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Leaf gas exchange physiology in rice genotypes infected with bacterial blight: An attempt to link photosynthesis with disease severity and rice yield

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Abstract

Bacterial blight (BB) of rice caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is one of the most devastating disease of rice causing significant yield reduction under serious infestations in many rice growing countries. BB interferes with leaf CO_2 exchange processes, enhances leaf senescence and reduces CO_2 assimilation. In the present study, 14 rice genotypes were initially studied to understand genotypic variability for resistance against infection by *Xoo* . Further, to assess the effect of BB on photosynthetic functions, three rice genotypes [*O. longistaminata; O. sativa* cv. IRBB21 and Pusa Basmati 1 (PB1)] possessing varied levels of resistance/susceptibility to BB were selected. IRBB21 and PB1 were also analyzed to study the effect of bacterial blight on grain yield. Exposure of plants to BB led to significant reductions in net photosynthetic rate (P_N), stomatal conductance to CO_2 (g_s), instantaneous water use efficiency (WUE_i), and leaf transpiration rate (E) in susceptible genotype PB1 when compared to resistant IRBB21 and highly resistant *O. longistaminata*. Under BB infection, *O. longistaminata* showed highest P_N (7.18 µmol CO_2 $m^{-2}s^{-1}$) compared to IRBB21 (4.35 µmol CO_2 $m^{-2}s^{-1}$) and PB1 (0.74 µmol CO_2 m^{-2} s^{-1}), on 216 h of post infection. Due to infection, g_s was strongly reduced to 0.046 mmol $m^{-2}s^{-1}$ on 216 h in PB1 followed by IRBB21 (0.16 mmol m^{-2} s^{-1} on 216 h), whereas *O. longistaminata* maintained highest g_s of 0.22 mmol m^{-2} s^{-1} on 216 h, indicating better CO_2 exchange capacity and resistance against bacterial blight. Regression plots showed significantly positive relationships between P_N vs g_s and P_N vs C_i for all the tested genotypes. Down-regulation in leaf CO_2 assimilation physiology as well as loss in photosynthetically active leaf tissue was observed with increment in disease severity, which resulted in substantial yield loss (61.75%) in susceptible genotype PB1. Yield loss was mostly attributed to reduced productive tillers, less number of seeds per panicle, decreased panicle weight and less number of filled grains. Relatively less variation in yield traits was recorded in resistant IRBB21.

Keywords: Bacterial infection, leaf gas exchange, net photosynthetic rate, stomatal conductance to CO_2, yield trait.
Abbreviations: BB-bacterial blight; CRD- completely randomised design; E- leaf transpiration rate; g_s-stomatal conductance to CO_2; NILs- near isogenic lines; P_N- net photosynthetic rate; PWC- pot water holding capacity; PAR- photosynthetically active radiation; SWC- Soil water content; WUE_i-instantaneous water use efficiency; Xoo-*Xanthomonas oryzae* pv. *Oryzae.*

Introduction

The year 2004 was declared as the International Year of Rice (IYR) by the United Nations General Assembly (UNGA) with the slogan "Rice is Life". On a global basis, UNGA declaration underlines the significance of rice (*Oryza sativa* L.) as a staple crop for human consumption and accredited as a primary food source for more than half of the world's population. In developing countries, rice accounts for 715 Kcal/capita/day, which involves 27% of dietary energy supply, 20% of dietary protein, and 3% of dietary fat (FAO, 2004). 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). Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most serious diseases of rice that limits annual rice production in both tropical and temperate regions of the world. BB is largely restricted to subtropical Asia including southern China, Thailand, Malaysia, India, Vietnam, the Philippines and Indonesia and is also prevalent in some parts of West Africa. Recently, outbreak of BB was also reported in northern Australia, Latin America and in Caribbean. In India, BB epidemic is known to occur in many parts of the northern and coastal regions of the country (DRR, 1975–2003). Yield loss due to BB varied from 2% to 74% depending on location, season, crop growth stage and genotype (Rao and Kauffman, 1977; Reddy et al., 1978; Muralidharan and Venkatarao, 1979). Reduction in photosynthetic rate is considered to be one of the most important factors causing yield loss in BB infected rice (Rajarajeswari and Muralidharan, 2006). BB reduces photosynthesis in rice by causing necrosis of leaf tissue (reducing photosynthetically active area) and down-regulation of CO_2 fixation rates in the existing green leaf tissues nearby the necrotic lesion (Bastiaans, 1991). The necrotic regions retained on the leaves even though may capture some light, do not contribute to photosynthesis (Bingham et al., 2009). Reductions in photosynthetic efficiency have been attributed to numerous mechanisms,

such as self-shading, stomatal limitation and other metabolic impairments (Boote et al., 1983; Johnson, 1987; Bowden et al., 1990; Bassanezi et al., 2002). Not surprisingly, reductions in plant growth and yield under diseased conditions have also been correlated with loss of photosynthetic capacity (Schwartz et al., 1981; Widin and Schipper, 1981; Spitters et al., 1990; Jesus et al., 2001). Previous studies demonstrated that BB infected rice showed an increase in the rate of respiration and loss of light use efficiency concomitantly with photosynthetic down-regulation. Under bacterial infection, the transcriptional activity of photosynthesis / chloroplast synthesis genes are found to change more or less in one direction and majority of these genes are down-regulated (Bozso, 2009). There is an imminent need to investigate the detailed photosynthetic functions under BB infection for better understanding of photosynthetic physiology during infection. The present state of knowledge on photosynthetic leaf gas exchange traits in BB infected rice is preliminary and the responses of some of the important gas exchange characteristics including stomatal conductance to CO_2, leaf transpiration rate and internal CO_2 concentration have not yet been investigated in detail during progressive stages of BB infection in rice. A comprehensive study that incorporates detailed photosynthetic leaf gas exchange analysis under various stages of BB disease progression can be helpful in order to understand CO_2 assimilation physiology in infected rice leaves which can also be linked further to plant growth and grain yield related responses. The present study has, therefore, been designed to study the impact of BB infection on photosynthesis in order to understand dynamic responses in leaf assimilation physiology with increment in disease severity. In the initial phase of our study, we tested 14 rice genotypes with an objective to screen them for varied levels of tolerance to BB infection. In the second phase, we performed leaf gas exchange studies with selected genotypes using sensitive infra-red gas analysis technique with an objective to study and compare CO_2 fixation characteristic in rice genotypes differing in their resistance to BB. Our third objective was to link the growth and yield responses in BB-infected rice with the observed changes in their photosynthetic traits and CO_2 fixation efficiencies.

Results and discussion

Genotypic variability in resistance against BB infection

The present study has revealed substantial genotypic variation among the 14 rice genotypes in terms of resistance against BB (Table 1). Two accessions of the wild species, *O. longistaminata* and *O. rufipogon* were found to be highly resistant and one NIL IRBB21, resistant to the disease, with minimum disease infection intensity of 4.66% (score-1) and 7.69% (score-3) and 12% (score-3) , respectively, whereas, IRBB13 and *O. nivara* were moderately resistant with the disease score of 5 (Table 2). The remaining genotypes, including IRBB1, IRBB3, IRBB4, IRBB5, IRBB7, IRBB10, IRBB11, PB1 and TN1 were highly susceptible with infection intensity ranging from 31.25% to 95.2%. PB1 exhibited highest susceptibility and showed 95.2% infection intensity (score-9). The resistance reaction of rice wild relatives to bacterial blight as observed in the present study was found as per the findings of Jena and Khush, 1990; Amante et al., 1992; Multani et al., 1994; Brar et al., 1996. Bacterial blight infection directly influences the photosynthetic activity which includes photophosphorylation (Kosuge and Kimpel, 1982), photoassimilation, photorespiration and disruption of orderly flow of carbon throughout the plant due to changes in mechanisms controlling carbon balance which finally results in yield loss (Bastiaans, 1991). Hence, we undertook the present study to understand the physiology of the rice plant, under attack by the BB pathogen.

Photosynthetic leaf gas exchange responses

In the second phase of our study, photosynthetic CO_2 fixation characteristics were analyzed using three contrasting genotypes, viz., *O. longistaminata* (highly resistant), IRBB21(resistant) and PB1 (susceptible) (Figure 1) based on resistance reaction. Effects of BB infestation depicted contrasting variability in P_N, g_s and E among the tested genotypes. Initially an elevation in P_N was observed (24 h, post infection) in all the three genotypes, consistent with previous report of Jonson (1984) where the same pattern was observed due to elevation in phytohormone, stomatal opening and ion transport in vesicular arbuscular mycorrhiza (VAM) infected *Citrus aurantium* plants. P_N of the healthy leaves was significantly higher than that of the infected leaves in each of the three genotypes, when measured periodically with disease progression (Figure 2). The control plants of *O. longistaminata* showed maximum P_N of ~19.26 µmol CO_2 $m^{-2}s^{-1}$ on 216 h, whereas IRBB21 and PB1 showed a maximum P_N of 12.5 µmol CO_2 $m^{-2}s^{-1}$ on 216 h and 48 h (11.83 µmol CO_2 $m^{-2}s^{-1}$) respectively. The P_N values in infected plants of each genotype declined progressively during the course of experiment. PB1 and IRBB21 showed rapid and substantial reduction in P_N compared to *O. longistaminata*. After 216 h of infection, the leaves of *O. longistaminata* showed P_N of 7.18 µmol CO_2 $m^{-2}s^{-1}$, whereas in IRBB21 P_N was 4.35 µmol $CO_2m^{-2}s^{-1}$ followed by PB1 (0.74 µmol CO_2 $m^{-2}s^{-1}$). Photosynthetic down-regulation under bacterial infection as recorded in our present study could be attributed to several factors. Lower activity of Rubisco and carbonic anhydrase (Goodwin et al., 1988; Saeed et al., 1999), reduction in mesophyll conductance to CO_2 diffusion (khairi and Hall, 1976), increase of respiratory and photorespiratory activities (Laisk et al., 1998) and biochemical damages (Ribeiro et al., 2004) were reported to cause loss in photosynthetic activities under BB infection. Some recent reports demonstrated that the reduction of P_N in infected leaves is connected with the decrease of g_s and also a result of transpiration rate inhibition (Chołuj et al., 2011). With the down-regulation of P_N in infected leaves, we observed that g_s and E were also concomitantly reduced in all tested genotype when compared to their control counterparts. The healthy leaves of *O. longistaminata* showed highest g_s (0.61 mmol m^{-2} s^{-1}) on 144 h followed by IRBB21 and PB1 (0.32 mmol m^{-2} s^{-1}on 24 h). During infection, g_s was strongly reduced to 0.046 mmol m^{-2} s^{-1} on 216 h in PB1 followed by IRBB21 (0.16 mmol m^{-2} s^{-1} on 216 h), whereas *O. longistaminata* maintained highest g_s of 0.22 mmol m^{-2} s^{-1} on 216 h. In comparison to the early stage of infection, the g_s of infected leaves decreased more drastically at later stage of infection from 48 h of infection till the end of experiment. Our results with BB infected rice genotypes suggest that one of the most probable cause of reduction in P_N of infected plants must be loss in g_s. Petit et al., (2006) reported that the simultaneous reduction of P_N and g_s in symptomatic leaves indicate a global water stress in plant. Pathogens like *Xoo* (as in our experiment) which can move through vascular tissues of plants, enters the xylem vessels and then proliferate within the vessels causing water stress in host plants body enhancing resistance to hydraulic conductance (Tyree et al., 1989). Reduced water conductance results in leaf moisture deficits and cause stomatal closure,

Table 1. List of rice genotypes used in the present study to assess genotypic variability in response to bacterial blight (BB). The International Rice GenBank Collection accession (IRGC acc) and pedigree of each genotype are mentioned according to the information available in International Rice Research Institute, Philippines database: (http://www.irgcis.irri.org:81/grc/irgcishome.html) and (http://www.iris.irri.org/).

Genotype	IRGC acc.	Pedigree
IRBB1	115095	IR24*5/Kogyoku (Xa1 gene from cultivar Kogyoku)
IRBB3	115100	IR24*5/Chugoku (Xa3 gene from cultivar Wase Aikoku)
IRBB4	115101	IR24*5/IR20 (Xa4 gene from cultivar TKM6)
IRBB5	115102	IR24*5/1545-339 (xa5 gene from cultivar DZ192)
IRBB7	115119	IR24*5/DV85 (Xa7 gene from cultivar DV85)
IRBB10	115606	IR24/Cas 209 (Xa10 gene from cultivar Cas 209)
IRBB11	115096	IR24*5/IR8 (Xa11 gene from cultivar IR8)
IRBB13	115097	BJ1/5*IR24(xa13 gene from cultivar BJ1)
IRBB21	115099	IR24*8/*O. longistaminata* (*Xa21* gene from *OL*)
O. longistaminata[1]	104500	Potential source of new alleles (wild genotype)
O. rufipogon	81892	listed as a noxious weed (wild genotype)
O. nivara	81849	Wild progenitor of Asian rice (*O. sativa*)
Pusa Basmati1[2] (PB1)	78422	Pusa167 / Karnal local (IET No: 10364)
Taichung Native1 (TN1)	105	Dee Geo Woo Gen/TSAI-YUAN-CHAN

1: Resistant check 2: Susceptible check

Fig 1

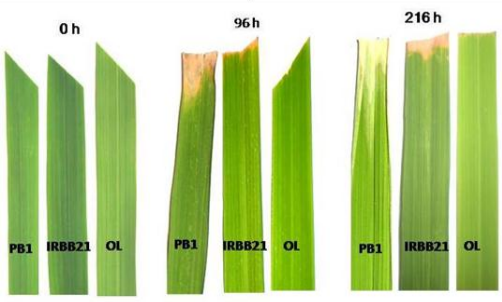

Fig 1. The effect of *Xoo* inoculation on the leaf blades of three rice genotypes (PB1, IRBB21 and *O. longistaminata*). Rice seedlings were inoculated with isolate DX133 of *Xoo* by leaf clipping method. The progress in disease infestation as observed on the infected leaf blades was photographed on 0 h, 96 h and 216 h of post inoculation. No necrotic symptoms and senescence were detected in the leaf blade of BB highly resistant *O. longistaminata* when compared to resistant IRBB21 and susceptible PB1.

down- regulation in g_s and concurrently leads to reduction in P_N (Saeed et al., 1999). As g_s and E are strongly coupled, we observed the same degree of down-regulation in E along with g_s. The lowest E was recorded in infected PB1 (0.69 mmol m^{-2} s^{-1} on 216 h) followed by IRBB21 (2.27 mmol m^{-2} s^{-1} on 216 h). However, *O. longistaminata* exhibited relatively higher E of 2.31 mmol m^{-2} s^{-1} on 216 h when compared to other genotypes (Figure 3). Our findings are in agreement with the earlier studies where similar reduction in E was recorded following pathogen infection (Scholes and Rolfe, 1995; Fleischmann et al., 2005; Guo et al., 2005). Meyer et al., (2001) and Bassanezi et al., (2002) also showed that the reduction of E in other pathosystems (rust in bean leaves) was directly related to g_s and therefore it was associated with stomatal closure. As, P_N was highly sensitive to disease severity in PB1 and IRBB21, a reduction in E could not improve photosynthetic *WUEi* in those genotypes. Moreover, with the progression in disease severity, *WUEi* was significantly reduced in PB1 (1.07 µmol CO_2 / mmol H_2O on 216 h), followed by IRBB21 (1.91 µmol CO_2/ mmol H_2O on 216 h) when compared to their control counterparts (Figure 4). In infected *O. longistaminata*, as the reduction in P_N was comparatively less when compared to E, hence the photosynthetic *WUEi* was significantly enhanced during the later stage disease progression. Previous reports have suggested that the reduced rate of P_N is also attributed to non-stomatal limiting factors including damage to primary photochemical (inhibited functional activity of PSII and reduced amount of photosynthetic pigments) and biochemical processes (Lawlor, 2002). Besides down-regulation in P_N, the concomitant decrease in g_s and E values in our study indicates strong existence of stomatal limitations besides the non-stomatal factors (Shangguan et al., 1999) which was also reported in case of water-stressed in alfalfa and sorghum (Li et al., 2011). The stomata in the symptomatic leaves might be largely closed, since infected plants were accompanied, especially in later stage of infection, by lower g_s and E. Moreover, the reduced C_i values in the infected rice plants as observed in our study, especially in later stages of infection, strongly supports stomatal limitation to CO_2 movement. Unlike other bacterial infection, where high C_i values (mostly due to patchy stomata, cuticular transpiration etc) were recorded in plants (Ribeiro et al., 2004), our study clearly indicates the presence of stomatal effect on the reduction of P_N. The lowest C_i value (263 µmol mol^{-1}) was detected in resistant IRBB21 on 216 h, whereas in susceptible genotype PB1 (277.16 µmol mol^{-1}) and highly resistant genotype *O. longistaminata* (283.66 µmol mol^{-1}) the values were comparatively higher on 216 h (Figure 3). Across the time period from 24 h to 216 h, IRBB21 has lowest C_i value (206.16 µmol mol^{-1}) on 72 h. To understand the association of P_N with g_s and C_i in the BB-infected symptomatic rice leaves, we performed linear regression analysis using the variables obtained from infected plants of all three genotypes. In BB-infected condition, the regulation of P_N by g_s followed a linear function and resulted in a significantly positive correlation between P_N and g_s for all the rice genotypes (Figure 5). The regression slopes were steeper for *O. longistaminata* ($r^2 = 0.81$, $p < 0.001$) and PB1 ($r^2 = 0.87$, $p < 0.001$) when compared to IRBB21 ($r^2 = 0.75$, $p < 0.001$).

Table 2. Disease score and percentage of disease infection in 14 tested rice genotypes. The genotypes were classified into three groups depending upon their level of resistance against BB *viz*., resistant (R), moderately resistant (MR) and susceptible (S).

Rice genotypes (R genes present)	Disease score	Disease infection (%)	Level of resistance
IRBB1 (*Xa1*)	9	94.00	S
IRBB3 (*Xa3*)	9	58.82	S
IRBB4 (*Xa4*)	7	31.25	S
IRBB5 (*xa5*)	9	90.50	S
IRBB7 (*Xa7*)	9	83.60	S
IRBB10 (*Xa10*)	9	90.46	S
IRBB11 (*Xa11*)	9	78.90	S
IRBB13 (*xa13*)	5	18.60	MR
IRBB21 (*Xa21*)	3	12.0	R
O. longistaminata[1]	1	4.66	R
O. rufipogon	3	7.69	R
O. nivara	5	14.86	MR
Pusa Basmati 1(PB1)[2]	9	95.20	S
Taichung Native1(TN1)	9	88.80	S

1: Resistant check 2: Susceptible check

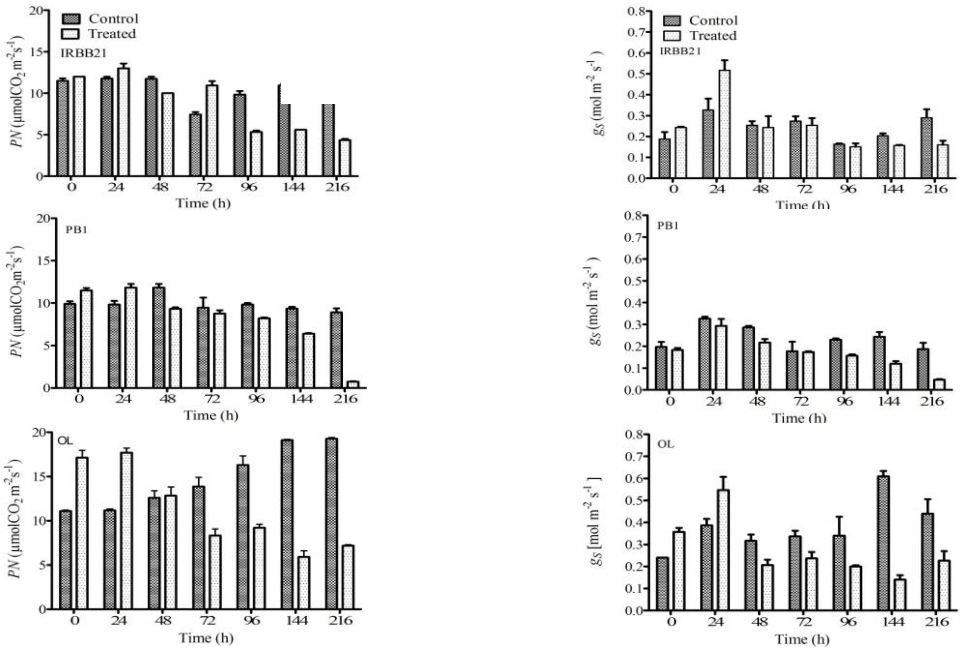

Fig 2. Net photosynthetic rate (P_N) and stomatal conductance to CO_2 (*gs*) in PB1, IRBB21 and *O. longistaminata* from 0 h to 216 h after inoculation with *Xoo*. Data are mean ± SD. (Treated=BB-treated).

The relationships between P_N vs C_i were linear and positively correlated for all the tested rice genotypes (Figure 5). The correlation was strong in IRBB21 (r^2=0.4, < 0.001), and *O. longistaminata* (r^2=0.3, p < 0.001) however, it was comparatively weak though significant in PB1 (r^2=0.2, p < 0.001). Thus from our results, it is evident that apart from existing non-stomatal limiting factors, significant stomatal limitations also coexist in BB-infected rice genotypes which can substantially limit the photosynthetic efficiency of the infected leaves with disease progression.

Yield and yield components

There was no significant effect of stress caused by BB pathogen on stem length, panicle length, plant height and total tillers in control as well as in treated plants of PB1 and IRBB21. These growth features remained unaffected because the infection was done at maximum tillering stage. However, upon infection, productive tillers (-29.3%), seeds per panicle (-18.4%), panicle weight (-39.3%), number of filled grains (-34%) and yield per plant (-61.75%) were significantly reduced, while semi-filled (+12.0%) and unfilled grains (+63.9%) increased significantly in susceptible genotype PB1 (Table 3). Comparatively less reduction in the above mentioned yield traits was recorded in the resistant genotype, IRBB21 under infected condition. Reduction in productive tillers per plant in infected PB1 can be linked to the fact that plant under stress condition could not produce required assimilates due to low photosynthesis and loss in photosynthetically active tissues (Savary et al., 2000). *Xoo* blocks conductive vessels and restricts water conduction and nutrient supply from roots to leaves and shoots. Such function of *Xoo* in BB-infected rice might have contributed in lowered seeds per panicle, panicle weight and increased unfilled and semi-filled grains as well (Tan et al., 2007). Few biochemical disturbances due to production of pathogen-induced enzymes, toxins and extracellular polysaccharides under stress conditions have also been reported (Tan, 1993). Photosynthesis in rice plants contributes 60-80% of the final carbon content during the grain filling period (Yoshida, 1981) and hence metabolic activity within the grain must coincide with maximum activity of source leaves during the grain-

Table 3. Effect of BB on various yield characteristics of resistant (IRBB21) and susceptible (Pusa Basmati 1) rice genotypes. Values are expressed as mean ± SD. *, ** and ***: Significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. ns: Not significant.

Parameters	Pusa Basmati 1			IRBB21		
	Control	BB-Treated	%increase(+)/ decrease (-)	Control	BB-Treated	%increase(+)/ decrease (-)
Plant height(cm)	92.75±4.68	90±4.37	- 2.96 ns	68±4.28	65±3.84	- 4.41 ns
Tillers/plant	10.66±2.16	9.16±1.32	- 15.52*	10.5±1.37	9.5±1.97	- 9.52 ns
Productive tillers/plant	9.66±1.63	6.83±0.75	- 29.29**	10.5±1.37	9.16±1.60	- 12.76 ns
Panicle length(cm)	24.75±0.93	23.91±1.06	- 3.39 ns	17.91±0.62	17.65±0.62	- 1.45 ns
seeds/panicle	155.16±21.12	126.5±19.58	- 18.47*	117.5±8.66	114±6.16	-2.97 ns
Filled grains/panicle	126±17.07	83±25.07	-34.12**	80.66±8.06	76±4.28	-5.77 ns
semi-filled grains/panicle	8.33±3.14	9.33±2.73	+10.71 ns	9.16±2.78	11.16±1.16	+17.92*
Unfilled grains/ panicle	20.83±7.05	34.16±7.35	+39.02**	23.33±5.20	26.16±3.97	+10.81 ns
Panicle weight with seed (g)	3.33±0.59	2.02±0.61	- 39.33**	3.04±0.31	2.80±0.19	-7.89 ns
1000 -grain weight (g)	28.69±3.24	24.16±2.62	- 15.78*	24.3±3.01	22.52±2.11	-7.72 ns
Yield per plant	26.33±4.5	10.19±3.7	- 61.29***	26.49±4.2	21.6±3.1	-18.45*

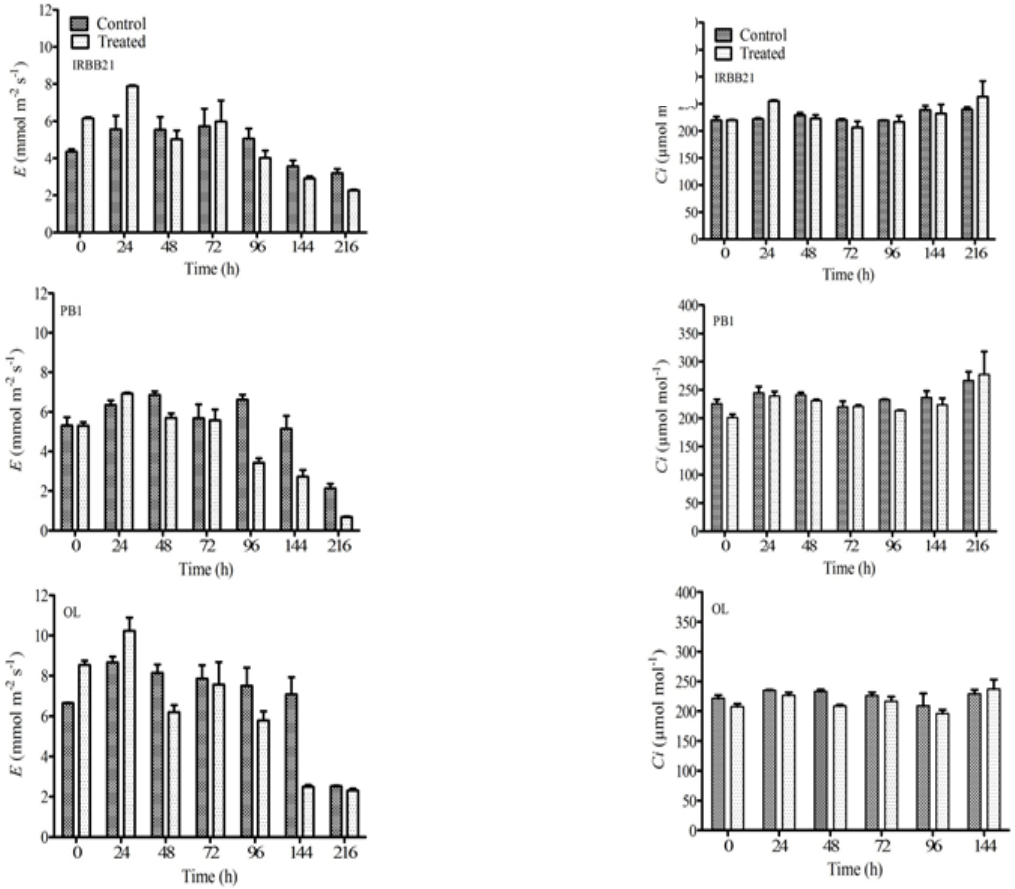

Fig 3. The influence of *Xoo* infection on the intensity of transpiration (*E*) and intracellular CO_2 concentration (C_i) in PB1, IRBB21 and *O. longistaminata* from 0 h to 216 h. Data are mean ± SD. (Treated=BB-treated).

filling stage (Murchie et al., 1999). Since, grain-filling is also closely related to whole plant senescence (Mi et al., 2002; Yang et al., 2006), the control plants of both the genotypes PB1 and IRBB21 showed high 1000-grain weight as compared to infected plants. This difference was significantly high in PB1. BB infection reduces the nutrient availability in the diseased tissue because pathogen uses the nutrient which is available for plant leading to reduced grain filling and grain weight in infected rice plants (Reddy et al., 1979a). IRBB21 showed less yield loss (18.45%) when compared to PB1 (61.75%), and the presence of *Xa21* in IRBB21 might have redeemed/rescued 43.3% of the yield loss. Since a single gene, *Xa21* can reduce yield significantly, pyramiding of multiple resistance genes with varied defence mechanism can even decrease the yield loss further and provide yield stability.

Materials and Methods

Experiment 1

Selection of rice genotypes

In this first phase of study, 14 rice genotypes were selected which included 9 near isogenic lines (NILs) developed in the genetic background of rice cultivar 'IR24' (IRBB1, IBBB3, IRBB4, IRBB5, IRBB7, IRBB10, IRBB11, IRBB13 and IRBB21 carrying genes *Xa-1, Xa-3, Xa-4, xa-5, Xa-7, Xa-10, Xa11, xa-13* and *Xa-21*, respectively); three wild rice genotypes (*O. longistaminata*, *O. rufipogon* and *O. nivara*)

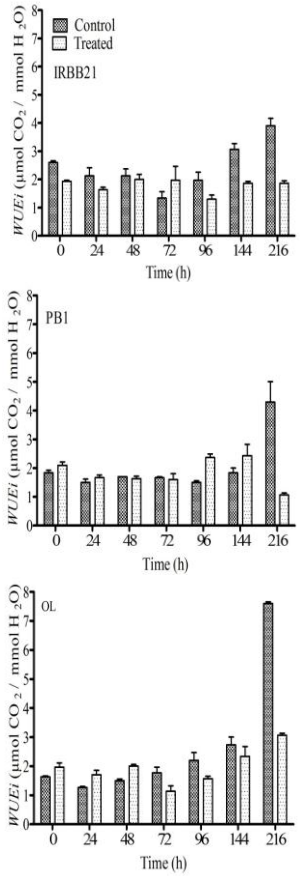

Fig 4. The influence of *Xoo* infection on instantaneous water use efficiency (*WUEi*) in PB1, IRBB21 and *O. longistaminata* from 0 h to 216 h. Data are means ± SD.

and two high yielding, popular cultivars: Pusa Basmati 1 (PB1) and Taichung Native 1 (TN1) (Table 1). Seeds of the tested rice genotypes and the *Xoo* strain (DX133) used in the present study were obtained from Directorate of Rice Research (DRR), Hyderabad, India. PB1 was used as a susceptible check and *O. longistaminata* was included as a resistant check for the *Xoo*.

Plant growth and experimental set up

The experiment was carried out at the glasshouse complex of University of Hyderabad, Hyderabad, India (17.20 N latitude, 37.30 E longitude, and 536 m above sea level). Seeds of all genotypes were germinated on separate seed beds maintaining the temperature of the growth room at 25/17^{o}C (day/night). Seed beds were uniformly watered and fertilized with a half-strength Hoagland nutrient solution. On 30th day after sowing, the healthy uniform seedlings were selected and used for transplantation in 20 litres earthen pots (four pots for each genotype with three plants in each pot) filled with a mixture of clay and peat (1:1, v/v). Single seedling was transplanted in each hill. The experiment was conducted in three replications (n=3) following a completely randomised design (CRD). Seedlings were uniformly irrigated and fertilised using Hoagland nutrient solution as required. Soil water content (SWC) was kept at 100% pot water holding capacity (PWC) and periodically measured (gravimetrically) at different points of the pot to check the homogeneity of moisture content in soil. Inside the glasshouse, photosynthetically active radiation (PAR) ranged from 900 to 1200 µmol m^{-2} s^{-1} between 10:00-11.00 AM, air temperature was 25/17^{o}C (day/night), relative humidity was 60 ± 5% and ambient CO_2 concentration was 360 to 370 µmol mol^{-1}.

Inoculum preparation, infestation and disease scoring

Bacterial pathogen was cultured on modified Wakimoto's agar medium with agitation at room temperature for 72 hours, and then suspended in distilled water into approximately 10^9 cfu/ml. Fully developed leaves of each plant were clip-inoculated when the plants were in maximum tillering to booting stage, as described by Kauffman et al. 1973. The control treatment consisted of clipping with scissors dipped in sterile water (Ghazi et al., 2009). Disease scoring was done after 15 days of inoculation according to the IRRI standard evaluation system for rice (IRRI, 2002). Percent leaf area infected on inoculated leaves was measured manually with scale and graph paper. Disease scores of the genotypes to bacterial infection was determined based on percentage of lesion length according to the standard evaluation system for rice (IRRI, 2002) (score 0=immune; score 1=1-5%; score 3=6-12%; score 5=13-25%; score 7=26-50% and score 9=51-100%). Genotypes were categorised as resistant (R: score 0-3), moderately resistant (MR: score 5-7) and susceptible (S: score > 7).

Experiment 2

Plant materials, growth of seedlings and bacterial inoculation

In this second phase of study (October-November 2010), one highly resistant (*O. longistaminata*), one resistant (IRBB 21) and one susceptible (PB1) genotype was selected. Growth and maintenance of the plants and disease inoculation methods were followed as described in experiment 1.

Leaf gas exchange analysis

To study plant CO_2 assimilation physiology, leaf gas exchange and microclimatic data were measured using a portable infrared CO_2/H_2O gas analyser (IRGA) (LCpro-32070, ADC Bioscientific Ltd., UK) equipped with a detachable leaf chamber with PAR sensor (silicon based sensor, LCpro-32070) and leaf thermistor probe (ADC-M.PLC-011, LICOR) attached to it. The gas analyser was used to measure instantaneous net photosynthetic rate (P_N), stomatal conductance to CO_2 (g_s), and leaf transpiration rate (E) periodically during the study period (0 h to 216 h, between 10:00 to 11:00 AM). Instantaneous water use efficiency (*WUEi*) was calculated (*WUEi*=P_N/E) and plants were also analysed for internal CO_2 concentration (C_i). Microclimatic parameters such as irradiance (PAR: 900-1200 µmolm^{-2} s^{-1}), relative humidity (RH: 40%), air temperature (Ta: 24-25%), CO_2 concentration (360-370 µmol mol^{-1}) and flow rate (~500 µmol s^{-1}) were recorded by the instrument. Each measurement was made when P_N and g_s readings were stabilized; this process took 1-2 min. All photosynthetic measurements were performed on well-expanded leaves (3rd to 4th from apex). In case of infected plants, measurements were performed just below the visible necrotic leaf tissue region.

Measurements of yield and yield attributing components

Three plants each from three replications were randomly selected for measuring rice yield and different yield components including; stem length, panicle length, plant height, total tillers per plant and productive tillers per plant at

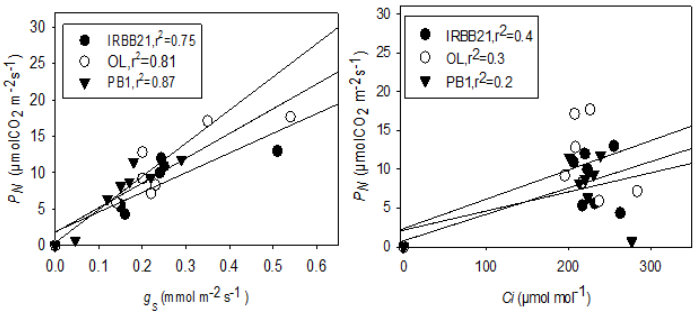

Fig 5. Relationships between (A) net photosynthetic rate (P_N) *versus* stomatal conductance to CO_2 (g_s) and (B) net photosynthetic rate (P_N) *versus* internal CO_2 concentration (C_i) in the BB infected leaves of three tested rice genotypes (PB1, IRBB21 and *O. longistaminata*). Each data point represents the mean of 6-8 independent measurements from three plants in each genotype. The correlation were significant at $p<0.001$.

appropriate growth stages. Post harvest data on number of filled, semi-filled, unfilled grains per panicle, percentage of ripened grains, panicle weight and 1000 grain weight were measured (Yoshida et al., 1976). *O. longistaminata* could not be studied for agronomic trait as sufficient seeds were not obtained due to its sterile nature.

Statistical analyses

Differences among genotypes were analysed using multivariate analysis of variance (MANOVA), significance of the differences between the treatments was determined by paired *t*-tests and correlation coefficient (*r*) and coefficient of determinations (r^2) of linear relationships between the investigated parameters and regression slopes were analysed using bivariate correlation coefficient and linear regression analysis using Sigma plot 11 software. The graphical representation of results were prepared with the help of Graphpad prism 5 software and all data represented as mean ± standard deviation (SD) .

Conclusion

Our results indicate that the leaf gas exchange physiology is widely affected in BB-infected susceptible rice genotypes when compared to the resistant ones. Besides known non-stomatal limitations, stomatal restrictions to CO_2, low leaf transpiration rates and less C_i clearly play significant roles in the intensity of photosynthetic down- regulation. The resistant genotype IRBB21 which maintained better gas exchange functions also exhibited greater seed filling and grain yield when compared to susceptible PB1. Thus photosynthetic down- regulation could lead to a deprivation of yield and vigour affecting plant productivity, demonstrating that the chronic form of bacterial blight infection will lead to carbon starvation and is deleterious for rice. To extend the utility of these results for a better comprehension of the host-pathogen physiological relationships the quantification of the impact of BB disease should be done using further techniques like P_N vs C_i response, P_N vs PPFD response and study of chlorophyll fluorescence transients coupled with leaf gas exchange analysis.

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