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Expression of bacterial blight under different levels of drought stress condition in rice

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Abstract

Xanthomonas oryzae pv. *oryzae* (*Xoo*), the causal agent of rice bacterial blight is a common reason for severe economic yield losses in rice. Plant response to one type of stress can be affected by simultaneous exposure to a second stress, for example when abiotic and biotic stresses occur together. In this study, two genotypically contrasting genotypes were challenged inoculated under different drought levels (based on field capacity). In compatible interaction, the susceptible genotype TN-1 showed great response to infection and expressed highly under all drought conditions. Symptoms appeared firstly at 6 DPI (day post inoculation) and gradually increased up to 14 DPI. Maximum lesion length was observed at drought level of 60% field capacity (12.02 cm) and minimum (5 cm) was recorded at no drought condition (100% field capacity) on 14 DPI. Same in case of bacterial multiplication rate, maximum CFUs (colony forming units) were recorded at drought level of 60% field capacity and minimum were recorded at no drought condition. In incompatible reaction, BPT5204 genotype showed no symptoms, on the contrary bacteria multipled in the host and observed maximum numbers of colonies at drought level of 60% field capacity. This study has shown the direct responses of the two contrasting genotypes under different drought stress.

Keywords: Xanthomonas oryzae pv. oryzae, drought, CFUs, symptom expression

Introduction

Rice is a staple food for a majority of the human population and it is the preferable source of carbohydrate all over the world. The large acreage of rice around the world has led to its cultivation in diverse ecosystems where it is exposed to diverse stresses. Planting rice has its own challenges due to frequent simultaneously exposure to a number of biotic and abiotic constraints, Under field conditions. Among these stress factors, bacterial blight caused by Xanthomonas oryzae pv. oryzae (Xoo) and drought are major constraints causing substantial yield losses worldwide. The bacterial blight disease is favored by leaf surface wetness, high relative humidity, and high temperature (25-30°C) and is highly prevalent in irrigated and rainfed lowland systems. *Xoo* is a vascular pathogen, and enters through hydathodes, leaf margins and wounds, before moving to the xylem vessels for systemic infection it multiplies into the intercellular spaces beneath epithelial tissues (Noda, T. et al. 1999) [25] Initially type III secretion system gets activated and injects transcription activator like-effector (TALE) protein into the host cell. In incompatible reaction it activates the susceptible gene to promote the disease progression and in compatible reaction it activate the resistance gene that triggers the defense mechanism (Boch, J et al. 2009; Wang, L et al. 2017)^[7, 35]. Against this pathogen rice has evolved a two-layered innate immune system that includes pathogen-associated molecular pattern-triggered immunity and effector-triggered immunity (Jones and Dangl 2006)^[17]. After infection the host cells may initiate diverse signaling pathways including calcium signaling, mitogen-activated protein kinase (MAPK) cascades, phytohormone pathways and ionic fluxes in a resistant plant host, at the early stage (Chu and others 2004; Grewal and others 2012)^[9, 13]. Host resistance remains the most economically effective control measure against bacterial blight disease, and 39 rice resistance genes have been identified to control the disease in Asia (Natraijkumar et al. 2012; Khan et al. 2014; Zhang et al. 2015)^[24, 19, 38].

Drought stress is another important constrain and may be a great challenge to agricultural production all over the world (Ashraf, M. 2010)^[2] and 42 million hectares of rice is subject to occasional or frequent DS in Asia, resulting in significant yield loss (Venuprasad, R.,2009)^[34]. The occurrence of drought is the consequence of increasingly unexpected fluctuations in precipitation. Rice is a drought-sensitive crop and requires a large quantity of water. In drought stressed condition, water content will be limited in plant that affects growth through its direct influence on plant water status (Anjum *et al.* 2011)^[1] and also leads to severe yield loss especially at the reproductive stage (Venuprasad *et al.* 2009)^[34]. Imposing drought stress on rice plants can decrease fresh and dry biomass, plant height, tiller number, and panicle number

(Bahattacharjee *et al.* 1973; De Datta *et al.* 1973; Rahman *et al.* 2002) ^[4, 10, 29]. Drought-responsive genes like encoding dehydration responsive element binding (DREB) protein, late embryogenesis abundant (LEA) proteins and protein phosphatase 2C (PP2C) have been characterized as key components in the molecular network of DT in plant (Schweighofer and others 2004; Sharoni and others 2012; Yamaguchi-Shinozaki and Shinozaki 2006) ^[30, 31, 38]. However, the drought stress mechanism in plant is still unclear because they involve complex metabolic and morphological pathways (Krasensky and Jonak 2012; Price *et al.* 2002) ^[20, 28]. Even though many disease-responsive genes and drought stress-responsive genes have been identified, the crosstalk between these in rice is still obscure.

Climate change is predicted to increase the simultaneous occurrence of abiotic and biotic stresses which may act synergistically in damaging the plant, depend on the host resistance or susceptibility and also on the race of pathogens (Tippmann et al., 2006)^[32]. The response of the host plant may also influence by the occurrence of multiple stress and microclimate of plant microbe interactions. Overlapping plant responses to drought and bacterial stress have been reported in Arabidopsis, rice, chickpea, and sunflower (Prasch and Sonnewald, 2013; Atkinson et al., 2013; Vemanna et al., 2016; Choudhary et al., 2016)^[27, 3, 33, 8]. The water content in leaf may be correlated with the host plant defense response to pathogens and may restrict the bacterial growth in intercellular spaces in limited water condition (Wright and Beattie (2004) ^[36]. During effector-mediated defense it has been observed that the apoplastic water availability for bacteria is reduced and have a negative impact on bacteria growth (Beattie (2011)^[6]. Other physiological responses like increase of ABA, aquaporin expression, stomatal closure, accumulation of solutes and turger pressure are directly related to water stress (Bartels and Sunkar 2005)^[5]. These responses may help in reduction in foliar diseases. A higher susceptibility of sorghum and common bean to Macrophomina phaseolina under drought stress has been reported (Diourte et al. 1995; Mayek-Perez et al. 2002)^[12, 23]. There are several common changes in morphological, physiological traits and biochemical responses of plants to drought and pathogen stresses (Pandev et al., 2017)^[26] and also unique responses observed in response to both stresses when exposed independently. ABA and ethylene increases in plants with concomitant reduction of photosynthetic ability under combined stresses (Grimmer et al., 2012; Zhang and Sonnewald, 2017)^[14, 40]. Under limited water condition in the apoplast bacterial movement may affect, which is regulated by its flagellae and this is favored by water availability in the leaf apoplast. Pseudomonas syringae on bean has been observed better colonization and movement that promotes spread of the bacterium (Leben et al. 1970)^[21], and an abundance of free water has been reported to favor phyllosphere tissue entry by bacteria (Beattie 2011)^[6]. Apart from these it is also reported that under limited water content, bacteria can still move by swarming ability (Hattermann and Ries 1989: Beattie 2011)^[15, 6].

In rice simultaneous effects of drought stress and blight on rice resistance (R) gene-mediated resistance is still unknown.

Therefore, we hypothesized that the development of bacteria may varies, depend on water stress level in the host plant understanding this complex interaction will provide the information on how the host plant responds on bacterial spread under different water stress level. In this study, two genotypically different genotypes were evaluated for their effects on blight expression under different moisture level.

A lot of studies had been done on drought and blight of rice, separately or combine but till now the development of blight of rice in different gradient of drought levels has not been done. Hence, we studied the relation of disease progress at different levels of drought stress in compatible and incompatible genotypes.

Materials and methods

Disease severity assay based on lesion lenght on susceptible and resistant genotype

All the *Xoo* isolates were grown on NB medium for two days at 28 °C. The bacterial cells were resuspended in MES buffer at an optical density 0.5 (600 nm). Bacterial blight inoculation was carried out on 35-40 days old plants on susceptible rice varieties TN-1 and resistant genotype BPT5204, using the leaf-clipping method (Kaufmann 1973). This experiment was conducted under greenhouse conditions. For disease severity assay, the lesion length was measured in 6, 8, 10, 12 and 14 days after inoculation with 5 leaves for each isolate.

Quantification of Bacterial multiplication rate *in planta* in susceptible and resistant genotypes

In planta assay was carried out on both genotype and bacterial multiplication counted at six time points after infection by leaf clipping on 6, 8,10, 12 and 14 days post inoculation. The *in planta* was assayed as described by Hu *et al.*, (2007) ^[16]. The complete leaf was ground in autoclaved distilled water and the leaf homogenate was diluted in same. Serial dilutions were made three times and spread onto NA agar plates. The plates were incubated at 28 °C until the number of colony-forming units (CFU) per leaf per ml was counted.

Results

Disease severity of bacterial blight under different drought conditions

Different levels of drought stress was maintained by maintaining its field capacity at 60% filed capacity, 70% filed capacity, 80% filed capacity, 90% filed capacity and 100% filed capacity means fully irrigated or control, with three biological replications and these are treated as treatments. The filed capacity of different levels with respective to the percentage was gradually decreased and manage all these different levels of filed capacity with different percent in such a way that all the respective drought level come at particular day and on the same day inoculation was done.

Two genotypically different genotype was taken for different reaction. BPT5204 is the resistant to xoo infection and TN-1 which is susceptible to the same bacteria and through out the experiment humidity was maintained at 90% till the last observation. Disease severity was counted based on lesion length in cm.

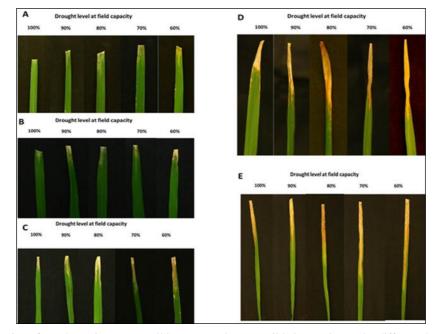


Fig 1: symptomatic expression of *Xoo* bacteria on susceptible genotype in compatible interaction under different drought stress level (different field capacity). A) Disease severity at 6 Day post inoculation (DPI), B) disease severity at 8DPI, C) disease severity at 10 DPI, D) disease severity at 12 DPI, E) disease severity at 14 DPI

Compatible interaction

In compatible interaction the pathogen infects and establishes in host plant and spread all over the plant and cause disease. In this type of interaction plant do not have any gene as a source of resistance. Symptoms started appearing on 6DPI (day post inoculation) and water soaked lesions were observed with little yellowing on all the leaf at 6DPI. surprisingly the drought level which is having filed capacity at 60% showed maximum length of lesion 2.92 cm among all and the least lesion length (0.46 cm) was recorded in the plant whose filed capacity was maintained at 100% at 6 DPI. Next observation was taken at 8DPI and lesion size was measured as at 70% filed capacity 2.96 cm, at 80% filed capacity 2.78 cm, at 90% filed capacity 2.54 cm and at 100% field capacity the lesion lenght was observed 1.5 cm. Same trend was continued till 14 DPI where the maximum lesion size was recorded 12.02 cm at 60% filed capacity and minimum was recorded 5cm at 100% filed capacity. As the drought level increases the blight severity was increases and vise-versa.

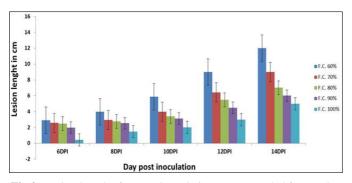


Fig 2: Lesion length after *Xoo* inoculation were recorded from 6 day post inoculation up to 14 day post inoculation

Incompatible interaction

For incompatible interaction the genotype taken is BPT5204, which is having resistance against this bacteria (*Xoo*), that is because of presence of three Xa genes Xa-5, Xa-13 and Xa-21, which confers resistance against this bacteria. After gradually decreasing the level of drought of respective treatment the *Xoo* infection where done. As such there was no

symptom where observed in any of treatment till 12 DPI. On 14 DPI little symptom of 0.4 cm was observed at 60% field capacity. On the contrary there was no any symptoms at 100% field capacity. This may be because of Xa genes present in the genotype.

Bacterial multiplication rate in-plant in both genotype

We have also investigated the role of drought stress on bacterial multiplication rate in-plant and its spread. The spread of the bacteria is along the vein and margin. The bacteria spread through the xylem vessels and multiply in inter cellular spaces. For bacterial multiplication rate the colony forming units (CFUs) has been calculated based on the formula CFU/leaf = (no. of colonies x dilution factor) / volume of culture plate.

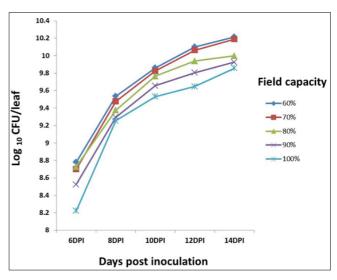


Fig 3: Quantification of bacterial multiplication rate in resistant genotype under different drought stress level (different field capacity)

Compactible interaction

In susceptible genotype TN-1, *Xoo* spread parallel to leaf. Drought stress positively effect on the spread of this bacteria,

it induced the multiplication of the bacteria. The maximum CFU count was recorded in 60% field capacity, where necrotic lesion size was observed maximum. Drought stress made the genotype susceptible to biotic stress and the genotype do not have any Xa genes as a source of resistance against this bacteria. The bacterial multiplication rate decreases as the field capacity increases and at 100% field capacity least bacterial multiplication has been observed among them.

Incompatible interaction

The genotype BPT5205 has already having 3 Xa genes and provide resistance to it. Even though the bacteria multiply inside the host was observed at 10, 12 and 14 DPI. Similar results were obtained in the resistant genotype also like susceptible genotype. On 10 DPI bacterial colonies were observed in 60% and 70% field capacity drought stress: on 12 DPI colonies were observed in 60%, 70%, 80% and 90% field capacity drought stress and at 100% filed capacity drought stress colonies were observed only at 14 DPI.

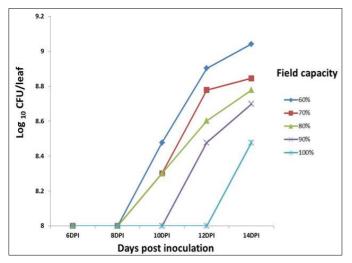


Fig 4: Quantification of bacterial multiplication rate in resistant genotype under different drought stress level (different field capacity)

Discussion

In this study, the lesion size in rice leaves varied by different drought stress level as well as genotype after the *Xoo* strain inoculation. The disease severity was generally increase when drought stress increased from the level of 60% field capacity to 100% field capacity in compatible interactions. The use of a two genotypically different genotype of rice in this study has shown that plant response to pathogens following a gene for gene interaction could be affected by drought stress.

In the compatible interaction, *Xoo* strain virulence was increased under high drought stress and induced longer lesion length, especially in the susceptible genotype TN-1. The growth of foliar pathogen has been previously reported to be restricted by low water availability (Wright and Beattie 2004) ^[36] and our results indicate that decrease in soil water content increase the lesion size, and additionally the different disease responses could be related to the genetic background of rice genotypes. Furthermore, the lesion size observed under 100% field capacity conditions compared to lesion size under high drought stress indicate that virulence of the bacteria was enhanced by drought stress.

Although lesion size was generally reduced with reducing drought stress, more colony forming units (CFUs) were recorded from different segments of inoculated leaves under high drought stress compared to the no drought stress treatments (100% field capacity), suggesting that *Xoo* multiplication, spread *in planta* and the lesion size depended on the resistance gene. In compatible interaction high drought stress may favor *Xoo* multiplication and spread *in planta*, possibly through lack of host immunity leading to increased bacterial multiplication and allowing *Xoo* movement *in planta*.

Although the disease symptoms were more developed under drought stress, this study showed that *Xoo* spread *in planta* extended beyond the symptomatic area under compatible interactions.

Simultaneous occurrence of abiotic and biotic stresses enhance host susceptibility or resistance, and that the outcome may depend on the stress and pathogen (Tippmann *et al.* 2006) ^[32]. Our study further suggest that the outcome of multiple stress interaction may also be influenced by host plant genetic background. Moreover, the microclimates in which the plants are growing can also influence the plantpathogen interaction.

Rice genotypes BPT5204 showed less disease development with *Xoo* infection, it's resistance were effective under simultaneous application of drought and bacterial blight stresses, demonstrating the genetic background effect on the interaction. Moreover, both increase and decrease of disease lesion size under drought stress revealed the complex interaction leading to physiological and molecular responses occurring in plants exposed to simultaneous abiotic and biotic stresses. This result is correlated to the report from Demirevska *et al.* (2009) ^[11] who suggested that plant tolerance to water deficit depends on stress level, plant species, and also developmental stage.

In summary, the lesion size development was generally increased under drought stress conditions along with bacterial blight multiplication and spread, varied according to rice genetic background. Furthermore, mechanistic understanding gained on the impact of drought stress on R gene mediated resistance to bacterial blight would provide better insights into the rice and bacterial blight pathosystem for rice varieties' improvement under climate change.

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