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Evaluating the Impact of Bakanae Disease on Leaf Gas Exchange Physiology of Rice in Resistant and Susceptible Genotypes

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ABSTRACT: Rice is the one main source of nutrition for a larger part of world population but a significant proportion of rice production is lost every year due to various and biotic stress factors. Bakanae disease is an emerging and serious threat to rice production and the remedies are needed of the bakanae disease otherwise it can be a destructive disease. The leaf gas exchange activities are seriously affected by any stress to normal plants. In present study the impact of bakanae disease on various leaf gas exchange activities of rice in two contrasting (resistant & susceptible) genotypes was studied. It was observed that pathogen inoculation inhibited the germination, increased the plant height and decreased the root length in susceptible genotype with no significant changes in resistant genotype. The leaf gas exchange activities were was initially increased in inoculated plants but later it was decreasing as compared to control. The reduction was very high in susceptible genotype with very less reduction in resistant genotype. No significant changes were observed in any activity in control plants of both genotypes at each time interval.

Keywords: Bakanae disease, inoculation, leaf gas exchange, stress, susceptible.

INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food crop for about half of the population of world. It is a major source of food along with wheat and maize throughout the world. Despite the significant increase in rice production and productivity witnessed in the last five decades, a significant proportion of rice production is lost every year due to various abiotic (*viz.,* high or low temperature, drought and salinity) and biotic (*viz*., pathogen infection and insect herbivory) stress factors (Kreye *et al.,* 2009; Devine 2009). Diseases like bacterial blight, sheath blight, blast, brown spot, bakanae and false smut are of major economic significance. Among these bakanae disease caused by *Fusarium fujikuroi* is emerging as a serious threat to production of rice (Singh and Sunder 2012). Hori (1898) first time demonstrated the fungus *Fusarium heterosporium* Nees induced the bakanae symptom in rice plants. The disease is incited by *Fusarium fujikuroi* Nirenberg (anamorph) (teleomorph: *Gibberella fujikuroi* Sawada, Wollen worth). This disease is emerging as a major threat to rice cultivation in India, Japan, Taiwan and Thailand (Bashyal *et al.*, 2016; Saremi 2005; Kini *et al.,* 2002). In India, the prevalence and incidence of bakanae disease has been reported very recently particularly in basmati rice cultivars (Bashyal *et al*., 2014). The yield losses ranging from15-25% have been reported from UP, Assam, AP, TN, Haryana and Punjab states of India (Gupta *et al*., 2014; Pannu *et al*., 2012; Sunder *et al*., 2014). Bakanae disease reduces photosynthesis in rice by causing yellowing of leaves which results in reduction in chlorophyll content (reducing photosynthetically active area) and down-regulation of CO_2 fixation rates in the existing green leaf tissues (Bingham *et al.,* 2009). Reductions in photosynthetic efficiency have been attributed to numerous mechanisms such as self-shading, stomatal limitation and other metabolic impairments (Bassanezi *et al.,* 2002). Bakanae infected rice is known to show an increase in the rate of respiration and loss of light use efficiency concomitantly with photosynthetic down-regulation. The infection of pathogen leads to the down regulation of majority of photosynthesis /chloroplast synthesis related genes and their transcriptional activity (Bozso *et al*., 2009). Genotypic variation in rice has long been reported for WUE, measured either as intrinsic WUE, *i.e.* the ratio of net photosynthesis rate to stomatal conductance (A/gs) or by carbon isotope discrimination (Δ) (Samejima 1985; Dingkuhn *et al.,* 1991). Understanding the physiological changes of a pathogen's host during the infection process can help to predict the effects of diseases on crop growth and yield (Bastiaans 1993; Boote *et al.,* 1980). Therefore, the proper assessment of the photosynthetic performance of plants under pathogen infection can provide crucial insight into the mechanisms underlying their interactions, with the potential for identifying novel strategies for crop protection (Rolfe and Scholes 2010). There is an imminent need to investigate the detailed photosynthetic functions under bakanae infection for better understanding of photosynthetic physiology during infection in the rice genotypes having varying

degree of resistance against the pathogen. The present study has, therefore, been designed to study the impact of bakanae infection on various physiological parameters related to leaf gas exchange activities in order to understand dynamic responses in leaf assimilation physiology in rice genotypes having contrasting response to disease infection.

MATERIAL AND METHODS

A. Seed material and fungal culture

The study was conducted at division of Plant Pathology, ICAR- Indian Agricultural Research Institute, New Delhi. Two rice genotypes having contrasting disease resistance (Susceptible genotype PB1121 and Resistant genotype GP50) against the disease were used for the study. The pathogen *Fusarium fujikuroi* was isolated and purified from the infected samples collected from fields. The purified culture of pathogen was mass multiplied on autoclaved sorghum in BOD incubator at 25±2 ºC.

B. Inoculum preparation and inoculation of seeds

The pathogen inoculum was prepared by mixing the pathogen culture maintained on sorghum in distilled water and meshing it with hands. The inoculum was filtered through muslin cloth and the concentration of inoculum was diluted to 10^6 spores per ml. The seeds of both genotypes were inoculated with spore suspension by immersing them in suspension for 24 hours. Another set of seeds of both genotypes was immersed in distilled water for 24 hours to be used as control. After 24 hours of inoculation the seeds were sown in plastic pro trays filled with autoclaved soil. All the treatments were sown in three replicates.

C. Assessment of plant growth and disease scoring

After sowing the plants were kept under glass house conditions. The germination percentage in each treatment was recorded seven days after inoculation. The disease scoring was done at 7, 14 and 21 days post inoculation and disease incidence was calculated. The average height of plants and average root length were also measured at every interval *viz.,* 7, 14 and 21 days post inoculation. Each observation was taken in three replications.

D. Assessment of physiological parameters

The IRGA LI-6400XT Portable Photosynthesis System was used to record the physiological parameters. The physiological parameters such as stomatal conductance, photosynthesis rate and internal CO_2 concentration were recorded as per manufacturer's protocol. To setup the IRGA instrument, the sensor head was connected to the console by uncoiled cable and the CO_2 cartridge was attached. Before using the instrument, calibration was done and before each observation zeroing was done (Evans and Santiago 2014). The leaf area standard was set 2 ×3 cm leaf chamber, light source (6400-02B LED light) and the flow rate of 300-400 µmol m^{-2} s^{-1}. The CO_2 entering the leaf chamber was 415 µmol mol^{-1} that maintains 400 µmol mol^{-1} in the leaf chamber head (Mujawamariya *et al.,* 2018). The leaf was placed in the head chamber and enclosed properly without any leakage. The physiological parameters were allowed to reach the steady state to prevent the variability due to the fluctuating environment. The readings were recorded when the values for the photosynthesis and stomatal conductance stabilized.

E. Photosynthesis rate

The difference of CO_2 and water flux between reference and sample circuit is measured and is used to calculate rate of photosynthesis (A_N) or rate of CO_2 assimilation (Douthe *et al.,* 2018; Von Caemmerer and Farquhar 1981).

$$A_N = U_e \frac{C_e - C_o}{L_a} - C_o E A_n$$

Where, C_e and C_o are the CO_2 mole fraction at the chamber entrance and output, respectively; u_e is the incoming flow air (mol air s^{-1}), La is the leaf area surface (m^2), and E is the transpiration rate (mol H_2Om^{-2} s^{-1}).

F. Transpiration rate

The rate of transpiration depicted as E is calculated as differences in H_2O concentration, based on the readings of the IRGAs in the reference and sample circuits, as per following formula:

$$E = F (W_e - W_o)/L_a$$

Where, W_e and W_o are the H_2O mole fraction at the chamber entrance and output respectively; F-flow and L_a- the leaf area surface (^{m2}).

G. Stomatal conductance

The transpiration rate (E) and relative humidity in the substomatal cavity is used to calculate the stomatal conductance to water vapour (gs) by using the first Fick's law of diffusion (Douthe *et al.,* 2018), as per following formula:

$gsw = E/(Wi - Wa)$

Where, Wi -H_2O in the substomatal cavity, Wa- H_2O in the atmosphere (chamber head) and E- rate of transpiration.

H. Water use efficiency

The water use efficiency (WUE), which represents the CO_2 assimilation as biomass per unit of water used by plant WUE is simply calculated as ratio between A_N and E (Mujawamariya *et al.,* 2018), as per following formula:

$WUE = A_N/E$

Where A_N- rate of CO_2 assimilation or rate of photosynthesis and E-rate of transpiration.

I. Internal CO_2 concentration

Stomatal conductance is expressed as gsw in terms of H_2O and gsc in terms of CO_2. The relationship between the two depicted as gsw = 1.6 gsc. Where, 1.6 factor is derived from the variation in the diffusivity of H_2O and CO_2. The CO_2 concentration in the substomatal cavities (Ci) is calculated from CO_2 concentration in the atmosphere, rate of photosynthesis and stomatal conductance in terms of CO_2 by using following formula:

$Ci = Ca - A_N/gsc$

Where, Ca is the atmospheric CO_2 (in chamber head) (Von Caemmerer and Farquhar 1981).

J. Statistical data analysis

The differences between means of the treatments for the different growth and physiological parameters were tested using one-way ANOVA using the student's t-test. Differences were considered statistically significant at $p < 0.05$. Statistical analysis of physiological data was performed using OPSTAT software.

RESULTS AND DISCUSSION

A. Germination percentage and growth parameters

The germination percentage was recorded at seven days post inoculation. Highest germination (93.33%) was recorded in control plants of resistant genotype (GP50) and lowest germination (78.67%) was recorded in *Fusarium fujikuroi* inoculated susceptible genotype (PB1121) (Table 1). There was no significant difference observed in germination of inoculated and non-inoculated plants in resistant genotype but a significant reduction in germination of inoculated plants of susceptible genotype was observed in comparison to non-inoculated plants. Yadav *et al.,* (2020) also reported similar findings where they observed that the *Fusarium* inoculation lead to colonization of seeds and inhibited the germination resulting in reduction in seed germination. The plant height was recorded highest in inoculated plants of susceptible genotype PB1121 at every time interval as compared to the control of same genotype. There was significant difference in the height of plants of pathogen inoculated and control of PB1121. This difference in height was due to the elongation of stems caused by the pathogen infection. There was no significant difference was observed in the average plant height of inoculated and non-inoculated plants of GP50 genotypes there was no disease observed in it. The root length was significantly reduced in pathogen inoculated plants of PB1121 as compared to the control plants of same genotype. The infection of *Fusarium fujikuroi* leads to blockage of root tissues and leads to reduced root growth. There was no significant difference was recorded in the root length of the control and inoculated plants of GP50 genotypes (Table 1). Similar set of findings were also recorded by Yadav *et al.* (2020).

Table 1: Germination percentage and different growth parameters of two rice genotypes (PB1121 and GP50) inoculated with *Fusarium fujikuroi* and control at different time interval.

Treatment	Germination %	Plant Height (cm)			Root length (cm)		
		7 DPI	14 DPI	21 DPI	7 DPI	14 DPI	21 DPI
PB1121 (I)	78.67	19.67	25.33	27.67	5.33	7.67	8.33
PB1121 (C)	91.67	15.33	20.33	23.33	6.67	9.33	10.33
GP50 (I)	91.33	13.33	17.67	20.33	4.67	6.33	7.33
GP50 (C)	93.33	13.67	17.33	19.67	4.33	6.67	7.67
S.E. (m) ±	2.73	0.49	0.62	0.78	0.34	0.36	0.36
CD	8.21	1.47	1.86	2.34	1.02	1.08	1.08

B. Disease incidence and Area Under Disease Progress Curve

The disease incidence was calculated by dividing the number of diseased plants by total plants and multiplying by 100. The AUDPC was also calculated from the data of disease incidence. Highest disease incidence was observed in the PB1121 inoculated with *Fusarium fujikuroi* at all time intervals. At 21 DPI pathogen inoculated PB1121 plants showed a disease incidence of 85.91%. Whereas no to very little disease was observed in pathogen inoculated GP50 plants (Fig. 1). At 21 DPI pathogen inoculated GP50 plants showed a disease incidence of 3.33%. No disease incidence was observed in the non-inoculated plants of both genotypes. After 21 days post inoculation highest AUDPC (885.88) was observed in pathogen inoculated PB1121 plants whereas at same time point an AUDPC of 23.34 was observed in pathogen inoculated GP50 plants (Table 2).

Table 2: Disease incidence and Area under Disease Progress Curve of two rice genotypes (PB1121 and GP50) inoculated with *Fusarium fujikuroi* and control at different time interval.

Genotypes	Disease Incidence			AUDPC
	7DPI	14DPI	21DPI	
PB1121 (I)	29.78	68.71	85.91	885.88
PB1121 (C)	0	0	0	0
GP50 (I)	0	1.67	3.33	23.34
GP50 (C)	0	0	0	0
S.E. (m) ±	1.67	2.78	3.22	25.687
CD	5.00	8.34	9.66	77.061

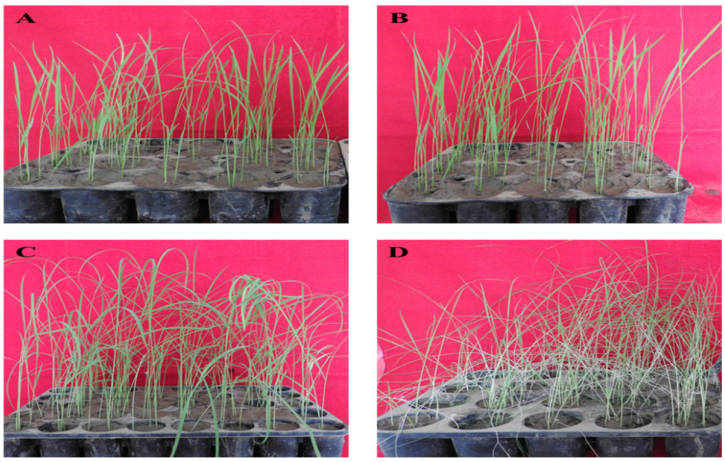

Fig. 1. Two rice genotypes (PB1121 and GP50) showing contrasting reaction to *Fusarium fujikuroi* infection. Where, A= GP50 control, B= GP50 inoculated, C= PB1121 control and D= PB1121 inoculated.

C. Physiological parameters

IRGA readings were taken at five different time intervals *viz.* 7, 10, 14, 17 and 21 days post inoculation in between 10-11 a.m. and on the basis of that different physiological parameters such as photosynthesis rate, transpiration rate, stomatal conductance, water use efficiency and internal CO_2 concentration etc were measured in inoculated and control plants of both genotypes (GP50 and PB1121).

D. Photosynthesis rate

The rate of photosynthesis was initially found to be increased in inoculated plants of both genotypes at 7 DPI but later it was found to be reduced in inoculated plants as compared to the control plants. The photosynthesis rate or net carbon assimilation rate was found to be decreased drastically in pathogen inoculated susceptible genotype (PB1121) as compared to the control plants. Lowest photosynthesis rate was observed at 21 DPI (5.27 µmol m^{-2} s^{-1}) in inoculated plants of PB1121 genotype (Fig. 2). This is in correlation with the severity of disease. In the GP50 genotype (resistant) the rate of photosynthesis was initially increased slightly and later it was found to be reducing however the reduction was not that significant as compared to the susceptible genotype. No significant changes in the photosynthetic rate was observed in the non-inoculated plants of both resistant and susceptible genotypes. Similar results were also observed by Kumar *et al.* (2013); Tatagiba *et al.* (2015). The reduction in photosynthesis rate in inoculated plants can be attributed to Lower activity of Rubisco and carbonic anhydrase, reduction in mesophyll conductance to CO_2 diffusion, increase of respiratory and photorespiratory activities and biochemical damages (Ribeiro *et al.,* 2004). The increased disease severity leads to the reduced photosynthesis rate.

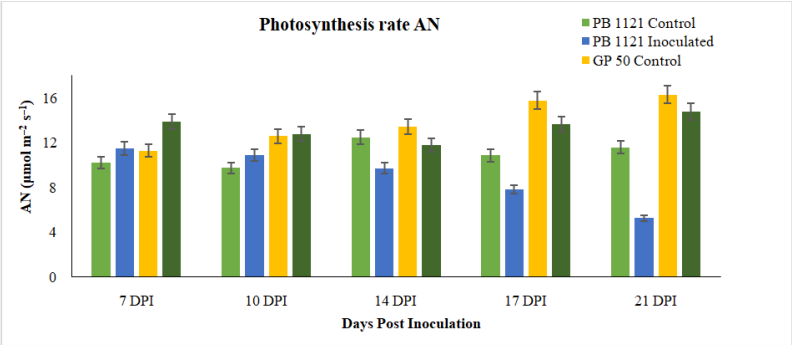

Fig. 2. Photosynthesis rate of two rice genotypes (PB1121 and GP50) inoculated with Fusarium fujikuroi and control at different time interval.

E. Transpiration rate

It was observed that the rate of transpiration was increasing initially in pathogen inoculated plants as compared to their non-inoculated counterparts at 7 DPI. Afterwards the transpiration rate started to decline in inoculated plants as compared to their control counterparts in both resistant and susceptible genotypes. However, the reduction in resistant genotype was very low but in susceptible genotype it was found to be decreasing at a very high rate. Lowest transpiration rate was observed at 21 DPI (0.26 mmol m^{-2} s^{-1}) in pathogen inoculated susceptible genotype. The control plants of both resistant and susceptible genotypes showed no significant changes in transpiration rate. The findings of the study are in accordance with the previous studies (Kumar *et al.,* 2013; Alves *et al.,* 2011; Resende *et al.,* 2012).

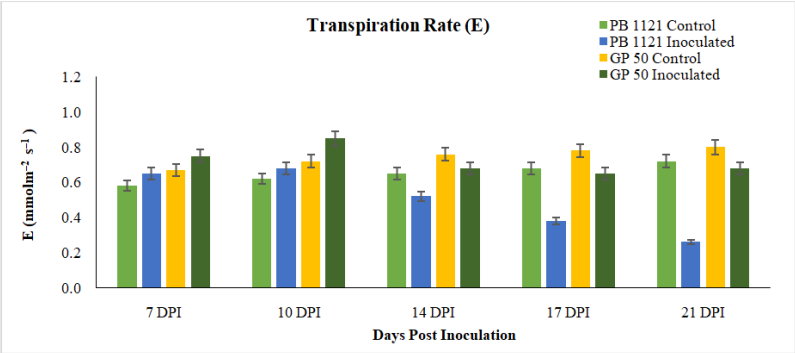

Fig. 3. Transpiration rate of two rice genotypes (PB1121 and GP50) inoculated with *Fusarium fujikuroi* and control at different time interval.

F. Stomatal conductance

The stomatal conductance was observed to be decreasing in inoculated plants as compared to their non-inoculated counterparts in both genotypes. However the rate of decrease was low in resistant genotype whereas, it was very high in susceptible genotype. The lowest stomatal conductance activity was recorded in pathogen inoculated PB1121 plants at 21 DPI (0.14 mol m^{-2} s^{-1}) (Fig. 4). No significant difference was observed in control plants of both resistant and control genotypes. Our findings are in accordance with the findings of Kumar *et al.* (2013).

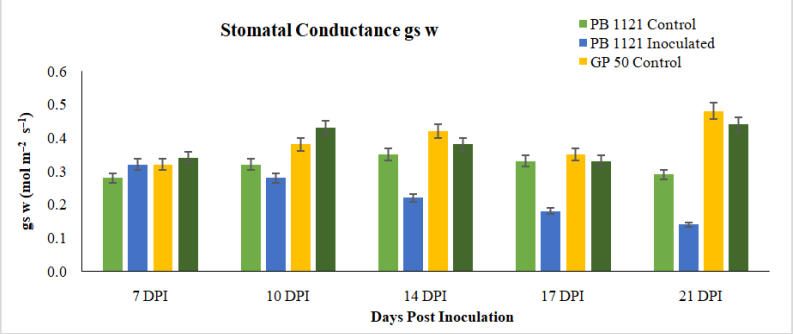

Fig. 4. Stomatal conductance of two rice genotypes (PB1121 and GP50) inoculated with *Fusarium fujikuroi* and control at different time interval.

G. Water use efficiency

It was observed that water use efficiency was increasing in inoculated plants of susceptible genotype as compared to their control counterparts. However in resistant genotype no significant difference was observed in the water use efficiency of both inoculated and non-inoculated plants (Fig. 5). Similar type of results were also observed by Kumar *et al.* (2013).

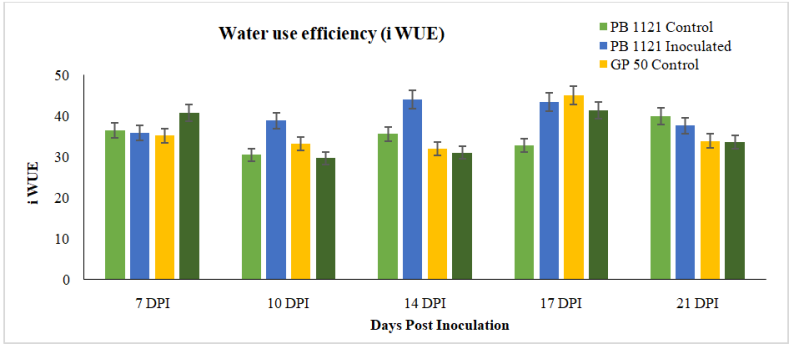

Fig. 5. Water use efficiency of two rice genotypes (PB1121 and GP50) inoculated with *Fusarium fujikuroi* and control at different time interval.

H. Internal CO_2

Highest internal CO_2 (275 µmol m^{-2} s^{-1}) was observed in PB1121 inoculated plants at 21 DPI. The internal CO_2 activity was initially decreasing in inoculated plants but it was reported to increase at later stages. In control plants no significant changes were observed in both genotypes (Fig. 6). Tatagiba *et al.* (2015) also observed similar results in their experiment.

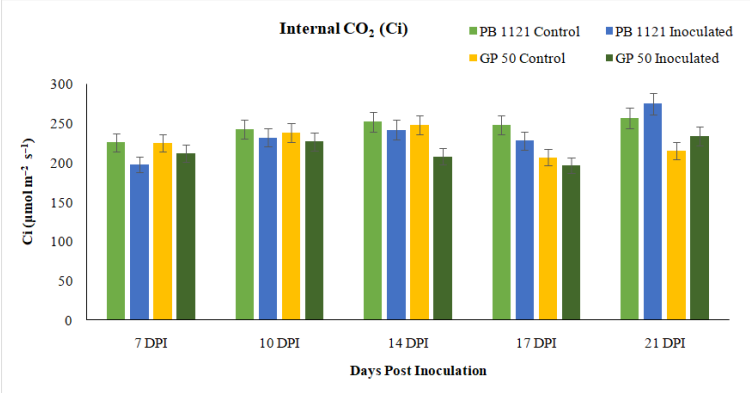

Fig. 6. Internal CO_2Water use efficiency of two rice genotypes (PB1121 and GP50) inoculated with *Fusarium fujikuroi* and control at different time interval.

CONCLUSIONS

The findings of the study reveals that the leaf gas exchange activities of plants is affected by disease severity. The genotypes varying in their response to the disease infection show different changes in their physiological parameters when challenged with the pathogens. The photosynthesis rate or net carbon assimilation rate generally tends to decrease in the infected plants because of many reasons associated with it. But in resistant genotypes as there is no or very less disease, these factors do not affect that much and not much significant change is observed. Similarly, the other activities like transpiration rate, water use efficiency, internal CO_2, stomatal conductance etc are also affected differently by pathogen infection in different genotypes according to their susceptibility towards the pathogen.

FUTURE SCOPE

Furthers studies are needed to better understand the mechanism involved in each physiological parameter and the factors involved in theses physiological processes are needed to be studied which could help in better understanding the defence mechanism of host and to develop better resistance sources.

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Conflict of Interest. None.

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