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BIOCHEMICAL AND ANATOMICAL CHARACTERISTICS OF BASMATI AND NON-BASMATI RICE (*ORYZA SATIVA* L.) FOR RESISTANCE TO FOOT ROT

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Abstract

Fusarium fujikuroi, the causal agent of foot rot disease, infects all the parts of rice, causing serious yield losses in different parts of the world. In order to understand the basis of disease tolerance, various biochemical and anatomical traits of ten days old healthy and infected basmati and non-basmati rice seedlings were compared. Total soluble sugar content was higher in healthy seedlings, while total phenols, orthodihydroxy phenols and total soluble proteins were higher in non-basmati rice seedlings post infection indicating the initiation of defence against the pathogen. Increase in the total antioxidant activity, enzymes and α-tocopherols in basmati rice cultivars post infection indicate the oxidative stress created by pathogen. Histopathological observations revealed hyphal and conidial growth in infected tissues of basmati cultivars whereas a complete and intact tissue was observed in healthy and infected counterparts of non-basmati cultivars.

Introduction

Rice (*Oryza sativa* L.) is the major cereal crop belonging to *Poaceae* having an average yield of 6193 Kg/ha in Punjab. Foot rot also called as Bakanae or foolish seedling disease caused by fungus *Fusarium fujikuroi* is probably the first disease of rice that has been causing yield losses with a range between 3 and 95.4 per cent depending on region and cultivar. The most characteristic symptom of this disease is the appearance of tall thin tillers in rice plants, hyper-elongation of shoots, pale green flag leaf and yellowish green leaves, dry and limited leaves at later stages of infection. The pathogen secretes the gibberellic acid that in turn causes the abnormal elongation of rice seedlings (Chhabra *et al.* 2019).

Fusarium fujikuroi is a polycyclic ascomycete which reproduces sexually and asexually through conidia and ascospores within a sac known as ascus (Duncan and Howard 2010). This fungus grows intercellularly in all plant parts and finally reaches and spreads in the field. It initiates infection in the rice seedlings through roots and the base of the stem; later becomes systemic by multiplying within the infected tissues (Puyam *et al.* 2017). The pathogen might enter the host by piercing the epidermis forcefully or through natural openings, wounds and insect punctures. Various phylloplane characters such as wax and cuticle act as barriers for penetration of the pathogen and must be broken by the latter if it has to invade the host. The aromatic or scented cultivars have been previously rated as highly susceptible in a study undertaken on screening of various rice cultivars towards foot rot disease (Ghazanfar *et al.* 2013). The pathogen survives in the seed and produces a few phytotoxic chemicals which induce seed deterioration. The *F. fujikuroi* colonisation starts 24 hrs after pathogen inoculation but the tissue gets fully colonised after 48 hrs of inoculation. The host pathogen interactions are often described as defence responses which are usually associated with the activity of various defence related enzymes that generally inhibit the pathogen growth and contributes to plant disease resistance.

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Plants protect themselves against the pathogen by defence response pathways (Saleh and Pleith 2009). Reactive Oxygen species (ROS) are involved in resistance mechanism as they induce activation of defence related genes. Production and accumulation of secondary metabolites particularly phenols and synthesis of defence related proteins takes place. Altered biochemical and enzymatic activities have been reported in plants with various biotic stresses (Chhabra *et al.* 2020). Many technologies have been proposed to reduce yield losses caused by foot rot, but disease management is yet largely dependent upon chemical control measures which may impose hazardous threats to the human health and environment (Poolsawat *et al.* 2012). Due to such ecological challenges, the focus is on cultivars that are tolerant to disease for a sustainable alternate (Boso *et al.* 2010). Hence, the present investigation was undertaken to decipher structural and biochemical traits of basmati and non-basmati rice cultivars for unravelling the basis of disease tolerance and to implement it for breeding programmes through genetic interventions.

Materials and Methods

The study was carried out in the laboratories of the Department of Botany and Seed Pathology laboratory of Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana during the year 2018-19. Two basmati (PUSA 1121 and PUSA 1509) and non-basmati (PR 121 and PR 126) rice cultivars were selected for the present investigation. The seeds of the experimental rice cultivars were inoculated with the virulent isolate of *F. fujikuroi* obtained from Seed Pathology Laboratory, Punjab of the same University. Fourteen days old culture of *F. fujikuroi* grown on PDA was flooded with sterile water and scraped with a sterile spatula separately. The resulting suspensions were pooled and filtered through two layers of sterile muslin cloth and the final concentration was adjusted to 1×10^6 spores/ml and used for inoculation. Before inoculation, rice seeds were treated with hot water for 15 min at 60ºC to remove any surface contaminants (Pannu *et al.* 2012). A total of 1000 seeds of each cultivar were soaked in spore suspension and shaken for 10 min, while control seeds were soaked in autoclaved distilled water for 10 minutes and air dried. The healthy and artificially infested seeds of the rice cultivars were germinated by paper towel method (ISTA 1999) in three replications as per standard procedures.

For proper identification, Pink coloured fungal growth was teased out and placed on the slide and observed under compound microscope at a magnification of 45X. The pathogen was identified morphologically on basis of mycelial and conidial characters using identification keys given in manual for identification of *Fusarium* species (Nelson *et al.* 1983). For anatomical studies, the ten day old healthy and infected seedlings of selected rice cultivars were collected and hand sections of shoot were cut by placing the tissue in the potato pith. These sections were mounted in DPX and were observed under Leica Bright Field Research Microscope fitted with digital camera and computing imaging systems using software NIS Elements F 3.0.

Different biochemical parameters *viz.,* total soluble sugars, total soluble proteins, total phenols, total antioxidant activity, ortho-dihydroxy phenols, α-tocopherol, and enzymatic antioxidants (peroxidase, superoxide dismutase and polyphenol oxidase) were analysed in ten days old healthy and foot rot infected seedlings of the selected rice cultivars. The method of Dubois *et al.* (1956) was followed for estimation of total soluble sugar. Total soluble proteins content was estimated by the standard method given by Lowry *et al.* (1951). Total antioxidant activity was determined according to the method of Patel *et al.* (2010). Alpha tocopherols were assayed according to method of Asthir *et al.* (2009). The total phenol and ortho-dihydroxy phenol content were determined according to the standard methods of Swain and Hills (1959) and Mahadevan and Sridhar (1986) respectively. Polyphenol oxidase was assayed according to method of Zaubermann *et al.* (1991). Peroxidase (POD) and superoxide dismutase (SOD) activity were assayed by the method of Shannon *et al.* (1966) and Marklund and Marklund (1974), respectively.

The experimental design of the study was kept as completely randomised design (CRD). The statistical analysis of physiological and biochemical data had been analysed by applying one way analysis of variance (ANOVA) using SAS software. The means were compared for significance using post hoc analysis by Tukey's honestly significant difference test and the values are presented are mean of three replicates. All pictures were combined by Adobe Photoshop (Adobe Systems Incorporated).

Results and Discussion

Anatomical and biochemical aspects are integral part of host-pathogen interaction studies. An insight into the cross-sections of shoot of 10 days old seedlings indicated non-stratified epidermis constituting cubical cells covered by thick cuticle; the cortex formed by layers of parenchymatous cells. Fungal hyphae were not visible in vessels and cortical region of seedlings with no cellular disintegration or dissolution in healthy tissues and infected tissue of non-basmati seedlings which are tolerant to this disease (Fig. 1). In the non-basmati rice seedlings, the colonization between the adjacent xylem vessels and meristematic tissues was restricted. It might be due to some chemical and structural alterations like tyloses, deposition of wall material, gel plugs and infusion of these structures with phenols and other secondary metabolites (Duncan and Howard 2010). The pathogen is capable of entering intact tissue usually through wounds or natural openings.

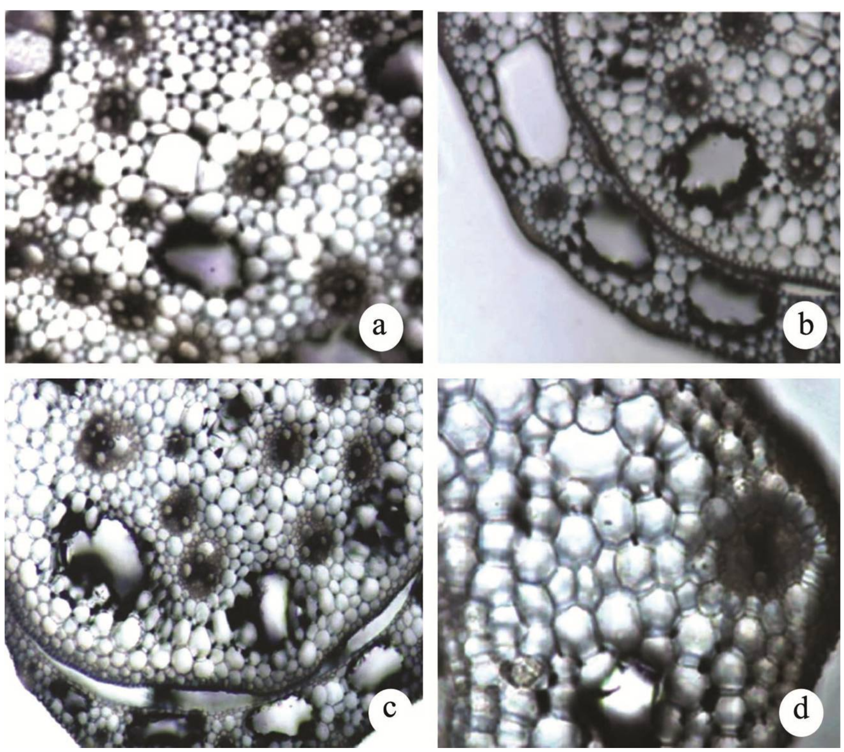

Fig. 1. Transverse sections of healthy and infected seedlings of non-basmati rice cultivars, namely PR 121 and PR 126. (a) PR 121 healthy (b) PR 121 infected (c) PR 126 healthy (d) PR 126 infected

The scented or basmati rice cultivars are more susceptible to foot rot disease as compared to other rice cultivars as revealed by previous varietal screening studies. In infected shoot of PUSA 1121 (Fig. 2), discoloration followed by disintegration of cortical cells and disruption of endodermis was observed. The fungus grew in intercellular spaces of root cortex to reach central root cylinder to enter vascular system. Further colonization might have taken place by hyphal growth and microconidia which are carried in vessels as observed in PUSA 1509 (Fig. 3). Infected seedlings revealed hyphal colonization in some of the xylem vessels. Discoloration of cortex and endodermis disruption was observed only in infected seedlings of basmati cultivars. Infected tissue showed infection and distortion of epidermal (Fig. 3) and mesophyll cells as the pathogen formed microconidia. Presence of fungal mycelium may impede upward translocation of water and minerals, reduction of the photosynthetic rate and yellowing of affected parts during later stages. This may cause premature death of affected tillers and improper filling of grains resulting in chaffy panicles (Chhabra *et al.* 2019). In healthy basmati rice seedlings, there was no damage of cortical cells and epidermal region.

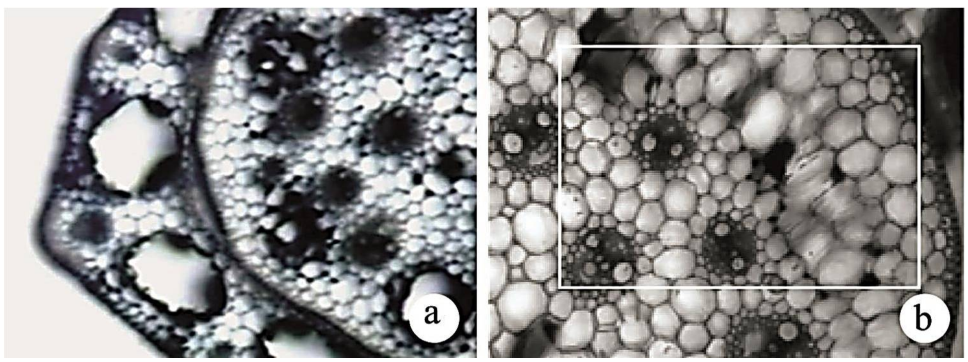

Fig. 2. Transverse sections of healthy and infected seedlings of basmati rice cultivar, namely PUSA 1121 (a) A complete healthy and intact tissue was seen in healthy seedlings (b) Cellular disintegration and dissolution was observed in infected seedlings

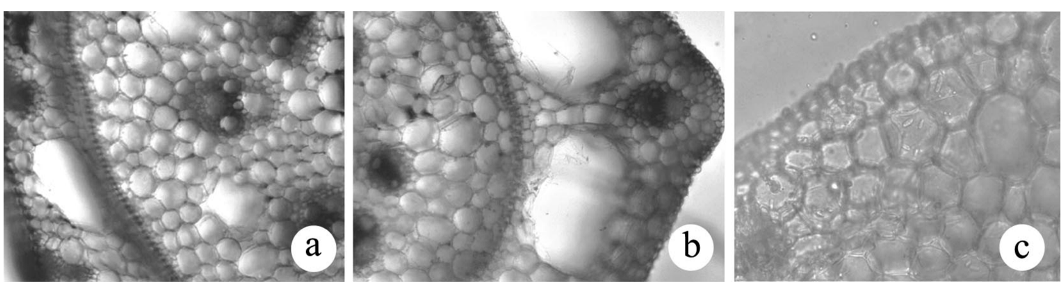

Fig. 3. Transverse sections of healthy and infected seedlings of basmati rice cultivar, namely Pusa 1509 (a) note the complete healthy tissue in uninfected seedlings (b) note the presence of fungal hyphae in cortex and areas adjoining vascular bundles (c) presence of microconidia in epidermal cells.

Data on total phenol content of healthy and infected seedlings of the basmati and non-basmati rice cultivars is presented in Table 1. It is evident from the table that the total phenolic content was remarkably high in the healthy and infected seedlings of non-basmati rice cultivars. Further, it was observed that in post infection, there was reduction in phenolic content in basmati rice seedlings, whereas, an increase in non-basmati rice cultivars. Maximum and significantly higher total phenols were observed in infected seedlings of non-basmati cultivars (54.00 mg/g DW in PR 121 and 52.18 mg/g DW in PR 126) which exhibited tolerant disease response to pathogen infestation. Phenolic compounds are widely distributed in healthy and infected tissues and increase in phenolics can be observed after infection (Ruelas *et al.* 2006) in resistant cultivars. Higher level of both constitutive and inducible phenols was observed by Madhavi *et al.* (2005) in the resistant cultivars of wild sunflower as compared to susceptible cultivars upon infection by *Alternaria helianthi*. The presence of higher phenol content in basmati cultivars indicates their protective role in non-basmati rice cultivars and provides disease resistance.

Table 1. Total phenols, orthodihydroxy phenols. Total antioxidant activity and alpha-tocopherols of healthy and infected rice seedlings.

Cultivars	Total phenols (mg/g DW)		Orthodihydroxy phenols (mg/g DW)		Total antioxidant activity (mg/g DW)		α-tocopherols (mg/g FW)	
	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected
Basmati								
Pusa 1121	13.97^{d}	4.43^{f}	4.59^{b}	2.48^{d}	3.26^{c}	4.22^{abc}	3.73^{e}	5.89^{cde}
Pusa 1509	12.75^{de}	6.45^{ef}	5.61^{a}	3.62^{c}	4.04^{abc}	5.12^{ab}	4.25^{ed}	7.47^{dc}
Non-basmati								
PR121	45.99^{bc}	54.00^{a}	1.25^{e}	2.25^{d}	5.03^{ab}	3.66^{bc}	12.63^{a}	9.19^{abc}
PR 126	42.29^{c}	52.18^{ab}	1.20^{e}	2.16^{d}	5.56^{a}	2.93^{c}	11.38^{ab}	9.07^{bc}
Means	28.75^{b}	29.26^{a}	3.16^{a}	2.62^{b}	4.47^{a}	3.98^{b}	7.99^{a}	7.90^{a}

Least Squares-means with the different letter are significantly different ($P < 0.05$).

Results of Orthodihydroxy phenol content is presented in Table 1 and it was observed the orthodihydric phenol content was high in the healthy seedlings of basmati rice cultivars. As pathogenesis progressed, there was a significant reduction in orthodihydroxy phenols in the infected seedlings of basmati cultivars as compared to the healthy seedlings. In the non-basmati rice cultivars, the infected seedings retained significantly higher orthodihyroxy phenol content as compared to the healthy seedlings (2.25 mg/g DW in PR 121 and 2.16 mg/g DW in PR 126). Orthodihydroxy phenols are considered as potentially toxic substances associated with reduction of multiplication of pathogen. This is in an agreement with the results where the basmati cultivar PUSA 1121 and PUSA 1509 recorded the minimum total phenols and orthodihydroxy phenol content. Bajaj *et al.* (1983) have also reported the role of phenolic compounds in the disease resistance via quinone oxidation mechanism which is more lethal to pathogens.

The data on total antioxidant activity indicate that the total antioxidant activity of the rice seedlings was higher in the infected seedlings of basmati (PUSA 1121 and PUSA 1509) as compared to their healthy counterparts whereas, in the non-basmati rice cultivars (PR 121 and PR 126) the healthy seedlings had higher total antioxidant activity content as compared to their infected counterparts (Table 1).The results further revealed increase in total antioxidant activity post infection in seedlings of basmati rice cultivars PUSA 1121 (4.22 mg/g DW) and PUSA 1509 (5.12 mg/g DW). Any kind of stress causes a number of variations in plant metabolism such as production of reactive oxygen species (ROS) and these are responsible for damaging cell membranes and organelles which eventually leads to cell death. The generation of ROS is scavenged by antioxidant system composed of antioxidant compounds and antioxidant enzymes. Higher antioxidant activity post infection in basmati seedlings may be attributed to the biotic stress created by the pathogen (Saleh and Pleith 2009).

The α-tocopherol content was found to be remarkably low in the healthy seedlings of basmati rice cultivars whereas, infected seedlings of basmati cultivars had higher α-tocopherol as compared to the healthy seedlings (Table 1). Further, the healthy seedlings of non-basmati rice cultivar PR 121 had higher α-tocopherol (12.63 mg/g FW) content which was at par with PR 126 (11.38 mg/g FW). Tocopherols collectively called Vitamin E are primarily of four forms i.e. α, β, γ and δ and among these, α-tocopherol is a form of Vitamin E. The inducers of disease resistance in plants like jasmonic acid modulate the endogenous level of tocopherols in plants (Antognoni *et al.* 2009). The infected seedlings of both the basmati cultivars exhibited higher α-tocopherol as compared to the healthy seedlings.

The total soluble protein content of the healthy and the infected seedlings of basmati and non-basmati rice cultivars is presented in Table 2. The results indicate that in the non-basmati rice cultivars, total soluble protein content was higher in the infected seedlings compared to their healthy counterparts; whereas, in the basmati cultivars, the healthy seedlings had higher total soluble protein content as compared to their infected counterparts. The protein biosynthesis of the host is widely assumed to be a significant feature of pathogenesis, particularly during incompatible reactions (Murria *et al.* 2018). The increases in protein content in basmati cultivars post infection could be attributed to accumulation of pathogenesis related proteins (PRs) which are proteins coded by the host plant and are related with the systemic acquired resistance against further infection by the disease. Induction of PRs suggests their role in adaptation to biotic stress.

Table 2. Total soluble proteins and total soluble sugar content of healthy and infected rice seedlings.

Cultivars	Total soluble proteins (mg/g DW)		Total soluble sugars (mg/g DW)	
	Healthy	Infected	Healthy	Infected
Basmati				
PUSA 1121	97.63^{a}	64.89^{b}	1.52^{ab}	0.117^{c}
PUSA 1509	42.99^{bc}	26.96^{c}	1.32^{ab}	$.872^{abc}$
Non-basmati				
PR 121	65.63^{b}	103.85^{a}	$.992^{ab}$	$.890^{abc}$
PR 126	45.22^{bc}	98.53^{a}	1.57^{a}	$.737^{bc}$
Means	62.87^{b}	73.56^{a}	1.35^{a}	0.654^{b}

Least Squares-means with the different letter are significantly different ($P < 0.05$).

Data on total soluble sugars presented in the Table 2 revealed that there was decline in sugar content of seedlings post pathogen infection in all the rice cultivars under study. On the basis of the mean of total soluble sugar content, it is inferred that healthy seedlings had significantly higher (1.35 mg/g of DW) mean TSS as compared to infected seedling mean (0.654 mg/g of DW). This decrease in sugar content after infection may be due to rapid hydrolysis of sugars during pathogenesis or due to utilization of sugars by pathogen for its establishment and sporulation (Kulkarni *et al.* 2009).

Antioxidant enzymes play an important role in disease reaction and the data on peroxidase (POD), superoxide dismutase (SOD) and polyphenol oxidase (PPO) of healthy and infected seedlings of the rice cultivars (Table 3) revealed that the enzymatic activity in basmati cultivars was higher in the infected seedlings as compared to the healthy ones. However, in non-basmati rice seedlings, a decline in activity of antioxidant enzymes was observed in infected seedlings as compared to healthy ones. In the infected seedlings of basmati cultivars, SOD was significantly higher with (7.32 units/min/mg protein in PUSA 1121 and 10.88 units/min/mg protein in PUSA 1509. The activity of POD was also high observed in infected seedlings of PUSA 1509 (3.22

units/min/mg protein) which was at par with PUSA 1121 (2.60 units/min/mg protein). A similar trend of increase in PPO activity was observed in foot rot infected seedlings of basmati cultivars.

Table 3. Activity of antioxidative enzymes (POD, SOD and PPO) of healthy and infected rice seedlings.

Cultivars	Peroxidase (units/ min/mg/protein)		Superoxide dismutase (units/ min/mg/protein)		Polyphenol oxidase (units/ min/ mg/protein)	
	Healthy	Infected	Healthy	Infected	Healthy	Infected
Basmati						
PUSA 1121	1.42^abc^	2.60^abc^	3.23^c^	7.32^ab^	29.94^abc^	33.04^abc^
PUSA 1509	1.44^abc^	3.22^ab^	4.92^bc^	10.88^a^	25.75^bc^	41.20^a^
Non-basmati						
PR 121	1.44^abc^	1.25^c^	6.27^ab^	2.39^c^	22.05^c^	20.26^c^
PR 126	3.34^a^	1.31^bc^	7.25^ab^	2.26^c^	39.63^ab^	19.18^c^
Means	1.91^b^	2.09^a^	5.41^a^	5.71^a^	29.34^a^	28.42^b^

Least Squares-means with the different letter are significantly different ($P < 0.05$).

The activity of enzymes Peroxidase, Polyphenol oxidase and superoxide dismutase is directly related to the resistance of the host and in infected seedlings of basmati cultivars there was higher activity than in the corresponding healthy seedlings. This might be due to biotic stress created by the pathogen attack (Gurjar *et al.* 2015). Peroxidase and superoxide dismutase are the major antioxidant enzyme directly involved in arresting pathogen development, preventing the advancing of infection or effect on the synthesis of compounds responsible for conferring resistance to the disease. Khatun *et al.* (2009) studied the role of antioxidative enzymes in defence against black spot in *Rosa centifolia* and reported a higher peroxidase and superoxide dismutase activity following inoculation in susceptible cultivar for preventing the spread for pathogen along with mitigating oxidative damage. Hence, the change in the activity of POD, SOD and PPO could be an ideal trait for predicting resistance to foot rot. These biochemical and anatomical traits might play an active role in imparting resistance to non-basmati cultivars and can serve as potential markers for early screening of rice cultivars resistant to this disease.

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