin and placing the tubes under two 15 W fluorescent lamps for 15 min. A complete reaction mixture without enzyme, which gives the maximal colour, served as control. Switching of the lights and putting the tubes into dark stopped the reaction. The non-irradiated complete reaction mixture served as blank.

The assay of POX activity (EC.1.11.1.7) was performed as described by Sadasivam and Manickam (1996). Enzyme extract for POX was prepared by grinding 0.5 g of controlled and inoculated leaf samples separately with 2 ml of 100 mM potassium phosphate buffer (pH = 7.5). The homogenate was centrifuged at 15,000 rpm for 15 min at 4 °C and the supernatant was used as the enzyme source. For the POX assay, the reaction mixture was prepared by adding 2.85 ml 0.1 M phosphate buffer (pH 7.0), 50 μl of 20 mM guaiacol solution, 50 μl of 12.3 mM hydrogen peroxide and 50 μl of enzyme extract. The reaction was allowed to proceed for 3 min. Absorbance at 470 nm was measured 30 s after adding the enzyme extract to the substrate, and change in the absorbance was recorded up to 3 min. The POX activity was determined using molar absorption coefficient \(U = 26.6\) mM⁻¹ cm⁻¹. Protein estimation – the protein content in the crude enzyme extract was estimated according to the method of Lowry et al. (1951).
All biochemical parameters were analysed in three replications. The data obtained by biochemical constituents and enzymes determination were subjected to factorial completely randomised design for the significance of various data using “F” test (Gomez & Gomez 1984). The vertical bar in Figures 1–7 indicates activity of profile of defence-related and antioxidative enzymes from control and infected leaf samples of blast-resistant and susceptible rice genotypes. The bar indicates SE± of each treatment (n = 3) at P ≤ 0.05 probability.

**Results**

**Total phenol content**

The total phenol content of the control leaf samples was recorded in the range of 6.36–13.56 mg/g dry wt and it was increased to 12.30–21.51 mg/g dry wt in the infected leaf samples. The highest total phenol content in the infected leaf samples was recorded in NLR-20104 (21.51) followed by KJT-2 (18.62 mg/g dry wt) and KJT-5 (18.00 mg/g dry wt) and lowest in both susceptible genotype EK-70 and Chimansal i.e. 12.92 and 13.70 mg/g dry wt respectively (Figure 1). The level of induction of total phenol content on infection with the pathogen was in the order NLR-20104 (0.96-fold) > RAU-631-9-10 (0.93-fold) > Tetep (0.88-fold) and least in Chimansal (1.01) and EK-70 (1.08)-fold, respectively. Higher endogenous level of total phenol recorded in control leaf samples of two susceptible rice genotypes.

![Figure 1. Total phenol content.](image)

Notes: The vertical bar in the figures indicates activity of profile of antioxidative enzymes from control and infected leaf samples of blast resistant and susceptible rice genotypes. The bar indicates SE± of each treatment (n = 3) at p ≤ 0.05 probability.
**Activity of antioxidative enzymes**

The activity of SOD recorded in range from 3.91 to 14.15 U mg\(^{-1}\) protein in the uninfected leaf samples of 11 rice genotypes and in infected it was ranged between 8.06 and 27.12 U mg\(^{-1}\)protein (Figure 2). The activity of SOD increased by 3.19-fold in infected leaf of KJT-5 followed 2.63-fold in CN-1447-9-4-2 and 2.36-fold in NLR 20104 and least in EK-70 (1.33-fold) and Chimansal (1.41-fold), these are the blast susceptible varieties of rice.

The POX activity profile ranged between 5.67 and 132.61 n moles H\(_2\)O\(_2\) oxidised min\(^{-1}\) mg\(^{-1}\) protein in the infected leaf samples of all 11 rice genotypes (Figure 2). The activity profile of POX in the infected leaf samples ranged between 51.16 and 725 n moles H\(_2\)O\(_2\) oxidised min\(^{-1}\) mg\(^{-1}\) protein. The POX activity significantly induced by 28.96-fold and 22.82-fold in the infected leaf samples of NLR-20104 (725.85 n moles H\(_2\)O\(_2\) oxidised min\(^{-1}\) mg\(^{-1}\) protein) and KJT-5 (466.63 n moles H\(_2\)O\(_2\) oxidised min\(^{-1}\) mg\(^{-1}\) protein) followed by TeTep (21.62-fold). The least level of induction was recorded in Chimansal (3.92-fold), a blast susceptible rice. It thus appears that the level of induction higher than 20-fold over the uninfected control (constitutive) may be considered as a criteria for selection, as some of the blast-resistant variety viz. CB-06-55 also exhibit lower level induction than susceptible EK-70 and Chimansal.

Ascorbate POX activity profile ranged between 42.48 and 296.59 n moles ascorbate oxidised min\(^{-1}\) mg\(^{-1}\) protein in the uninfected leaf samples of 11 rice genotypes (Figure 4). Whereas in the infected leaf samples, it was ranged between 184.43 and 587.67 n moles ascorbate oxidised min\(^{-1}\) mg\(^{-1}\) protein. The highest APX activity was recorded at control as well as in the infected leaf samples of NLR-20104 (296.59 to 587.67 n moles ascorbate oxidised min\(^{-1}\) mg\(^{-1}\) protein) followed

![Figure 2. SOD activity in resistant and Susceptible rice genotypes.](image)

**Figure 2.** SOD activity in resistant and Susceptible rice genotypes.

Notes: The vertical bar in the figures indicates activity of profile of antioxidative enzymes from control and infected leaf samples of blast resistant and susceptible rice genotypes. The bar indicates SE± of each treatment (n = 3) at \( p \leq 0.05 \) probability.
by KJT-5 (93.56 to 476.67 n moles ascorbate oxidised min$^{-1}$ mg$^{-1}$ protein) and Swaranthand (131.58 to 463.23 n moles ascorbate oxidised min$^{-1}$ mg$^{-1}$ protein), respectively. Lowest induction was recorded in Chimansal and EK-70 but these blast susceptible genotypes recorded constitutive higher levels of APX as against other resistant genotypes.

**Activity profile of defence related enzymes**

The PAL activity profile 11 rice genotypes ranged between 3.21 and 4.24 $\mu$ moles cinnamic acid released min$^{-1}$ mg$^{-1}$ protein in the uninfected leaf samples and in the infected leaf samples ranged between 3.39 and 5.01 $\mu$ moles cinnamic acid released min$^{-1}$ mg$^{-1}$ protein (Figure 5). Highest increase of PAL activity in NLR-20104 (4.49 $\mu$ moles cinnamic acid released min$^{-1}$ mg$^{-1}$ protein). The order of induction (Fold increase) of PAL activity on infection with the pathogen was in the order Rp-BIOPATHO-3(R) (1.15-fold) > Swaranthand (R) (1.06) and the least level of induction was recorded in Chimansal (0.03-fold) a blast susceptible rice genotype. From Figure 5, it was also observed that the PAL activity constitutively recorded higher in both group of genotypes.

The leaf chitinase activity profile ranged between 11.53 and 33.35 $\mu$g N-acetylglucosamine (NAG) released min$^{-1}$ mg$^{-1}$ protein in the uninfected leaf samples of 11 rice genotypes. As compared to uninfected samples chitinase, activity significantly increased in infected leaf samples from 23.21 to 77.15 $\mu$g NAG released min$^{-1}$ mg$^{-1}$ protein (Figure 6). The maximum leaf chitinase activity both in the uninfected (constitutive) and infected (induced) was recorded in RAU-631-9-10 that is 33.35 to 77.15 $\mu$g NAG released min$^{-1}$ mg$^{-1}$ protein, The susceptible EK-70 and Chimansal recorded constitutively higher activity after infection but it was low as compared to other resistant rice genotypes. The level of induction of chitinase activity significantly ($p \leq 0.05$) higher and fold increase was in the order of NLR-20104 (3.17) > Swaranthand (1.77) > RAU-631-9-10 (1.32) and least level of induction was recorded in blast susceptible genotype Chimansal (0.28).

In case of $\beta$-glucosidase, as compared to uninfected samples (6.45–15.42 n moles p-nitro phenol released min$^{-1}$ mg$^{-1}$ protein) the activity profile significantly induced from 12.93 to 43.08 n moles p-nitro phenol released min$^{-1}$ mg$^{-1}$ protein in infected leaf samples of all 11 rice genotypes (Figure 7). Highest $\beta$-glucosidase activity in the infected leaf samples was recorded NLR-20104 of 43.08 n moles p-nitro phenol released min$^{-1}$ mg$^{-1}$ protein followed by Rp-BIOPATHO-3R (39.87 n moles p-nitro phenol released min$^{-1}$ mg$^{-1}$ protein) and Swaranthand with 38.34 n moles p-nitro phenol released min$^{-1}$ mg$^{-1}$ protein. Constitutive higher $\beta$-glucosidase activity recorded in both group of rice genotypes. The level of induction (fold increase) of $\beta$-glucosidase activity on infection with the pathogen was in the order Swarnadhan (3.78) > TeTep (3.64) NLR-20104 (2.55) respectively.
Discussion

Understanding the nature and mechanisms of resistance to infection caused by *M. Oryzae* is important to know the related metabolic changes. In plant–pathogen interactions, defence mechanisms involves both structural and biochemical barriers (Jones & Dangl 2006). Synthesis of new proteins includes a heterogeneous group of proteins collectively defined as pathogenesis-related (PR) proteins such as PAL, β-glucosidase and chitinase have been suggested to be involved in plant resistance against fungal pathogens (Kini et al. 2000; Van Loon 2007). The ROS can be second messengers in resistance mechanisms leading to the activation of defence-related genes and interferes with other important signalling molecules such as phytohormones and nitric oxide (Chen et al. 2013). Enzymatic antioxidants such as SOD and POX are participating in scavenging various types of ROS (Barna et al. 2012).

The enzyme SOD constitutes the first line of defence against ROS by catalysing the dismutation of $O_2^{-}$ to $O_2$ and $H_2O_2$ (Alscher et al. 2002). In our study, SOD activity was significantly induced in infected leaf of both blast-resistant and susceptible rice genotypes (Figure 2). POX enzymes remain active in elimination of ROS and catalyse the oxidoreduction of various substrates using hydrogen peroxide. Many reports have suggested POX plays a role in resistance to pathogens (Kawaoka et al. 2003). In our study, POX activities also increased significantly over controls suggesting that POX could be important for resistance. SOD and POX showed similar trends (Figures 2 and 3), thus these two enzymes might be more efficiently control ROS. POX activity increased significantly over control in all resistant and susceptible genotypes except Swarandhan (Figure 3) suggesting their activation more efficiently in for scavenging of ROS during pathogen attack.

Enzymatic antioxidant APX is present in all subcellular compartments. In rice, Agrawal et al. (2003) reported cytosolic APX genes are up-regulated upon wounding suggesting that the cytosolic APX isozymes play a protective role against stressful conditions. In our study, constitutive higher APX activity has been recorded in both groups of rice genotypes and it was induced on infection (Figure 4). This indicates their participation in the removal of $H_2O_2$ produced by induced SOD activity. Similar increase in the APX activity with glutathione reductase activity is reported in pearl millets *Sclerospora graminicola* by Kumar et al. (2015).

Overall, this study revealed that priming in the ROS production and the activity of antioxidant enzymes such as SOD, POX and APX occurred during interaction with the pathogen more in blast-resistant rice compared to susceptible genotypes. Furthermore, the greater resistance of NLR-20104 (R), KJT-5 (R) and Swarandhan (R) genotypes might be associated with greater lignin contents in this genotypes. Together, these findings suggest the critical role of SOD- and POX- and APX-dependent lignifications as a defence mechanism involved in basal resistance in our pathosystem. Many reports showed induced levels of antioxidants after post infection in rice might be due to pathogen-associated molecular pattern-triggered
immunity and or effectors-triggered immunity that accompanied by ROS generation and that might also strengthening of plant cell wall to limit proliferation of pathogen under control condition as a basal defence and during the pathogen attack of *M. Oryzae*. (Chisholm et al. 2006; Jones & Dangl 2006; Filippi et al. 2011; Taheri et al. 2014). Higher endogenous levels of ROS scavenging enzymes in control leaf samples in this study might be associated their basal defence mechanism.

**Figure 3.** Peroxidase activity in resistant and susceptible rice genotypes. Notes: The vertical bar in the figures indicates activity of profile of antioxidative enzymes from control and infected leaf samples of blast resistant and susceptible rice genotypes. The bar indicates SE± of each treatment (*n* = 3) at *p* ≤ 0.05 probability.

**Figure 4.** APX activity in resistant and susceptible rice genotypes to *M. Oryzae*. Notes: The vertical bar in the figures indicates activity of profile of defense related enzymes from control and infected leaf samples of blast resistant and susceptible rice genotypes. The bar indicates SE± of each treatment (*n* = 3) at *p* ≤ 0.05 probability.
In addition to this, phenolics are strong non-enzymatic antioxidants due to availability of their phenolic hydrogen. Some phenolics are constituents of lignin and these phenolics are oxidised by POX using \( \text{H}_2\text{O}_2 \) (Sharma et al. 2012; Nikraftar et al. 2013). In our study, post infection increase of total phenol (1–1.93-fold) with similar intensity in both groups of genotypes might be due to oxidation of phenols by POX using \( \text{H}_2\text{O}_2 \) produced by induced SOD (Sharma et al. 2012). Increase in total phenols indicates their positive correlation against disease resistance. Concentration of phenolics compounds is usually higher in

**Figure 5.** PAL activity.
Notes: The vertical bar in the figures indicates activity of profile of defense related enzymes from control and infected leaf samples of blast resistant and susceptible rice genotypes. The bar indicates SE± of each treatment \((n = 3)\) at \( p \leq 0.05 \) probability.

**Figure 6.** Leaf chitinase activity.
Notes: The vertical bar in the figures indicates activity of profile of defense related enzymes from control and infected leaf samples of blast resistant and susceptible rice genotypes. The bar indicates SE± of each treatment \((n = 3)\) at \( p \leq 0.05 \) probability.
resistant than in susceptible genotypes of different crop plants. Sindhan and Parashar (1991) reported that total phenols, ortho-dihydric phenols, and total reducing and non-reducing sugars were higher in the resistant rice varieties IET 4141 and IR-36 than in the susceptible Bennibhog and TN-1. In our study, resistant and susceptible genotypes showed higher amount of total phenols in both control and infected leaves. However, susceptible genotypes EK-70(S) and Chimansal(S) recorded constitutively higher total phenols than the resistant TeTep-(R), Rp-BIOPATHO-3(R), RAU-631-9-10(R).

The PR protein PAL involved in the synthesis of both phytoalexins and lignins which prevent cell wall penetration by the pathogen (Dixon 2001). In this study, higher constitutive level of PAL in both groups of rice genotypes showed similar increment in the levels in infected samples (Figure 5). Earlier positive correlation of PAL in six rice cultivars differing in resistance to *Pyricularia Oryzae* with the degree of resistance has been reported (Zhang et al. 1987). PAL activity in highly resistant cultivars was 63.5 per cent higher than in susceptible cultivars which cause hardening of infection sites, thus preventing pathogen entry into the host plant reported by Hsieh et al. (2010).

In this study, constitutively higher activity of Chitinase in control leaf of all rice genotypes indicates their importance and involvement at basal defence response in rice (Figure 6). Earlier research reports showed low constitutive expression of chitinases in healthy plants and induced to much higher levels upon infection or wounding (Boller 1988). In *M. Oryzae* infected rice genotypes, chitinase activity increased due to pattern recognition receptor and chitin elicitor binding protein which is required for chitin-triggered immunity in rice acting in cooperation with the LysM receptor-like kinase Os-CERK1 (Shimizu et al. 2010). Transgenic over expression lines, using family 19 chitinases from bean, tobacco and rice confirmed...
that higher constitutive expression of some chitinases indeed does contribute to increased fungal resistance (Datta et al. 2001).

β-glucosidase enzymes is a microbial cell wall degrading enzymes and have relationship to pathogenicity (Takeda et al. 2010). In our study KJT(5),CN-1447-9-4-2 and CB-06-55 a resistant rice genotypes recorded lowest activity than susceptible EK-70 and Chimansal both in control and infected leaf samples indicates these enzymes showed more activation to avoid infection but their action might insufficient to limit the spread of M. oryzae than the resistant PR protein showed more activation (Figure 7). Yang et al. (2008) reported microbial and fungal β-glucosidase (EC 3.2.1.21) are produced extra cellularly and intra cellularly and are thought to play a significant role in saccharifying cellulosic materials and acquiring nutrients by producing glucose. Earlier, Whetten et al. (1998) reported that the plant β-glucosidases may be involved in the processing and release of fungal glucan elicitors, triggering a chain of reactions in the host, including phytoalexin formation and the biosynthesis of phenylpropanoids and lignin-like phenol aglucones by hydrolyzing B-phenyl glucosides. These aglucones are basically fungi toxic and fungi static in action and may limit the spread of M. oryzae in resistant plants. A similar result in accumulation of β-1-3 glucanase, PAL and chitinase enzymes in incompatible interactions of pearl millets has been reported recently by Kumar et al. (2015). Suppression of blast infection M. Oryzae has been reported with induction of this enzyme by soil drenching of rhizobacteria in rice field by Filippi et al. (2011). In resistant rice genotypes, the activity of defence-related and antioxidative enzymes increased might associated with hypersensitive response and or M. Oryzae has robust the defence system. Our result is in agreement with the results of above quotation.

**Conclusions and future prospects**

A significant variations were observed in the activity profile of defence-related enzymes i.e. pathogenesis-related protein in blast-resistant and susceptible genotypes upon pathogen infection. However, the activity profile of these enzymes in the control leaf samples of some of the resistant genotypes was less than the activity recorded in blast susceptible genotypes (Chimansal). The post infection induction in activity level of defence-related and antioxidant enzymes could be effectively used to screen the genotypes. The lower activity profile in some of the resistant genotypes than the blast susceptible checks warrants needs field screening as well as at molecular level.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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