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Defense reaction and biochemical interpretation of rice genotypes to sheath blight, *Rhizoctonia solani* Kuhn

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Abstract
Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world. Sheath blight disease of rice caused by *Rhizoctonia solani* Kuhn AG-1 IA (Telomorph, *Thanatephorus cucumeris* Frank. Donk) is a devastating disease in all rice-growing regions of the world. The yield losses ranging from 4-50% have been reported depending on the crop stage at the time of infection, the severity of the disease, and environmental conditions. Landraces are valuable genetic resources to explore novel genetic variations and they are highly adaptive. Considering the significance of this disease the present study was conducted at College of Agriculture, V.C. Farm, Mandya during *Kharif* 2021 and *Summer* 2022. Ninety landraces and ten popular varieties of rice were screened against sheath blight disease by artificial inoculation method. Disease scoring was done by the standard evaluation system (SES) as per IRRI (2002). The mean percent disease index (PDI) ranged from 11.11 to 79.56%. Based on AUDPC values, rice genotypes were categorized into 5 groups *i.e.*, resistant, moderately resistant, moderately susceptible, susceptible, and highly susceptible. Further to know the resistance mechanism, 34 selected genotypes were analyzed for biochemical constituents by using standard procedure and protocols and it was found that phenol (-0.79), total soluble sugar (-0.84), reducing sugar (-0.87), crude protein (-0.84), tannin (-0.91), peroxidase (-0.67), polyphenol oxidase (-0.83), and phenylalanine ammonia lyase (-0.84) was negatively correlated with sheath blight disease severity and these biochemicals were higher in resistance genotypes *viz*., Kalanamak and Sidda sanna compare to susceptible genotypes.

Keywords: Sheath blight, rice, artificial inoculation, screening, resistance mechanism, *Rhizoctonia solani*

Introduction
Rice (*Oryza sativa* L.) is a member of the Poaceae family and belongs to the genus *Oryza*. Rice is grown in about 113 countries around the world. It is the primary food crop of the countries of the south and southeast. As the theme "Rice is Life" suggests, rice is the single most important staple food crop for more than one-third of the world's population and more than half of India's population. It is the staple food for more than two-thirds of the Indian population, and as such, it holds the key to food security and plays an important role in the national economy. Rice demand is expected to rise indefinitely as the world's population grows (Mahajan *et al*. 2017) [29]. In India, rice is grown under a variety of agro-ecological conditions (Maclean 2002) [28]. Insect pests, bacterial, viral, and fungal pathogens, as well as abiotic stresses such as ozone, heat, UV-B, heavy metals, and others, all pose a significant threat to rice yield. Rice sheath blight (ShB) is a fungal disease that has become a major concern in recent decades (Molla *et al*. 2020) [35]. The rice sheath-blight (ShB) pathogen, *Rhizoctonia solani* (Kuhn) *Thanatephorus cucumeris* (Frank.) (Donk), is a major production constraint for rice in India and most rice-growing Asian countries (Devi *et al*. 1989) [12]. Sheath blight of paddy is one of the most common paddy diseases. Miyaki was the first to report this disease from Japan in 1910. However, this disease was first reported in India by Paracer and Chahal in Gurdaspur (Punjab) in 1963. The disease's severity has increased due to intensive and changed cultivar practices. It is a potentially devastating rice disease in all temperate and tropical rice production regions, particularly in irrigated systems

(Dath 1990) [10]. There have been several estimates of yield reduction due to sheath blight, ranging from 5.2 to 69%. (Hori and Anraku 1969) [18]. It is a significant production constraint in highly tillering, fertilizer-responsive high yielding varieties and hybrids grown in intensive rice production systems. Depending on the disease's severity and the environment, yield loss of 4-50% has been reported (Singh *et al*. 2004) [44].

Despite being helpful in treating disease, frequent use of chemical control negatively affects both people and the environment by contaminating soils, above- and below-ground water resources, and the entire food supply chain. The creation of disease-resistant cultivars is one strategy for long-term disease management without the use of pesticides. Disease-resistant cultivars have advantages such as decreased disease incidence and higher grain yields. The absence of sources for resistance in farmed rice or in closely related wild species is mainly responsible for the only moderately successful resistance breeding efforts against ShB to date (Yellareddygari *et al*. 2014) [54]. Numerous researchers have screened thousands of germplasm samples for the sheath blight pathogen, but so far there has been no evidence of absolute resistance in rice germplasm. *Oryza* species, both wild and landrace, have untapped alleles that may hold great promise for the development of Asian rice. It is thought that landraces are a rich source of features that are both commercially significant and resistant to a variety of stresses (Willocquet and Savary 2011) [53].

It is high time to find solutions to combat the disease in order to reduce rice yield losses and, which in turn, reduce the threat to global food security. One alternative method for avoiding the use of hazardous and toxic chemical fungicides is the search for resistant germplasm. Landraces are traditional rice genotypes maintained and cultivated by farmers. They are potential sources of resistant donors and they possess traits potentially adaptable to a wide range of biotic and abiotic stresses, which can be used for breeding rice varieties with durable resistance. So, there is a need to screen local varieties and landraces to explore novel genetic resistance source. In addition to these the proper understanding of biochemical changes during infection process of a pathogen helps to know the crucial information in combating the disease. With these mandates, the present investigation was designed to screen rice genotypes and to estimate possible biochemical changes during sheath blight infection in healthy and inoculated leaves in local landraces and popular rice cultivars.

Materials and Methods

Experimental design

The field experiment was carried out at 'A' block, College of Agriculture, V. C. Farm, Mandya, Karnataka, India during *Kharif* 2021 and summer 2022 to screen ten popular cultivars and ninety landraces of rice for resistance against sheath blight with artificial inoculation of *R. solani*. A total of 90 land races were collected from All India Coordinated Research Project, (AICRP) on rice, ZARS, V. C. Farm, Mandya which were used for the study.

Artificial Inoculation

The inoculum of *R. solani* was applied (mass multiplied in sorghum grains) at tillering stage (30 days after transplanting). For the mass multiplication of inoculum, the sorghum grains were boiled until the grains were half opened and from these 500 g of boiled sorghum were taken in 1000 ml conical flask. The flasks were sterilized in an autoclave at 1.1 kg / cm^2 (121 °C) pressure for 20 minutes. Two to three, 5 mm mycelia bits of *R. solani* were transferred to the flask containing sterilized sorghum grains under aseptic conditions and kept for incubation at 27±1 °C for 10 days. The flasks were agitated regularly to obtain a uniform growth all over the flask. The sorghum grains with fungal growth were used for field inoculations on 30 day old plants (depending on the maturity group of the rice genotypes) by dropping five grains coated with fungal growth and sclerotial bodies gently in the middle of the hill. High humidity (>90%) was maintained throughout the disease development period by constantly maintaining water logged condition and closer spacing of 15 x 10 cm. The disease incidence was studied on 10 plants for each entry.

Disease assessment

Data on disease severity were recorded on five different dates at 7 days intervals *i.e*., 35, 42, 49, 56, and 63 days after transplanting (DAT) by using field key 0-9 scale Where, 0 = free from infection; 1 = Vertical spread of the lesions up to 20% of plant height; 3 = 21–30%; 5 = 31-45%; 7 = 46–65%; 9 = more than 65% (IRRI 2013), these scales was converted to percent disease index (PDI) by using the formula given by Wheeler (1969) and computed for area under disease progress curve (AUDPC). Based on mean disease severity and AUDPC, all genotypes were categorized into five different reactions, resistant (R) for scale 1, moderately resistant (MR) for scale 3, moderately susceptible (MS) for scale 5, susceptible (S) for scale 7 and highly susceptible (HS) for scale 9 (Pavani *et al*. 2020) [42].

$$\text{Percent disease index} = \frac{\text{Sum of the individual rating}}{\text{No. of plants examined} \times \text{Maximum disease scale}} \times 100$$

Area under disease progress curve (AUDPC)

The "Area under Disease Progress Curve" would be calculated by using the formula suggested by Johnson and Wilcoxson (1982) [22].

$$AUDPC = \sum_{i=1}^{n-1} [\{(X_i + X_{(i+1)}) / 2\} \times (t_{(i+1)} - t_i)]$$

X_i = disease index expressed as a proportion at the i^{th} observation.

t_i = time (days after transplanting) at the i^{th} observation

Estimation of bio-chemicals and enzymes

Collection of plant samples

This experiment was carried out to know the different bio-chemicals associated with resistance in rice genotypes against sheath blight. After grading for disease, 34 rice genotypes (*viz*., 2 R, 10 MR, 10 MS, 10 S, and 2HS) were selected based on resistance categories representing each group with varied level of infection for biochemical component *viz*., total sugar, reducing sugar, total phenols, tannins and crude protein, and also for enzymes *viz*., peroxidase, polyphenol oxidase, phenylalanine ammonia lyase activity by using standard procedure and protocols. The biochemical constituents in the test genotypes were correlated with disease severity to establish the relationship. The samples of inoculated and healthy leaves were collected

and dried at 35 °C in hot air oven for 24 to 48 hrs. The dried samples were ground using mixer grinder. The powdered samples were stored in plastic covers until analysis, whereas, fresh samples were used for enzyme analysis.

Estimation of total and reducing sugars

The total and reducing sugars in each test genotype was estimated by the method suggested by Somogyi (1952) [46]. For estimating the total sugars, hydrolysis of non-reducing sugars to reducing sugars was done by adding one ml of 1.0N hydrochloric acid to one ml of plant extract and was heated on a boiling water bath at 50 °C for 20 minutes. Later, it was cooled and a drop of phenolphthalein indicator solution was added. Then 1.0N sodium hydroxide was added drop wise till the solution turned pink due to excess alkali. The excess alkali was re-neutralized with 0.1N hydrochloric acid, which was added drop wise till the solution turns colourless.

One ml of hydrolysate for total sugars and one ml of plant extract for reducing sugars was taken separately in boiling tubes to which one ml of freshly prepared alkaline copper reagent was added and boiled in a water bath for exactly 20 minutes. After cooling, one ml of arsenomolybdate reagent was added with immediate mixing. The volume was made up to 15 ml with distilled water and the blue colour developed was read at 510 nm using UV double beam spectrophotometer. A standard curve was prepared with glucose, which was used to calculate the unknown. The quantities were expressed as milligrams per gram of the plant sample.

Estimation of total phenols

Estimation of total phenols in leaf samples of test genotypes was done by following Folin-Ciocalteau method suggested by Bray and Thorpe (1954) [7]. One ml of Folin-Ciocalteau reagent was added to 1.0 ml of the alcohol extract of the plant sample in a test tube followed by 2.0 ml of 20 percent sodium carbonate solution and the mixture was heated on a boiling water bath for exactly 1 minute. It was later cooled and made up to known volume (20 ml) with distilled water. The blue colour developed was measured in a UV double beam spectrophotometer at 650 nm. The standard curve was prepared using catechol and concentration of phenols present in different samples of the genotypes was calculated using the standard curve and expressed as milligrams per gram of the plant sample.

Estimation of tannins

Estimation of tannins in leaf samples of test genotypes was done by following Folin-Denis method suggested by Bray and Thorpe (1954) [7]. 0.5g of the powdered material was weighed and transferred to a 250ml conical flask and 75ml water was added. The flask was gently heated and boiled for 30 min and the mixture was centrifuged at 2000 rpm for 20 min. The supernatant was collected in 100 ml volumetric flask and the volume was made up to 100 ml. 1ml of the sample extract was transferred to a 100 ml volumetric flask containing 75 ml water. Five ml of Folin-Denis reagent, 10 ml of sodium carbonate solution were added and diluted to 100 ml with water and shake well. The absorbance at 700 nm was read after 30 min. Tannin content was calculated as tannic acid equivalents from standard graph and expressed in percent.

Estimation of crude protein content

0.5 g of finely powdered oven dried samples was taken in the digestion tubes. To this 1-2 g of digestion mixture and 10-15 ml conc. H_2SO_4 and digested samples were added in Kjheldahl digestion assembly till a light bluish green residue was obtained. Then the content was cooled and some distilled water was added. Receiving flask was placed at the receiving end of distillation unit. The digestion was loaded on tube along with digested sample to the distillation apparatus one at a time. By keeping all reserve tanks loaded with appropriate reagents such as 4% boric acid with mixed indicator and 40% NaOH the content was distilled for 6 minutes and the released ammonia is collected in boric acid solution by programming the instrument. Once the distillation was completed, the receiving flask was removed and titrated against standard H_2SO_4 till the colour changes from green to pink.

Crude protein was calculated by the formula

Crude protein (g %) = % N x 5.95 (conversion factor for rice)

Then, the percentage crude protein was expressed in terms of mg/gm of the sample.

Estimation of Peroxidase (PO) activity

The peroxidase enzyme activity was assayed by spectrophotometer method as described by Hartee (1955). One gram of leaf sample was homogenized in 3 ml Phosphate buffer (0.1 M), at 4 °C and pH 6.5. This blend was filtered through the muslin cloth of 4 layers. The filtrate was centrifuged at 4°C at 12000 rpm for 20 min. The supernatant was collected and used to estimate the activity of peroxidase. The activity of the enzyme was expressed as a change in fresh weight absorption at 420 nm min^{-1} g^{-1}.

Estimation of polyphenol oxidase (PPO) activity

The polyphenol oxidase activity was determined as per the procedure suggested by Mayer *et al*. (1965). 0.2 ml of enzyme extract was taken and 0.1 ml of sodium phosphate buffer (pH 6.5) was added and 0.2 ml of 0.01 M of catechol was added. The absorbance was recorded at 495 nm after an interval of 30 seconds up to 3 minutes.

Estimation of phenylalanine ammonia lyase (PAL) activity

Leaf tissue (100 mg) was homogenized with 2 ml ice cold 0.1 M Tris HCl buffer (pH 7.5) contained 1mM EDTA, 1% PVP, 10 mM β-mercaptaethanol with pestle and mortar. The homogeneous was then centrifuged at 10,000 g for 15 minutes at 4°C and the obtained supernatant was used for enzyme assay. The reaction mixture contained 2.7 ml of 0.03 M of L-phenylalanine prepared in a buffer of 0.05 M of sodium borate and 0.3 ml of enzyme. The combination of the reaction was incubated for 1 hour at 30 °C. The one-hour incubation free reaction mixture served as control. The absorbance was recorded at 290 nm after stopping the reaction with 0.3 ml of 1N HCl.

Statistical analysis

The data obtained from biochemical analysis for resistance to sheath blight disease were subjected to correlation and regression analysis (Panse and Sukhatme 1967) [39].

The correlation and regression coefficients and p values thus obtained were used to interpret the relationship between biochemical and resistance in different rice genotypes. Further, the mean data on biochemical constituents were processed after suitable transformation and subjected to ANOVA (Gomez and Gomez 1984) [15] and means were separated by Tukey's HSD (Tukey 1965) [49].

Results and Discussions

Screening of rice genotypes for sheath blight resistance under field conditions during *Kharif* 2021 and summer 2022

In the present study, the 10 popular cultivars and 90 landraces of rice were screened for sheath blight resistance under open field conditions with artificial inoculation of *R. solani*. On the basis of mean PDI and area under disease progress curve (AUDPC), all the genotypes were grouped into five categories *i.e*., resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible.

Reaction of popular rice cultivars and landraces during *kharif* 2021

During *kharif* 2021, the mean PDI ranged from 11.11 to 79.56. The PDI ranging from 11.11to 19.11 were categorized as resistant genotypes with scale 1. Whereas, in moderately resistant categories (scale 3), the PDI ranged between 19.11 and 33.33. Likewise, in moderately susceptible categories (scale 5) the PDI varied from 33.33 to 55.56 percent. However, PDI ranging from 55.56 to 59.10 percent were categorized as susceptible (scale 7) and PDI ranged from 59.10 to 79.56 percent were regarded as highly susceptible (scale 9).

Out of 100 genotypes were screened for sheath blight disease resistance, AUDPC ranged from 318.89 to 2434.44. Among them two genotypes with scale 1 showed less AUDPC ranging from 318.89-552.22 which depicted significantly resistant among all the genotypes. Likewise, AUDPC ranged from 552.22-987.78 were categorized as moderately resistant with scale 3. Whereas, in moderately susceptible categories (scale 5), the AUDPC ranged from 987.78-1656.67. However, AUDPC ranging from 1656.67 - 1765.56 were categorized as susceptible (scale 7) and AUDPC ranged from 1765.56-2434.44 reacted as highly susceptible (scale 9).

Reaction of popular rice cultivars and landraces during *summer* 2022

During *summer* 2022, the mean PDI ranged from 11.11 to 78.67 percent. The PDI ranging from 11.11 to 17.33 percent were categorized as resistant genotypes with scale 1. Whereas, in moderately resistant categories (scale 3), the PDI ranged between 17.33 and 33.33. Likewise, in moderately susceptible categories (scale 5) the PDI varied from 33.33 to 54.67 percent. However, PDI ranging from 54.67 to 57.33 percent were categorized as susceptible (scale 7) and PDI ranged from 57.33 to 78.67 percent were regarded as highly susceptible (scale 9).

Out of 100 genotypes were screened for sheath blight disease resistance, AUDPC ranged from 318.89 to 2403.33. Among them two genotypes showed resistance with AUDPC ranged from 318.89-551.11. Likewise, AUDPC ranging from 551.11-972.22 were categorized as moderately resistant with scale 3. Whereas, in moderately susceptible categories (scale 5) the AUDPC ranged from 972.22-1641.11. However, susceptible genotypes recorded AUDPC in the range of 1641.11-1703.33 and highly susceptible showed AUDPC ranging from 1703.33-2403.33 (scale 9).

Landraces are excellent genetic resources for researching novel genetic variation that addresses crop production challenges. They are highly adaptable and valuable genetic resources for pathogen resistance, as well as potential donors, disease tolerance, and various abiotic stresses for breeding (Newton *et al*. 2011) [38].

A similar result was observed by Nagaraju (2013) [37], who screened 139 genotypes under natural epiphytotic conditions at ARS, Siruguppa, and he found that none of the genotypes were immune to *R. solani*. Only Aditya, Vikramarya, Swarnadhan, Ajaya, and Nidhi genotypes were resistant. Goswami *et al*. (2019) [17] screened 261 rice germplasm lines for resistance to sheath blight. Rice germplasm lines were classified as resistant 57 (262.93-957.92), moderately resistant 169 (957.93-1220.87), moderately susceptible 14 (1220.88-1490.81), susceptible 18 (1490.82-1753.75), and highly susceptible 3 (1490.82-1753.75) based on AUDPC values (1753.76–2016.69).

The current findings were also consistent with those of Turaidar *et al*. (2017) [51], who tested 30 landraces for sheath blight disease and found that none of the genotypes scored between 0 and 1. Three landraces had a moderately susceptible reaction on a scale of 5, fifteen landraces had a susceptible reaction on a scale of 7, and eleven landraces had a highly susceptible reaction on a scale of 9. Similarly, Singh and Borah (2000) [43] tested sixty Assamese upland rice cultivars against rice sheath blight in Titabar, India. They discovered that only one cultivar, Chingdar, was resistant. Seven cultivars (As 93-1, Mairan, N-22, Panjasali, Up-52, Upland-2, and 1/69-70) were moderately resistant, while the rest were susceptible.

Pavani *et al*. (2020) [42] used natural conditions to screen 196 germplasm after inoculation with a virulent isolate of *R. solani* (RS 49). No entries were found to be immune or resistant. Fifty-seven entries were found to be moderately resistant, moderately susceptible, and the remaining entries to be highly susceptible. In the current study, 100 genotypes were screened using *R. solani* inoculation. None of the genotypes tested positive for immunity. However, two genotypes, Kalanamak and Sidda sanna, exhibit resistant reaction with scale 1. With a scale of 3, 38 genotypes showed a moderately resistant reaction, while 33 genotypes were classified as moderately susceptible (scale 5). Twenty-five genotypes were found to be susceptible (scale 7), while two genotypes, Kottayyam and TRVs biladadi martiga, were found to be highly susceptible (scale 9).

Table 1: Disease reaction of rice genotypes against sheath blight

Scale	Reaction	No. of genotypes	Mean PDI		AUDPC		Genotypes
			Kharif	Summer	Kharif	Summer	
0	Immune	0	Nil	Nil	Nil	Nil	Nil
1	R	2	11.11-19.11	11.11-17.33	318.89-552.22	318.89-521.11	Kalanamak, Sidda sanna
3	MR	38	19.11-33.33	17.33-33.33	552.22-987.78	521.11-972.22	Ambe mohar, Bigan munji, Basumathi, Coimbatore, Duddoge, Gandha sale 1, Gangadale, Game, Jawahar, Kaduvelpe, Kundi pullan, Laalya, Muththina sanna, Mise batta, Moradda, Manjula sona, Maplilai samba 1, Murkhanna sanna, Nawali, Naland paddy, Narali, Padma rekha 2, Putta batta, Sanna mallige, Selam sanna, Sona masuri, Theerthalli local, Gud batta 2, Tulasiya, TRVs murkhana sanna, Ugi batta, White sticky, BR2655, MTU1001, MTU1010, IR 64, Gangavathi, Rajmudi
5	MS	33	33.33-55.56	33.33-54.67	987.78-1656.67	972.22-1641.11	Bangara sanna, Bheema sanna 2, Boo jaddu, Chinne ponni 2, Chinne ponni 5, Doddabyra, Dappa playa, Esdali, GK 1, Gk 9 light brown, Gujarath basamati, Gulwadi sannaaki, Jadda batta, Joopvadly, Karimndaga, Kempunellu, Kagisale 1, Kadulile, Kyasare, Kari swarna, Mysore mallige 1, Mallige, Mullu batta, Mapilai samba 2, Val bag sughanda, Navalisale, Nazar bat, Rajbhoga, Sarjana, TRVs valtgya gidda, Jaya, KRH 4, Rajmudi kempu
7	S	25	55.56-59.10	54.67-57.33	1656.67-1765.56	1641.11-1703.33	Aishwarya, Akkalu, Adri batta, Thanu, Bilikanna hegge, Bebbanna, Bili nellu, Bidagi kannappa, Budda, Coimbatore sanna 1, Dodda batta, Danggaia, Hasnudi, Ittan gidda, Jeerige batta, Malkod, Mobikar, PB Local, PSB 887, Raichur sanna, Rajakime, Jyothi, Sanna rajakime, TRV jasmine, Vanasu
9	HS	2	59.10-79.56	57.33-78.67	1765.56-2434.44	1703.33-2403.33	Kottayyam, TRVs biladadi martiga

Biochemical studies

This experiment was carried out to understand various biochemicals and enzyme associated with resistance in rice genotypes against sheath blight. Leaf samples were collected from 34 selected rice genotypes (*viz*., 2 R, 10 MR, 10 MS, 10 S, and 2HS) for biochemical and enzyme analysis.

Estimation of Total and reducing sugars

Total soluble sugars in healthy and sheath blight inoculated plants were found to range from 6.91 mg/g to 16.20 mg/g and 6.62 mg/g to 15.58 mg/g respectively. Resistant genotypes (Sidda sanna and Kalanamak) showed more soluble sugar of 14.96 to 16.20 mg/g in healthy leaves and less soluble sugar of 14.08 mg/g to 15.58 mg/g in inoculated leaves respectively. In moderately resistant genotypes, total soluble sugars in healthy leaves and inoculated leaves range from 9.89 mg/g to 13.58 mg/g and 8.83 mg/g to 12.28 mg/g respectively. Similarly, moderately susceptible genotypes showed soluble sugar ranges from 9.85 mg/g to 11.85 mg/g in healthy leaves and 9.04 mg/g to 11.94 mg/g in inoculated leaves. In susceptible genotypes, total soluble sugars in healthy and inoculated plans were found to range from 7.09 mg/g to 9.60 mg/g and 6.74 mg/g to 9.30 mg/g respectively. However, highly susceptible genotypes *viz*., Kottayam and TRVs Biladadi martiga showed more soluble sugar of 7.04 mg/g to 6.91 mg/g in healthy leaves and less soluble sugar of 6.92 mg/g to 6.62 mg/g in inoculated leaves respectively and it was found that increasing trend of total soluble sugar in resistant genotypes compare to highly susceptible genotypes. The findings of correlation studies showed that total soluble sugars had a negative significant influence (r = -0.84) on percent disease severity.

With regard to reducing sugar content in healthy and sheath blight inoculated plants were found to range from 2.29 mg/g to 11.46 mg/g and 2.54 mg/g to 9.31 mg/g respectively. Resistant genotypes, Sidda sanna and Kalanamak showed more reducing sugar of 11.46 to 10.42 mg/g in healthy leaves and less reducing sugar of 9.31 mg/g to 9.00 mg/g in inoculated leaves respectively. In moderately resistant genotypes, total reducing sugars in healthy leaves and inoculated leaves range from 8.69 mg/g to 9.54 mg/g and 7.62 mg/g to 8.85 mg/g respectively. Similarly, moderately susceptible genotypes showed reducing sugar ranges from 7.62 mg/g to 8.85 mg/g in healthy leaves and 6.23 mg/g to 7.62 mg/g in inoculated leaves. In susceptible genotypes, total reducing sugars in healthy and inoculated plants were found to range from 4.54 mg/g to 7.31 mg/g and 2.54 mg/g to 4.54 mg/g respectively. However, highly susceptible genotypes *viz*., Kottayam and TRVs Biladadi martiga showed reducing sugar of 3.31 mg/g to 3.36 mg/g in healthy leaves and less soluble sugar of 3.15 mg/g to 3.62 mg/g in inoculated leaves respectively and it was found that decreasing trend of reducing sugar in highly susceptible genotypes compare to resistant genotypes. The findings of correlation studies showed that total soluble sugars had a negative significant influence (r= -0.87) on percent disease severity.

A high level of total soluble sugars in plant tissues, such as sucrose and monosaccharides, boosts the plant's immune response to fungal pathogens. They serve as activators of pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) and effector-triggered immunity (ETI) in plants (Morkunas and Ratajczak 2014) [36]. The decrease in reducing sugar content in susceptible genotypes compared to resistant genotypes may be due to the preferential

utilization by the fungus and it is confirmed by the findings of Mains (1917) [30] who reported a similar process in corn rust.

The current findings of more total soluble sugar in resistant genotypes compared to susceptible genotypes agreed with Patil *et al*. (1985) [41], who reported that the decrease in sugar quantity in susceptible genotypes may be attributed to fungus utilisation for growth. Gopalakrishnan *et al*. (2010) [16] observed that 28 percent reduction in the total sugar content of rice seeds due to *Sarocladium oryzae* infection in susceptible varieties whereas it was 17 percent in resistant varieties. The results agreed with those of Lenka *et al*. (2018) [26], who found a decrease in total soluble sugar and reducing sugar in the inoculated leaf sheath tissues of a sheath blight susceptible variety 'Tapaswini' when compared to healthy leaf sheaths of rice. They also reported that the soluble sugar content of the tolerant variety 'Pankaj' was higher than that of the susceptible variety 'Tapaswini'.

Estimation of total phenol content

Total phenol content ranging from 0.26 to 1.82 mg/g and 0.29 to 1.91 mg/g in healthy and inoculated leaves respectively. Resistant genotypes (Sidda sanna and Kalanamak) showed less phenol content ranging from 1.66 to 1.82 mg/g in healthy leaves and more total phenol content of 1.81 to 1.91 mg/g in inoculated leaves respectively. An increasing trend of total phenol content was noticed in inoculated leaves compared to healthy leaves and they were found on par with each other. In moderately resistant genotypes, total phenol content in healthy leaves and inoculated leaves ranged from 0.67 to 1.06 mg/g and 0.73 mg/g to 1.16 mg/g respectively. Similarly, moderately susceptible genotypes showed lower total phenol content ranging from 0.32 to 0.75 mg/g in healthy leaves and higher total phenol content ranging from 0.50 to 0.91 mg/g in inoculated leaves.

In healthy leaves of susceptible genotypes, total phenol content was recorded which ranged from 0.26 to 0.82 mg/g. whereas, higher total phenol content was recorded in inoculated leaves ranging from 0.29 to 0.89 mg/g respectively. However, highly susceptible genotypes *viz*., Kottayam and Biladadi martiga showed least total phenol content of 0.28 to 0.29 mg/g in healthy leaves and 0.36 to 0.33 mg/g in in leaves and it was found that increasing trend of total phenol content in resistant genotypes compared to highly susceptible genotypes. The findings of correlation studies showed that total phenol content had a negative significant influence (r= 0.79) on percent disease severity.

The higher content of phenol in the resistant genotypes may be the possible reason for limiting the pathogenesis of the pathogen, thus reducing the disease, and vice versa in the susceptible genotypes, so that the disease was higher due to lower phenol content.

Phenol compounds play critical roles in plant growth and development, particularly in defence mechanisms, and the majority of phenolic compounds have potent antioxidant properties that help to mitigate the effects of oxidative stress (Tuladhar *et al*. 2021) [50]. Phenolic compounds are secondary metabolites produced by plants. They aid plants in their defence against pathogens. The accumulation of these secondary metabolites in plants may play an important role in plant defence response. Their activity is linked to antimicrobial properties, cell wall reinforcement, modulation, and the induction of plant responses (Aly *et al*. 2002) [2]. One of the major factors for incompatible host pathogen interactions is the presence of a high concentration of phenolic compounds (Farkas and Kirlay 1962). The plant cells synthesize phenol oxidising enzymes that oxidise phenols to toxic quinones upon pathogen infection, which play a key role in disease resistance (Ashry and Mohamed 2011) [4] and it was evident from the present investigation.

Table 2: Biochemical activity in healthy and sheath blight inoculated leaves of rice genotypes

Sl. No.	Genotypes	Reaction	PDI	Total sugar (mg/g)		Reducing sugar (mg/g)		Phenol (mg/g)		Tannin (mg/g)		Crude protein (mg/g)	
				Healthy	Inoculated	Healthy	Inoculated	Healthy	Inoculated	Healthy	Inoculated	Healthy	Inoculated
1.	Kalanamak	R	11.11	16.20^{a}	15.58^{a}	10.42^{b}	9.00^{ab}	1.66^{b}	1.81^{a}	2.01 ^{a}	2.29^{a}	85.22^{a}	89.65^{a}
2.	Sidda sanna		19.11	14.96^{ab}	14.08^{b}	11.46^{a}	9.31^{a}	1.82^{a}	1.91^{a}	1.94 ^{ab}	2.25^{ab}	80.47^{b}	88.30^{b}
3.	Ambe mohar	MR	23.56	13.58^{bc}	10.74^{ef}	9.54^{c}	8.08^{def}	0.86d^{e}	1.06^{b}	1.76 ^{abc}	2.18^{abc}	74.97^{d}	81.63^{c}
4.	Bigan munji		29.78	9.89^{ghijkl}	8.83^{ijk}	9.02^{cdef}	8.54^{bcd}	1.04^{c}	1.16^{b}	1.91 ^{abc}	2.20^{abc}	71.64^{f}	76.64^{f}
5.	Duddoge		25.78	12.28^{cde}	12.02^{cd}	9.15^{cde}	7.62^{fgh}	0.81^{ef}	0.93^{b}	1.90 ^{abc}	2.15^{abcd}	76.64^{c}	78.30^{e}
6.	Coimbatore		31.56	12.49^{cde}	12.28^{c}	9.30^{cd}	8.85^{abc}	0.75^{efg}	0.75^{e}	1.85 ^{abcd}	2.14^{abcd}	71.64^{f}	74.97^{g}
7.	Game		32.44	12.57^{cd}	11.89^{cde}	9.00^{cdef}	8.69^{bc}	0.81^{ef}	0.91^{bcd}	1.89 ^{abc}	2.16^{abcd}	73.30^{e}	81.63^{c}
8.	Mise batta		31.11	11.38^{defgh}	10.74^{ef}	9.15^{cde}	8.85^{abc}	1.06^{c}	1.12^{b}	1.77 ^{abcdefg}	2.14^{abcd}	74.97^{d}	79.97^{d}
9.	Narali		26.22	11.21^{degh}	11.09^{cdef}	8.85^{defg}	8.38^{cde}	0.67^{gh}	0.73^{ef}	1.80 ^{abcde}	2.04^{cdef}	76.64^{c}	81.63^{c}
10.	BR2655		36.00	10.43^{fghij}	10.32^{fgh}	9.00^{cdef}	7.92^{efg}	0.85^{def}	0.93^{b}	1.76 ^{abcdefg}	2.11^{bcde}	74.97^{d}	79.82^{d}
11.	Tulasiya		33.33	12.45^{cde}	10.36^{fgh}	8.69^{efg}	8.08^{def}	0.97^{cd}	1.08^{b}	1.75 ^{abcdefg}	2.09^{bcde}	72.02^{f}	76.85^{f}
12.	MTU1010		31.56	11.72^{def}	10.83^{ef}	8.85^{defg}	8.38^{cde}	0.68^{gh}	0.76^{ef}	1.76 ^{abcdefg}	2.04^{cdef}	67.14^{g}	73.30^{h}
13.	Bangara sanna	MS	34.22	11.85^{cdef}	10.87^{def}	7.77^{ij}	7.31^{h}	0.52^{jkl}	0.59^{ghi}	1.78 ^{abcdef}	2.05^{cdef}	56.64^{j}	64.97^{k}
14.	Bheema sale 2		43.56	9.89^{ghijkl}	9.04^{ij}	8.54^{fgh}	7.46^{gh}	0.52^{jkl}	0.57^{hi}	1.75 ^{abcdefg}	1.96^{efgh}	38.32^{s}	41.65^{u}
15.	Esdali		46.22	11.12^{defgh}	10.74^{ef}	8.08^{hi}	7.31^{h}	0.61^{hij}	0.66^{fg}	1.77 ^{abcdefg}	2.01^{ef}	49.98^{r}	58.31^{n}
16.	Kempunellu		36.89	10.83^{defghi}	10.57^{fg}	7.62^{ij}	6.23^{j}	0.63^{ghij}	0.71^{ef}	1.66 ^{bcdefg}	1.92^{fghi}	39.78^{r}	46.65^{s}
17.	Jaddabatta		37.78	10.70^{efghi}	10.45^{fgh}	8.38^{gh}	6.38^{j}	0.52^{jkl}	0.60^{ghi}	1.63 ^{bcdefg}	1.91^{fghi}	46.65^{o}	53.31^{p}
18.	Chinne ponni 2		44.00	10.15^{fghijk}	9.47^{ghi}	8.54^{fgh}	7.46^{gh}	0.72^{fgh}	0.76^{ef}	1.63 ^{cdefg}	1.89^{fghi}	41.65^{q}	46.65^{s}
19.	Jaya		43.56	9.85^{ghijkl}	9.04^{ij}	8.85^{defg}	6.69^{ij}	0.75^{efg}	0.80^{cde}	1.66 ^{bcdefg}	1.90^{fghi}	54.98^{k}	58.31^{n}
20.	KRH 4		38.67	11.62^{defg}	11.26^{cdef}	8.08^{hi}	7.48^{gh}	0.64^{ghi}	0.76^{ef}	1.65 ^{cdefg}	1.95^{efgh}	64.97^{h}	69.97^{j}

21.	Kadulile		39.11	12.30^{cde}	11.94^{cde}	7.62^{ij}	7.25^{hi}	0.45^{lm}	0.50^{ij}	1.76 ^{abcdefg}	1.97^{efg}	54.98^{k}	64.97^{k}
22.	Joopvadly		36.89	10.45^{fghij}	9.43^{ghi}	7.77^{ij}	7.62^{fgh}	0.32^{n}	0.91^{bcd}	1.65 ^{cdefg}	1.90^{fghi}	66.64^{g}	71.64^{i}
23.	Adri batta	S	56.00	9.60^{hijkl}	9.30^{hi}	4.85^{n}	2.77^{nop}	0.34^{mn}	0.38^{jk}	1.66 ^{bcdefg}	1.80^{hijkl}	45.32^{p}	51.65^{q}
24.	Aishwarya		56.00	8.15^{lmnop}	7.77^{klmn}	6.01^{m}	2.85^{nop}	0.33^{n}	0.40^{jk}	1.63 ^{cdefg}	1.84^{ghij}	38.32^{s}	43.32^{t}
25.	Bidagi kannapa		60.00	7.09^{nop}	6.74^{mno}	7.31^{jk}	4.54^{k}	0.30^{n}	0.43^{jk}	1.54 ^{efgh}	1.66^{klm}	53.31^{l}	63.31^{l}
26.	Mobikar		56.00	9.09^{ijklm}	8.83^{ijk}	6.08^{m}	3.00^{nop}	0.26^{n}	0.29^{l}	1.54 ^{efgh}	1.62^{m}	39.98^{r}	53.31^{p}
27.	Budda		56.00	8.47^{jklmn}	8.49^{ijkl}	6.54^{jk}	3.77^{l}	0.29^{n}	0.37^{k}	1.56 ^{defg}	1.65^{lm}	36.65^{t}	48.31^{r}
28.	Danggaia		57.33	8.19^{lmnop}	7.85^{jklm}	6.85^{kl}	3.31^{mn}	0.82^{ef}	0.89^{bcd}	1.50 ^{fgh}	1.63^{m}	39.78^{r}	46.65^{s}
29.	Thanu		56.00	7.60^{mnop}	7.44^{lmno}	6.38^{lm}	2.54^{p}	0.55^{ijkl}	0.65^{fg}	1.65 ^{cdefgh}	1.82^{hijkl}	48.31^{n}	51.65^{q}
30.	Vanasu		56.00	9.60^{hijkl}	7.81^{jklmn}	6.20^{m}	2.58^{op}	0.35^{m}	0.42^{jk}	1.57 ^{defgh}	1.76^{ijklm}	58.31^{i}	60.98^{m}
31.	Hasnudi		56.44	8.83^{jklmnop}	8.36^{ijkl}	4.69^{n}	2.69^{op}	0.45^{lm}	0.50^{ij}	1.52 ^{efgh}	1.61^{m}	48.31^{n}	54.98^{o}
32.	PB local		56.44	8.40^{klmnop}	8.43^{ijkl}	4.54^{n}	2.85^{nop}	0.37^{mn}	0.40^{jk}	1.63 ^{cdefgh}	1.77^{ijklm}	46.65^{o}	51.65^{q}
33.	Kottayam	HS	79.56	7.04^{op}	6.29^{o}	3.36^{o}	3.62^{lm}	0.28^{n}	0.36^{k}	1.45 ^{gh}	1.63^{m}	36.65^{t}	39.98^{v}
34.	TRVs biladadi martiga		69.78	6.91^{p}	6.62^{no}	3.31^{o}	3.15^{mno}	0.29^{n}	0.33^{k}	1.49 ^{h}	1.70^{klm}	35.49^{u}	36.65^{w}
	SE m ±			0.10	0.16	0.09	0.09	0.02	0.02	0.05	0.02	0.12	0.13
	CD @p=0.05			0.38	0.61	0.35	0.34	0.07	0.07	0.19	0.10	0.48	0.19

*Values in the column followed by common letters are non-significant at p=0.05 as per DMRT

The higher content of phenol in the resistant genotypes may be the possible reason for limiting the pathogenesis of the pathogen, thus reducing the disease, and vice versa in the susceptible genotypes, so that the disease was higher due to lower phenol content (Manjunatha *et al*. 2021) [31]. Similar findings were reported by Dahima *et al*. (2014) [9] and Anushree *et al*. (2016) [3], who found that total phenol accumulation was higher in inoculated rice genotypes compared to healthy ones, and it was significantly higher in blast-resistant genotypes. Following *Rhizoctonia solani* Kuhn f.sp. *sasakii* infection, Akhtar *et al*. (2011) [1] observed that resistant cultivars had higher phenolic content than susceptible cultivars.

Estimation of tannin content

Tannin content showed significant difference among genotypes for different categories and it ranged from 1.45 to 2.01 mg/g in healthy leaves and 1.61 to 2.29 mg/g in inoculated leaves. In resistant genotypes (Kalanamak and Sidda sanna) showed higher tannin content of 2.01 to 1.94 mg/g in healthy leaves and 2.29 to 2.25 mg/g in inoculated leaves respectively. However, tannin content was significantly increased in resistant genotypes compared to susceptible and highly susceptible genotypes. The tannin content in moderately resistant genotypes showed less in healthy leaves, ranging from 1.75 to 1.91 mg/g compare to inoculated leaves which ranged from 2.04 mg/g to 2.20 mg/g. Similarly, moderately susceptible genotypes follow the same trend where tannin content in healthy leaves and inoculated leaves ranged from 1.63 to 1.78 mg/g and 1.89 to 2.05 mg/g respectively. Further, susceptible genotypes recorded lower tannin content in healthy leaves which ranged from 1.50 to 1.66 mg/g. Whereas, higher tannin content was noticed in inoculated leaves from 1.61 to 1.84 mg/g. However, highly susceptible genotypes *viz.*, Kottayam and Biladadi martiga showed less tannin content of 1.45 to 1.49 mg/g in healthy leaves and 1.63 to 1.70 mg/g in inoculated leaves and it was found that tannin content was high in resistant genotypes compare to highly susceptible genotypes. Results from the correlation studies revealed that tannin content had a negative significant influence (r= -0.91) on percent disease severity. Hence, higher tannin content attributes to sheath blight disease resistance.

Tannins are a group of highly hydroxylated phenolic compounds present in the plant. The foremost purpose of tannins in plants is most likely for protecting a wide range of potential phytopathological microorganisms and their extracellular enzymes (Field and Lettinga 1992) [14]. Findings were similar to Kamalakannan *et al*. (2001) [23] recorded higher levels of proteins, glycoproteins, total phenol, and tannin in resistant plants which exhibited greater antifungal properties towards the rice blast pathogen.

Estimation of Crude protein

Crude protein content in healthy and sheath blight inoculated plants ranging from 35.49 to 85.22 mg/g and 36.65 to 89.65 mg/g respectively. Resistant genotypes, Sidda sanna and Kalanamak showed higher crude protein content of 80.47 to 85.22 mg/g in healthy leaves and 88.30 to 89.65 mg/g in inoculated leaves respectively. Likewise, moderately resistant genotypes showed crude protein content of 67.14 to 76.64 mg/g in healthy leaves and in inoculated leaves ranged from 73.30 to 81.63 mg/g. Similarly, moderately susceptible genotypes showed crude protein content ranging from 38.32 to 66.64 mg/g in healthy leaves and 41.65 to 71.64 mg/g in inoculated leaves. In susceptible genotypes, crude protein content in healthy and inoculated leaves was found to range from 36.65 to 58.31 mg/g and 43.32 to 63.31 mg/g respectively. However, highly susceptible genotypes *viz.*, Biladadi martiga and Kottayam showed lower crude protein content of 35.49 to 36.65 mg/g in healthy leaves and 36.65 to 39.98 mg/g in inoculated leaves respectively. It was found that highly susceptible genotypes showed lower crude protein content compare to resistant genotypes.

The findings of correlation studies showed that crude protein had a negative significant influence (r= - 0.84) on percent disease severity. These results showed a negative relationship between crude protein and disease severity in the genotypes. Plants respond to any type of stress, whether biotic or abiotic, by producing more defense-related proteins (Broz *et al*. 2010) [8]. Plants use active, passive, or both defense mechanisms to recognize pests and counter their attacks (Houterman *et al*. 2008) [20]. Proteins play a larger role in plant defense against invading pathogens in both mechanisms (Meena *et al*. 2021) [33]. The current study agreed with Kandan *et al*. (2010) [24], who reported that resistant rice plants had higher crude protein levels. Low disease incidence increases total phenol and soluble protein levels, and protein banding pattern is an important factor in developing resistance to the brown spot pathogen (Bisen *et al*. 2015) [5].

Estimation of Peroxidase enzyme activity (POD)

The peroxidase activity in resistant genotypes was 0.323 to 0.416 Δ Abs min^{-1} g^{-1} in healthy leaves. Whereas, in the inoculated leaves reported higher peroxidase activity ranging from 0.702 to 1.203 Δ Abs min^{-1} g^{-1}. Similarly, peroxidase activity in moderately resistant genotypes was lower in healthy leaves which ranged from 0.211 to 0.364 Δ Abs min^{-1} g^{-1} and it was higher (0.403 to 0.614 Δ Abs min^{-1} g^{-1}) in inoculated leaves. In healthy leaves of moderately susceptible genotypes the peroxidase activity ranged from 0.219 to 0.273 Δ Abs min^{-1} g^{-1} in healthy leaves and 0.271 to 0.416 Δ Abs min^{-1} g^{-1} in inoculated leaves. In susceptible genotypes, peroxidase activity in healthy and inoculated leaves were found to range from 0.155 to 0.235 Δ Abs min^{-1} g^{-1} and 0.297 to 0.414 Δ Abs min^{-1} g^{-1} respectively. However, highly susceptible genotypes *viz*., Biladadi martiga and Kottayam showed lower peroxidase activity of 0.128 to 0.131 Δ Abs min^{-1} g^{-1} in healthy leaves and 0.214 to 0.224 Δ Abs min^{-1} g^{-1} in inoculated leaves respectively. From these results, it reveals that highly susceptible genotypes showed lower peroxidase activity compare to resistant genotypes. Results obtained from correlation studies showed a negative association (-0.67) between peroxidase activity and rice sheath blight disease severity, which indicates that increasing trend of peroxidase activity reduces rice sheath blight disease severity.

Peroxidase is one of the first response enzymes, providing quick defense against pathogens (Sulman *et al*. 2001) [47]. Plant peroxidases participate in a wide range of physiological processes throughout the plant's life cycle. Peroxidases are enzymes that can produce reactive oxygen species, polymerize cell wall compounds, and control H2O2 levels (Passardi *et al*. 2005) [40]. By oxidising phenolics and related compounds, the oxidative enzymes were shown to increase their toxicity. These enzymes are active in inhibiting mycelial elongation, penetration, and colonisation, as well as in spore producing fungi, where they may also inhibit spore germination (Usenik *et al*. 2004) [52]. The findings were supported by Deborah *et al*. (2001) [11], where Peroxidases, Polyphenol oxidases, accumulation of phenolics and lignin were significantly increased in rice leaf sheaths after inoculation with *R. solani*. Similar study was also conducted by Liu *et al*. (2011) [27], who found that PO activity was more important for resistant wheat cultivars compare to susceptible cultivars when inoculated by *Rhizoctonia cerealis*. These reports are in agreement with the present data, which showed higher level of PO activity in resistant genotypes.

Estimation of Polyphenol oxidase (PPO) enzyme activity

The polyphenol oxidase activity in resistant genotypes ranged from 0.952 to 0.978 Δ Abs min^{-1} g^{-1} in healthy leaves. Whereas, in the inoculated leaves polyphenol oxidase activity ranged from 0.994 to 1.262 Δ Abs min^{-1} g^{-1}. Further, polyphenol oxidase activity in moderately resistant genotypes was lower in healthy leaves which ranged from 0.878 to 0.933 Δ Abs min^{-1} g^{-1} and it was higher in inoculated leaves with 0.945 Δ Abs min^{-1} g^{-1} in inoculated leaves. Moderately susceptible genotypes showed polyphenol oxidase activity ranged from 0.715 to 0.905 Δ Abs min^{-1} g^{-1} in healthy leaves and 0.732 to 0.912 Δ Abs min^{-1} g^{-1} in inoculated leaves. Whereas polyphenol oxidase activity in susceptible genotypes was found to range from 0.634 to 0.810 Δ Abs min^{-1} g^{-1} in healthy leaves and inoculated leaves ranged from 0.652 to 0.820 Δ Abs min^{-1} g^{-1}. Further, highly susceptible genotypes *viz.*, Kottayam and Biladadi martiga showed lower polyphenol oxidase activity of 0.603 to 0.610 Δ Abs min^{-1} g^{-1} in healthy leaves and more polyphenol oxidase activity of 0.621 to 0.593 Δ Abs min^{-1} g^{-1} in inoculated leaves respectively. An increasing trend of polyphenol oxidase activity was observed in resistant genotypes. Whereas, polyphenol oxidase activity was decreased in highly susceptible genotypes and these results were supported by correlation studies, which show that polyphenol oxidase activity was negatively associated (-0.83) with rice sheath blight disease severity.

Polyphenol oxidase is important in the early stages of plant defense when membrane damage causes phenol release. PPO catalyses the oxidation of phenolics to free radicals, which can then react with biological molecules, resulting in an unfavourable environment for pathogen development (Mohamed *et al*. 2012) [34]. PPO accumulates in wounded plants to resist pathogen attack, according to Bradley *et al*. (1992) [6]. Sivakumar and Sharma (2003) [45] supported the findings by studying the biochemical changes in banded leaf and sheath blight-affected maize plants caused by *Rhizoctonia solani* f. sp. *sasakii*. When leaf sheaths were inoculated with the pathogen, they found increased Peroxidase (PO), Polyphenol oxidase (PPO), Catechol oxidase (PPO), and Phenylalanine ammonia-lyase (PAL) activities.

Estimation of Phenylalanine ammonia lyase (PAL) enzyme activity

The Phenylalanine ammonia lyase activity was found to be higher in resistant genotypes ranging from 2.580 to 2.990 Δ Abs min^{-1} g^{-1} in healthy plants. Whereas, in the inoculated leaves Phenylalanine ammonia lyase activity ranged from 3.010 to 3.172 Δ Abs min^{-1} g^{-1}. Further, phenylalanine ammonia lyase activity in moderately resistant genotypes healthy leaves ranged from 1.520 to 2.110 Δ Abs min^{-1} g^{-1} and 1.980 to 2.590 Δ Abs min^{-1} g^{-1} in inoculated leaves. Lower phenylalanine ammonia lyase activity was observed in moderately susceptible genotype which ranges from 1.480 to 2.020 Δ Abs min^{-1} g^{-1} in healthy leaves and 1.520 to 2.230 Δ Abs min^{-1} g^{-1} in inoculated leaves. Whereas, phenylalanine ammonia lyase activity in susceptible genotypes was found to range from 1.330 to 1.590 Δ Abs min^{-1} g^{-1} in healthy leaves but in inoculated leaves, it ranged from 1.490 to 1.920 Δ Abs min^{-1} g^{-1}. Further, highly susceptible genotypes *viz*., Kottayam and Biladadi martiga showed least phenylalanine ammonia lyase activity of 0.920 to 0.980 Δ Abs min^{-1} g^{-1} in healthy leaves and 1.110 to 1.210 Δ Abs min^{-1} g^{-1} in inoculated leaves. Phenylalanine ammonia lyase activity was increased in resistant genotypes compare to highly susceptible genotypes and they were negatively associated (-0.84) with rice sheath blight disease severity.

Phenylalanine ammonia lyase (PAL) is the primary enzyme in the phenylpropanoid metabolism and plays a significant role in the synthesis of several defense-related secondary compounds such as phenol and lignin (Tahsili *et al*. 2014) [48]. The studies showed that PAL activity is essential for the accumulation of phenolics in an inoculated plant (Klessig and Malamy 1994) [25]. The study showed that phenol accumulation was also increased due to PAL activity which offererd protection against diseases (Jayaraj *et al*. 2010) [21]. Findings were in close agreement with Pavani *et al*. (2020)

[42], who conducted a field trial to screen 196 rice germplasm lines against sheath blight and recorded Moderately resistant cultivar IC281785 recorded higher peroxidase activity (2.990) in moderately resistant cultivar IC281785 than moderately susceptible cultivar IC282450 (1.232). Similarly, PAL activity was highest in moderately resistant cultivars than moderately susceptible and susceptible cultivars.

Table 3: Enzyme activity in healthy and sheath blight inoculated leaves of rice genotype

Sl. No.	Genotypes	Reaction	PDI	PO (Δ Abs min^{-1} g^{-1})		PPO (Δ Abs min^{-1} g^{-1})		PAL (Δ Abs min^{-1} g^{-1})	
				Healthy	Inoculated	Healthy	Inoculated	Healthy	Inoculated
1.	Kalanamak	R	11.11	0.416^{a}	0.702^{b}	0.952^{a}	0.994^{b}	2.990^{a}	3.172^{a}
2.	Sidda sanna		19.11	0.323^{bcd}	1.203^{a}	0.978^{a}	1.262^{a}	2.580^{a}	3.010^{b}
3.	Ambe mohar	MR	23.56	0.315^{cde}	0.500^{de}	0.918^{abc}	0.934^{bc}	1.980^{bcd}	2.120^{ef}
4.	Bigan munji		29.78	0.313^{bcdef}	0.513^{de}	0.902^{abc}	0.920^{cd}	1.520^{bcdef}	2.090^{efg}
5.	Duddoge		25.78	0.216^{ghi}	0.423^{fgh}	0.933^{ab}	0.945^{bc}	2.020^{bc}	2.150^{de}
6.	Coimbatore		31.56	0.211^{ghi}	0.614^{c}	0.925^{abc}	0.931^{bc}	1.820^{bcd}	2.100^{ef}
7.	Game		32.44	0.364^{ab}	0.614^{c}	0.904^{abc}	0.920^{cd}	1.860^{bcd}	2.230^{d}
8.	Mise batta		31.11	0.243^{defgh}	0.487^{def}	0.925^{abc}	0.930^{bc}	1.630^{bcde}	2.490^{c}
9.	Narali		26.22	0.248^{defg}	0.445^{efg}	0.911^{abc}	0.920^{cd}	2.110^{b}	2.590^{c}
10.	BR2655		36.00	0.343^{abc}	0.528^{d}	0.878^{bcd}	0.899^{cd}	1.890^{bcd}	2.090^{efg}
11.	Tulasiya		33.33	0.319^{bcde}	0.489^{def}	0.902^{abc}	0.915^{cd}	1.770^{bcd}	1.980^{hi}
12.	MTU1010		31.56	0.255^{cdefg}	0.403^{ghi}	0.915^{abc}	0.920^{cd}	1.960^{bcd}	2.030^{d}
13.	Bangara sanna	MS	34.22	0.263^{cdefg}	0.416^{gh}	0.715^{abc}	0.732^{ijk}	2.020^{bc}	2.110^{ef}
14.	Bheema sale 2		43.56	0.238^{defgh}	0.381^{ghijk}	0.820^{def}	0.850^{def}	1.960^{bcd}	2.230^{d}
15.	Esdali		46.22	0.235^{efgh}	0.385^{ghij}	0.850^{bcd}	0.880^{cde}	1.720^{bcd}	1.980^{hi}
16.	Kempunellu		36.89	0.238^{defgh}	0.322^{jklmn}	0.905^{abc}	0.912^{cd}	1.760^{bcd}	1.790^{klmn}
17.	Jaddabatta		37.78	0.238^{defgh}	0.329^{jklmn}	0.795^{ef}	0.801^{fgh}	1.730^{bcd}	1.820^{jklm}
18.	Chinne ponni 2		44.00	0.273^{cdefg}	0.363^{hijklm}	0.770^{fgh}	0.792^{fghi}	1.820^{bcd}	1.850^{jk}
19.	Jaya		43.56	0.263^{cdeg}	0.314^{klmn}	0.810^{def}	0.819^{efg}	1.800^{bcd}	1.980^{hi}
20.	KRH 4		38.67	0.247^{defg}	0.405^{ghi}	0.815^{def}	0.823^{efg}	1.700^{bcd}	1.990^{ghi}
21.	Kadulile		39.11	0.255^{cdefg}	0.271^{nop}	0.800^{def}	0.802^{fgh}	1.480^{bcdef}	1.520^{i}
22.	Joopvadly		36.89	0.219^{ghi}	0.319^{jklmn}	0.802^{def}	0.815^{efg}	1.590^{bcdef}	1.730^{lmno}
23.	Adri batta	S	56.00	0.235^{efgh}	0.31^{lmn}	0.810^{def}	0.820^{efg}	1.460^{bcdef}	1.920^{ij}
24.	Aishwarya		56.00	0.205^{ghi}	0.316^{klmn}	0.705^{hijk}	0.722^{jk}	1.500^{bcdef}	1.690^{no}
25.	Bidagi kannapa		60.00	0.217^{ghi}	0.333^{jklmn}	0.786^{ef}	0.810^{fg}	1.360^{cdef}	1.560^{p}
26.	Mobikar		56.00	0.223^{fgh}	0.285^{no}	0.634^{kl}	0.652^{lmn}	1.490^{bcdef}	1.730^{lmop}
27.	Budda		56.00	0.197^{ghi}	0.308^{mn}	0.716^{ghij}	0.722^{jk}	1.510^{bcdef}	1.830^{jkl}
28.	Danggaia		57.33	0.199^{ghi}	0.301^{mn}	0.709^{ghijk}	0.738^{hijk}	1.390^{cdef}	1.570^{p}
29.	Thanu		56.00	0.191^{ghi}	0.355^{hijklm}	0.689^{ijk}	0.697^{kl}	1.410^{cdef}	1.720^{mno}
30.	Vanasu		56.00	0.214^{ghi}	0.414^{gh}	0.711^{ghijk}	0.728^{ijk}	1.560^{bcdef}	1.680^{o}
31.	Hasnudi		56.44	0.223^{fgh}	0.37^{hijkl}	0.759^{fghi}	0.784^{ghij}	1.590^{bcdef}	1.720^{mno}
32.	PB local		56.44	0.155^{hi}	0.297^{mn}	0.664^{jkl}	0.678^{klm}	1.330^{def}	1.490^{p}
33.	Kottayam	HS	79.56	0.131^{i}	0.214^{p}	0.610^{l}	0.621^{mn}	0.920^{f}	1.210^{q}
34.	TRVs biladadi martiga		69.78	0.128^{i}	0.221^{op}	0.603^{l}	0.593^{n}	0.980^{ef}	1.110^{q}
	SE m ±			0.04	0.01	0.01	0.01	0.11	0.01
	CD @p=0.05			0.05	0.03	0.05	0.04	0.44	0.05

Conclusion

Till date there is no rice germplasm completely resistance to sheath blight disease caused by *R. solani*. Our study was an effort to screen the rice genotypes and to analyse the activity of total sugar, reducing sugar, total phenols, tannins, crude protein, peroxidase, polyphenol oxidase, phenylalanine ammonia lyase activity to understand the biochemical changes during infection process of pathogen which helps to know the mechanism of resistance development in rice plant. Out of 100 genotypes screened, 2 were resistant, 10 moderately resistant, 10 moderately susceptible, 10 susceptible and 2 highly susceptible genotypes. Resistant genotypes (Kalanamak and Sidda sanna) recorded higher level of phenol (-0.79), total soluble sugar (-0.84), reducing sugar (-0.87), crude protein (-0.84), tannin (-0.91), peroxidase (-0.67), polyphenol oxidase (-0.83), and phenylalanine ammonia lyase (-0.84) activity compared to susceptible genotypes (TRVs biladadi martiga and Kottayam). From the present investigation it was concluded that, the biochemical activity during rice-*Rhizoctonia* interaction serve as valuable tool for determining the resistance and susceptibility in rice genotypes.

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