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Study on expression of PR-proteins and defense related enzyme during interaction of rice differentials with *Magnaporthe oryzae* Cav.

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Abstract
Magnaporthe oryzae Cav. (Anamorph *Pyricularia oryzae* (Cooke) Sacc.) is a cause for a very serious and threatful biotic stress called rice blast. Managing the disease through PR protein was found to be an effective approach as over expression helps in antimicrobial control. An experiment was conducted using 25 rice differentials to study pathogenesis-related (PR) protein expression and activity of enzymes such as ascorbate peroxidase, guaiacol peroxidase, glutathione reductase and superoxide dismutase during pathogen attack as they are indication of defense activation. Among them, Tetep was found resistant where in HR 12 and CO 39 was found highly susceptible to rice blast. PR 11 was expressed in resistant and moderately resistant differentials but it was unnoticed in highly susceptible differentials (HR 12 and CO 39). Activity of above mentioned defense related enzyme was found higher after 48 hours of inoculation in prominent rice variety CO 39. Among other 24, RIL 10, a moderately resistant differential recorded maximum activity of ascorbate peroxidase and glutathione reductase. The higher reports of guaiacol peroxidase and superoxide dismutase activity was observed in IR 64 and Kanto 51 respectively (moderately resistant differentials). All four enzyme activity was lowest in susceptible to highly susceptible differentials.

Keywords: Rice blast, PR protein, defense enzymes

Introduction
Rice (*Oryza sativa* L.) is number one cereal crop that constitutes staple diet for more than 3.5 billion people all over the world (CGIAR, 2016) [7] and its role in global food and nutritive security is inevitable. Rice production in India accounts for 20 per cent of the world's production, thus standing in second position (172.58 million tonnes) following China and the productivity in India is 3878.2 Kg/ha (FAO, 2018) [10]. An account of 70 different diseases caused by fungi, bacteria, viruses or nematodes have been reported on rice (Zhang *et al*., 2009) [31]. Rice is a major produce for the people in the world. The role of rice is inevitable in the current and future global food security.

The spores produced at later stages of growing season result in collar blast and neck blast (Wang *et al*., 2014a, Wang *et al*., 2014b), which causes about 30% of yield loss (Spence *et al*., 2014). Rice blast (*Magnaporthe oryzae*) is a key concern in combating global food insecurity given the disease is responsible for approximately 30% of rice production losses globally—the equivalent of feeding 60 million people (Nalley *et al*., 2016) [15]. The blast disease caused by *Magnaporthe oryzae* Cav. (anamorph *Pyricularia oryzae* (Cooke) Sacc.) is a major disease of rice with typical spindle shaped lesion having grey white centre and brown border surrounded by yellow halo. The pathogen attack seed, leaf, neck, node etc. It is not only important diseases, but also a great hindrance for the cultivation of rice. The ever-changing climate turned this pathogen into a serious threat to the world production.

The commonly used approaches to deal with blast are to use fungicides or to generate resistant varieties. In India, rice blast management is majorly through use of health and environment hazardous chemicals, management of rice blast has become difficult due to great capacity of pathogen to survive under varied conditions, appearance of new virulent strains, failure of plant breeding and development of fungicide resistance. In addition, the fungus also gains fungicide resistance by mutating the target genes of fungicides (Kim *et al*., 2003). Several plant genes confer rice blast resistance. The use of resistant cultivars is the most effective, economical and environmentally friendly way of controlling rice blast (Tian *et al*., 2016) [29]. In plants with a resistant phenotype, early recognition of the pathogen by the resistance gene product in the host triggers rapid and effective defense responses, such as generation of reactive oxygen species, a localized hypersensitive response, accumulation of phytoalexins and

expression of pathogenesis-related (PR) proteins (Dixon and Harrison, 1990; Staskawicz *et al.*, 1995) [9, 28]. In general, an interaction is incompatible when the rice plant recognizes the invading pathogen early enough through activation of a host resistance gene, resulting in a hypersensitive response (HR) and the triggering of rapid and effective defense responses, including the production of pathogenesis-related (PR) proteins, oxidative enzymes and phytoalexins (Dixon and Harrison, 1990) [9]. The importance of PR proteins in plant defense has been related to: (*a*) their rapid and early accumulation often associated with incompatibility, (*b*) their antimicrobial activity and (*c*) their ability to reduce symptoms development (Schroder *et al*., 1992; Wang *et al*., 2005) [23, 30]. PR proteins accumulate locally in the infected and surrounding tissues and also in remote uninfected tissues. Production of PR proteins in the uninfected parts of plants can prevent the affected plants from further infection. Expression of PR proteins serve as an indicator of the activation of plant defense response. A number of differentially expressed proteins (DEPs) that may be involved in rice response to pathogens were identified, including pathogenisi related proteins. In this view, exploration of defense mechanism through PR protein was studied in twenty five rice differentials. The results are discussed in the paper.

Materials and methods

Isolation and purification of *M. oryzae*

M. oryzae, rice blast causing fungi was isolated from the symptoms showing leaves of susceptible variety, CO 39. The pathogen infected parts of leaves along with healthy portions were cut into pieces of around 1.5cm and surface sterilized using 0.1 per cent mercuric chloride solution followed by sterile distilled water wash. These leaf tissues were dried using sterilized filter paper and transferred into Petri plates plated with potato dextrose agar (PDA) medium. These plates were incubated at 26±2^{0}C for approximately seven days and mycelial growth were observed. Further purification was carried out by single spore isolation technique. The purified isolate were maintained on PDA slants for further studies (Ou, 1985) [16].

Challenge inoculation and sample collection

The study was carried out using twenty five rice differentials *viz.*, C 101 LAC, C 101 A51, C 104 PKT, C 105 TTP-4-L23, RIL 10, RIL 29, *O. minuta*, BL-122, BL-245, A-57, C 101-KPT, Raminad str-3, Zenith, NP-125, Usen, Dular, Kanto 51, Shia-tia-tsao, Calaro, Tadukan, IR 64, Tetep, HR 12, Rasi and CO 39. Seeds collected from Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore were raised in nursery bed. The pathogen, *M. oryzae* was mass multiplied on stem bits of *Paspalum spp.* weed. Later fifteen stem pieces with pathogen were put in 25 ml of sterile water, shaken well and spore suspension was decanted. The decanted spore suspension containing 2 x 10^3 conidia per ml was sprayed with a pinch of CMC (carboxymethyl cellulose) on 20 days old rice seedlings using an automizer. The seedlings were covered with polythene sheets during night hours to create humidity to favor disease development. Plants showing the symptoms of leaf blast were assessed as per the standard evaluation scale (SES) proposed by International Rice Research Institute, Philippines (IRRI, 2002) [11]. The leaf samples were collected 3 days after the occurrence of initial symptom and stored at -20^{0}C.

PR protein extraction and sample preparation for SDS-PAGE

PR proteins were extracted using citrate-phosphate buffer, pH 2.8. One gram of the powdered sample was ground using 1ml of citrate-phosphate buffer, pH 2.8 in a pre-chilled pestle and mortar. The homogenate was centrifuged at 10,000 rpm for 10 min. The supernatant was collected and further used for sample preparations. Hundred microlitre of supernatant was taken and thirty microlitre of 5X sample buffer was added. This mixture was boiled for 5 minutes in water bath at 100^{0}C, cooled rapidly and used as sample.

SDS- PAGE

To study the expression of PR proteins of twenty five entries sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using 4 per cent stacking and 12 per cent separating gel. Each well was loaded with 100 µl of sample and medium range molecular marker (BioLit) was used. Electrophoresis was carried out at 100V. The gels were incubated overnight in the staining solution containing 0.2% Coomassie Brilliant Blue (R250) and then destained with destaining solution containing methanol, acetic acid and distilled water, until the gels turned colourless and the protein bands were clearly visible. Then the gels were documented (Sambrook and Russell, 2001) [21].

Bioassay of defense related enzymes

The blast susceptible variety CO 39 was challenge inoculated with *M. oryzae*. Samples were collected at 24 hours interval for 5 days after the pathogen inoculation. Four enzymes were estimated *viz*., Ascorbate peroxidase, Guaiacol peroxidase, Glutathione reductase and Superoxide dismutase. In the same way twenty four rice differential were challenge inoculated, samples were collected from each differential after 24 hours of inoculation and enzyme activities were measured.

Ascorbate peroxidase enzyme source was prepared by using potassium phosphate buffer and reaction mixture was prepared using hydrogen peroxide. The change in absorbance at 290nm was recorded at 30seconds interval in spectrophotometer. Reaction mixture without enzyme served as blank. Enzyme activity was expressed in units/min/g fresh weight of the sample (Sengar and Chaudhary, 2014) [24].

$$\text{Units/min/g fresh weight} = \frac{\text{Change in absorbance /minute X Total volume (ml)}}{\text{Extinction coefficient X Volume of sample taken (ml)}}$$

Extinction coefficient = 2.8 $mM^{-1}cm^{-1}$

Guaiacol peroxidase activity was assessed spectrophotometrically following the method given by Putter (1974) [17]. The enzyme activity was noted at 436nm**.** Enzyme activity was expressed in units/L of enzyme extract.

$$\text{Enzyme activity units/l} = \frac{3.18 \text{ X } 0.1 \text{ X} 1000}{6.39 \text{ X } 1 \text{ X minutes to increase the absorbance by } 0.1 \text{ X } 0.1}$$

Glutathione reductase enzyme activity was analysed spectrophotometrically. The enzyme was extracted by grinding 0.3g of sample in 1ml of 1% Bovine Serum Albumin and centrifuged at 12,000rpm at 4 ^{0}C at 15minutes. Supernatant was separated and served as enzyme source. The reaction mixture contained 0.65ml of distilled water, 1.5ml of reagent A (100mM potassium phosphate buffer with 3.4mM EDTA), reagent B (30mM Glutathione substrate solution), 0.35ml of reagent C (0.8mM β- NADPH), 0.30 ml of reagent D (1% BSA) and 0.1ml of reagent E (enzyme solution).

Change in absorbance was recorded at 340nm at 30seconds interval. The enzyme activity was expressed in units/ ml enzyme extract.

$$\text{Enzyme activity Units/ml of enzyme extract} = \frac{(\Delta A\ 340nm/min\ test - \Delta\ A\ 340nm/min\ blank) \times 3 \times df}{6.22 \times 0.1}$$

df = dilution factor
Extinction coefficient = 6.22

Superoxide dismutase activity was assayed using a procedure described by Beauchamp and Fridovich (1971) [5]. Enzyme was extracted by grinding 1g of fresh leaf tissue in 10ml of ice cold 50mm potassium phosphate buffer, pH 7.8 in a pre-chilled pestle and mortar. The homogenate was centrifuged at 10,000rpm at 4ºC for 10min. Supernatant was used as enzyme source within 12 h of extraction. The reaction mixture cocktail containing 50mM potassium phosphate buffer, pH 7.8, 13mM methionine, 2 µM riboflavin, 0.1mm EDTA, 75 µM NBT and crude enzyme extract was prepared. Reaction mixture without enzyme served as blank. All tubes were exposed to 40W bulb for 15min and absorbance was recorded at 560nm immediately. Fifty percent inhibition of the reaction between riboflavin and NBT in the presence of methionine was taken as 1 unit of SOD activity. Enzyme activity was expressed in units/g fresh weight.

Results and Discussion

Reaction of rice differentials to blast pathogen

Host behavior of twenty five differentials as mentioned above were assessed using SES, IRRI, 2002 [11]. Based on lesion size and color, the disease was scored. The susceptible checks CO 39 and HR 12 differentials were highly susceptible (grade 9) as more than seventy five percent area was covered by blast lesions. Differential, Tetep was found to be promising source of resistance as it expressed resistant reaction (grade 2.33) (Figure 1, Plate 1). The results are in accordance with Muralidharan *et al.* (2004) [14] who reported that the rice differentials, Tadukan, Rasi, Tetep and IR 64 are resistant checks for blast disease screening. The results revealing moderately resistant nature of C101LAC and C101A51differentials are in partial conformity with Sere *et al.* (2013) [25] where these entries were found consistently resistant to blast under natural infection over 13 months. In the present study, IR 64 entry expressed moderately resistant reaction, this is partially confirms the reports of Salimah *et al.* (2019) [20] revealing that among seven differentials screened, IR 64 was found resistant. In this study, maximum disease incidence was recorded in CO 39 and HR 12 (score 9). The similar findings of universal susceptibleness of CO 39 variety against blast diseases were recorded by Srinivasachary *et al.* (2002) [27].

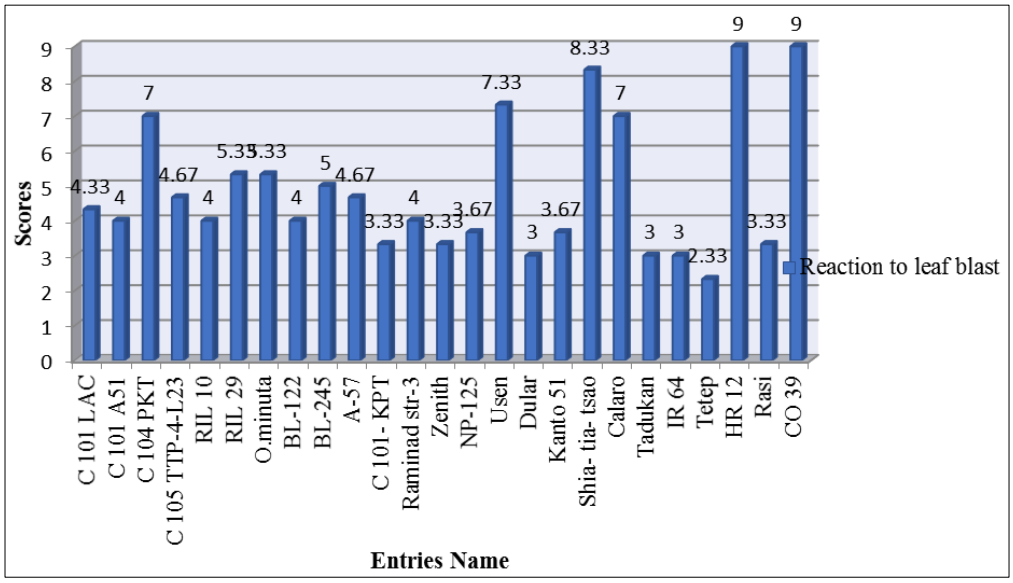
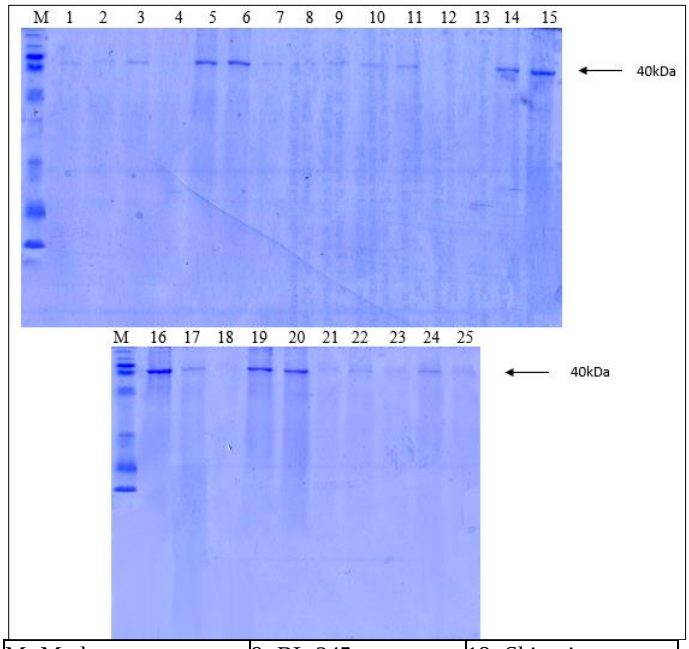


Fig 1: Screening of rice differentials against Coimbatore isolate of *M. oryzae*

Plate 1: Resistant and highly susceptible reaction of differentials

PR protein expression

PR protein induction was studied in twenty five differentials by performing SDS- PAGE. The expression of PR protein was in twenty five differentials varied from nil to medium. Expression of PR protein was observed in RIL 10, RIL 29, NP-125, Usen, Dular, Calaro, Tadukan at medium level (++) where as C104 PKT, A 57, C101- KPT, Kanto 51, Tetep and Rasi showed low expression (+). It was observed that there was no expression of PR protein in susceptible checks HR 12 and CO 39 (Plate 2). The molecular weight of the expressed PR protein was determined by comparing with standard molecular weight marker and it was found to be 40kDa. The detected PR protein had similarity with PR 11 since both have 40 kDa molecular weight. Thus the expressed PR protein was coinciding with Tobacco 'class V' chitinase (under chitinase class I type). Similar reports of expression of PR proteins in both resistant and susceptible variety was documented by Soh *et al*. (2011) [26] where PR protein expression was found one first day which declined drastically from the next day in case of *Colletotrichum acutatum* challenge inoculated susceptible variety of pepper, PBC 80, but higher and constant amount of PR 10 expession was noticed in resistant variety Yeoju. Sayari *et al*. (2014) [22] also recorded the higher expression of PR proteins (PR-3, PR-5, PR-9, PR-10 and PR-12) in resistant rice variety Tarom when challenge inoculated with *Rhizactonia solani* and expression was very low or unnoticed in susceptible variety Khazar.



M- Marker	9- BL-245	18- Shia- tia- tsao
1- C 101 LAC	10- A-57	19- Calaro
2- C 101 A51	11- C 101- KPT	20- Tadukan
3- C 104 PKT	12- Raminad str-3	21- IR 64
4- C 105 TTP-4-L23	13- Zenith	22- Tetep
5- RIL 10	14- NP-125	23- HR 12
6- RIL 29	15- Usen	24- Rasi
7- *O.minuta*	16- Dular	25- CO 39
8- BL-122	17- Kanto 51	

Plate 2: PR protein expression in price differential

Bioassay of defense related enzymes

Ascorbate peroxidase, guaiacol peroxidase, glutathione reductase and superoxide dismutase were analyzed in susceptible CO 39 variety at different interval (Table 1) and in twenty four differential after 24 hours (Table 2).

Ascorbate peroxidase enzyme activity was found to be highest on second day after pathogen inoculation (5.35 units/ min/ g fresh weight) to CO 39 variety. The enzyme activity was lowest on first day (1.08). Activity at third and fourth day did not have much difference. In present study, among 24 differentials, ascorbate peroxidase activity was highest in pathogen inoculated moderately resistant rice differential RIL 10 (2.29) followed by *O. minuta* (1.73) and lowest in susceptible varieties Usen (0.49) and HR 12 (0.50). The results are in concurring with Caverzan *et al*. (2012) [6] reporting the expression of *ascorbate peroxidase* genes in response to biotic and abiotic stresses. The reports of study conducted by Kumar *et al*. (2013) [12] revealing increased activity of peroxidase enzyme from initial phase of the infection of *P. oryzae* in PB-21 rice genotype is in accordance with the present study results. In rice, Agrawal *et al*. (2003) [1] reported cytosolic ascorbate peroxidase genes are up-regulated upon wounding suggesting that the cytosolic ascorbate peroxidase isozymes play a protective role against stressful conditions such as pathogen attack. Hence, ascorbate peroxidases play an important role in the defense response.

Guaiacol peroxidase activity was investigated in this study in pathogen inoculated CO 39 variety at different intervals. The activity was highest on second day (708.33 units/l of enzyme extract) and reduced as days passed. The lowest was recorded on fifth day (396.83) Guaiacol *peroxidase* responses are directly involved in the protection of plant cells against adverse environmental conditions. Generally, enhanced peroxidase activity was observed after a pathogen attack. Thus the enzyme activity was high at initial stage of pathogen attack. Present study on guaiacol peroxidase activity in twenty four rice differentials showed that the enzyme activity was highest in IR 64 (moderately resistant variety) (1805.56) and low in highly susceptible variety HR 12 (233.33). This result coincide with result of study conducted by Rajalakshmi and Lingaraju (2016) [19] where peroxidase activity was found higher in resistant pigeonpea variety WRP-1 and lower in susceptible variety GS-1 during interaction of *Fusarium udum* and *Heterodera cajani* pathogens. The present findings were also in conformity with Rai *et al*. (2011) [18] who noticed higher activity of peroxidase enzyme in resistant tomato cultivars inoculated with *Fusarium oxysoprum* f.sp. *lycopersici* when compared with susceptible cultivars. In plant systems, peroxidase plays a major role in synthesis of plant cell wall and the enzyme which cross-links phenolic residues in cell wall. Polysaccharides and glycoproteins used to strengthen the cell wall components. This action may represent a part of wound-healing response. Some peroxidase isoenzymes were induced by stress such as high salt, physical wounding and microorganisms. Stimulated peroxidase activity was also detected after plant treatment with pathogen metabolites and increase of activity coincides with high H_2O_2 level (Lebeda *et al*., 2001) [13].

Glutathione reductase enzyme activity in pathogen inoculated susceptible CO 39 variety was highest on second (0.47) and third (0.48) day after inoculation. The result of enzyme activity in pathogen inoculated twenty four differential showed that the activity was highest in moderately resistant differential RIL 10 (1.11) and lowest in susceptible differential Calaro (0.03), Usen (0.04) and HR 12 (0.05). The resistant differential had higher enzymatic activity than susceptible differential, thus by exhibiting higher enzyme activity it defends against blast pathogen. The results are in accordance with Debona *et al*. (2012) [8], analysis glutathione reductase activity in wheat infected with *Pyricularia oryzae*

revealed that enzyme activity was higher in partially resistant variety BRS 229. As reported by Anjum *et al*. (2010) [3] glutathione reductase plays an essential central role in cell defense against reactive oxygen metabolites.

Superoxide dismutase enzyme activity in pathogen inoculated CO 39 variety was high at third day after pathogen inoculation (44.71), thus it defends the plants immediately after inoculation of pathogen. Among 24 differentials, the enzyme activity was highest in moderately resistant differential Kanto 51 (48.05) and lowest in susceptible differential Usen (28.53). Resistant to moderately resistant differential had high enzymatic activity compared to susceptible differentials. Similar study on superoxide dismutase activity by Anushree *et al*. (2016) [4] reported concurring results to the present study revealing the highest superoxide dismutase activity in blast pathogen inoculated resistant rice variety KJT-5 and lowest in inoculated susceptible variety Ek-70. The enzyme superoxide dismutase constitutes the first line of defence against ROS by catalyzing the dismutation of O_2 to O_2 and H_2O_2 (Alscher *et al*., 2002) [2].

Table 1: Enzyme activity in pathogen inoculated susceptible rice variety CO 39

Days after pathogen inoculation	Ascorbate peroxidase activity* (units/min/g fresh weight)	Guaiacol peroxidase activity* (units/l of enzyme extract)	Glutathione reductase activity* (units/ml enzyme extract)	Superoxide dismutase activity* (units/g fresh weight)
Day 1	1.08^{c} (1.04)	416.67^{b} (20.34)	0.06^{b} (0.24)	11.76^{c} (3.43)
Day 2	5.35^{a} (2.29)	708.33^{a} (26.61)	0.47^{a} (0.68)	33.99^{ab} (5.80)
Day 3	3.26^{b} (1.80)	424.60^{b} (20.56)	0.48^{a} (0.69)	44.71^{a} (6.68)
Day 4	3.01^{b} (1.72)	424.60^{b} (20.56)	0.42^{a} (0.64)	33.20^{b} (5.73)
Day 5	2.25^{b} (1.50)	396.83^{b} (19.84)	0.40^{a} (0.61)	32.94^{b} (5.74)
CD(.05)	0.43	3.16	0.22	0.88
SEd	0.19	1.42	0.10	0.40

* Values are mean of three replications

Figures in parentheses represent square root transformation.

Table 2: Estimation enzyme activity in rice differentials challenge inoculated with *M. oryzae*

S. No.	Entry Name	Ascorbate peroxidase activity* (units/min/g fresh weight)	Guaiacol peroxidase activity* (units/L of enzyme extract)	Glutathione reductase activity* (units/ ml enzyme extract)	Superoxide dismutase activity* (Units/g fresh weight)
1.	C 101 LAC	1.14^{bcdefg} (1.05)	639.71^{ef} (24.59)	0.46^{bc} (0.67)	40.24^{efg} (6.42)
2.	C 101 A51	1.31^{bcd} (1.14)	916.67^{bcdef} (29.78)	0.07^{ghi} (0.27)	47.75^{hi} (5.58)
3.	C 104 PKT	0.68^{fgh} (0.81)	282.16^{g} (16.76)	0.08^{fghi} (0.30)	39.64^{fgh} (5.98)
4.	C 105 TTP-4-L23	1.21^{bcdef} (1.10)	1166.67^{b} (33.77)	0.41^{bc} (0.64)	44.74^{abcd} (7.11)
5.	RIL 10	2.29^{a} (1.48)	972.22^{bcde} (31.03)	1.11^{a} (1.05)	42.04^{fgh} (5.98)
6.	RIL 29	1.44^{b} (1.18)	839.95^{bcdef} (28.55)	0.13^{fgh} (0.36)	38.14^{fgh} (6.13)
7.	*O.minuta*	1.73^{ab} (1.29)	1166.67^{b} (34.11)	0.20^{def} (0.44)	42.04^{j} (4.25)
8.	BL-122	1.66^{ab} (1.26)	960.32^{bcdef} (29.97)	0.34^{cd} (0.59)	48.05^{fgh} (5.98)
9.	BL-245	0.77^{defgh} (0.87)	849.21^{bcdef} (29.07)	0.05^{ghi} (0.23)	45.65^{cde} (6.83)
10.	A-57	1.42^{b} (1.18)	791.67^{bcdef} (27.57)	0.29^{cde} (0.53)	40.24^{hi} (5.64)
11.	C 101- KPT	1.37^{bc} (1.16)	1097.22^{bcd} (32.48)	0.10^{fghi} (0.29)	47.75^{gh} (5.87)
12.	Raminad str-3	1.27^{bcde} (1.11)	1027.78^{bcde} (31.95)	0.66^{b} (0.75)	39.64^{bcde} (6.89)
13.	Zenith	0.68^{fgh} (0.82)	613.10^{ef} (24.70)	0.11^{fgh} (0.34)	44.74^{ef} (6.53)
14.	NP-125	1.62^{ab} (1.26)	631.61^{def} (25.10)	0.98^{a} (0.99)	42.04^{abcd} (7.29)
15.	Usen	0.49^{h} (0.70)	500.00^{fg} (22.36)	0.04^{hi} (0.21)	28.53^{ab} (7.45)
16.	Dular	0.71^{efgh} (0.84)	1000.00^{bcde} (31.62)	0.11^{fghi} (0.33)	38.14^{i} (5.22)
17.	Kanto 51	0.65^{fgh} (0.80)	1083.33^{bc} (32.87)	0.15^{efg} (0.39)	48.05^{a} (7.62)
18.	Shia- tia- tsao	0.51^{h} (0.71)	1111.11^{b} (33.19)	0.09^{fghi} (0.29)	45.65^{gh} (5.86)
19.	Calaro	0.56^{h} (0.75)	500.00^{fg} (22.36)	0.03^{i} (0.17)	40.24^{abcd} (7.29)
20.	Tadukan	0.64^{gh} (0.79)	666.67^{cdef} (25.45)	0.04^{hi} (0.20)	47.75^{de} (6.71)
21.	IR 64	1.29^{bcde} (1.13)	1805.56^{a} (42.06)	0.32^{cd} (0.57)	39.64^{abc} (7.39)
22.	Tetep	0.64^{gh} (0.80)	1027.78^{bcde} (31.95)	0.12^{fgh} (0.34)	44.74^{abcd} (7.16)
23.	HR 12 (Susceptible check)	0.50^{h} (0.70)	233.33^{g} (15.25)	0.05^{hi} (0.21)	28.53^{ab} (7.43)
24.	Rasi	0.78^{cdefgh} (0.88)	1111.11^{b} (33.19)	0.12^{fgh} (0.35)	38.14^{h} (5.84)
	CD(.05)	0.30	7.72	0.16	0.58
	SEd	0.15	3.84	0.08	0.29

* Values are mean of three replications

Figures in parentheses represent square root transformation

Conclusion

Blast, a severe threat to rice crop is very difficult to manage. Screening of differentials helped in identifying a resistant source Tetep, which can be further used in contemporary resistance breeding. Activation of defense mechanism was observed in resistant to moderately resistant crops by the production of PR proteins and higher activity of defense enzymes such as ascorbate peroxidase, guaiacol peroxidase, glutathione reductase and superoxide dismutase.

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