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Thermo-induction on Rice (*Oryza sativa* L.) Varieties and their Resistance Pattern-Foliage against Common Bacterial Leaf Blight Caused by *Xanthomonas*

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Abstract - Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* of the family Xanthomonadaceae is considered as one of the most serious diseases of *Oryza sativa* L. An experiment was conducted to assess the effect of temperature on the resistance pattern-foliage level of juvenile rice. Rice inbred cultivars PSB Rc82 (Peñaranda), NSIC Rc160 (Tubigan 14) inoculated with the isolated *Xanthomonas* sp. and controls were grown under two temperature conditions (34°C with humidity averaging 62.41% and 36°C with humidity averaging 59.17%) in an indoor lighting with 12h dark and 12 h light cycle. Evaluation of pattern-foliage response of inoculated plants revealed that PSB Rc82 is more susceptible to BLB than NSIC Rc160 under both temperature growth conditions especially in terms of length of the leaf from 0 DAI to 15 DAI. Also, at higher temperature of 36ºC, severity of BLB was limited.

Keywords: *bacterial leaf blight, resistance, rice, Xanthomonas*

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important crops and considered as main staple foods for most Filipinos. Philippines, although it is a small island nation, is also regarded as one of the top producers of rice [1], [2]. However, it is evident that there are a lot of problems that farmers encounter which are factors in limiting the yields from planted rice. Increasing global temperature, salt water intrusion, chilling temperatures resulting to water deficit, disease-causing microorganism and phytophagous insects are some factors which can cause damaging effects to crop plants thereby limiting their yield. Consequently, establishment of rice in the fields and obtaining the maximum benefit by having a good yield turns out to be a great challenge nowadays for our farmers.

Xanthomonas is one of the pathogenic microorganisms that cause devastating disease on rice plant belongs to kingdom Bacteria, Phylum Proteobacteria. It can infect rice from seedling to mature plant and the disease is manifested by either leaf blight or kresek symptoms [3]. Infection occurs at natural openings like in plants hydathodes, where excess water passes out of the leaf. Infection could also result from wound which made when seedlings are pulled from the seedbed, when leaves are cut or made by insects [4]. Bacterial leaf blight is a vascular disease in which lesions appear on leaf which may extend to the leaf sheath. The lesions enlarge in both length and width and may have wavy margin. From initial water-soaked greyish or yellowish hue it becomes whitish-straw color in 1-2 weeks [5], [6]. Bacterial ooze can be observed in freshly infected leaves which consist of small, yellowish, spherical masses [7]. Leaf blight may occur at all growth stages but it is more usual in tiller stage to maturity. Damage is due to wilting of tillers leading to unfilled grains or due to the partial or total blighting that might cause up to 50% yield losses [6]. The other phase of the bacterial blight is the Kresek phase where destructive manifestation can be observed and the phase where the entire plant wilts and become yellow at early tiller stage, resulting in total crop failure [8].

Xanthomonas oryzae pv. *oryzae* causes bacterial leaf blight (BLB) was proven to decrease annual rice production in both the tropical and temperate regions of the world [9]. The disease has become a serious threat to rice

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and several incidences of crop losses have been reported in several countries. Incidence has been reported from different parts of Asia, northern Australia, Africa and the United States [10]. In Japan, 25%-30% and often up to 50%-60% crop loss has been recorded while in India and Philippines up to 10%-81% in some genotypes [11]. The present yield losses of susceptible rice crops in the Philippines is about 22.5% in wet season and 7.2% in dry seasons while in resistant crops, it is about 1.8%-9.5% [9].

Temperature is a key determinant for microbial invasion and host evasion. Fluctuations in temperature due to changing climate can influence the initiation of plant diseases by promoting favorable conditions for growth and development of microorganisms [12], [13]. The global temperature is expected to rise from 1.8ºC lower scenario to a maximum estimated temperature of 4ºC in 2050s [14]. Thus, knowing the effect of temperature in the initiation of disease caused by microorganisms like *Xanthomonas* is critical in minimizing the adverse effects of the pathogen. The objectives of this study, therefore, are to evaluate the effects of temperature on resistance patterns in the foliage of two common rice varieties (PSB Rc82/ Peñaranda and NSIC Rc160/Tubigan 14) against bacterial leaf blight (BLB) caused by isolated *Xanthomonas* sp. in terms of its morphological character, the width and length, exhibited disease index and, disease severity.

METHODOLOGY

Description of the study area

Isolation and characterization of the microorganism was performed at the Don Mariano Marcos Memorial State University-South La Union, Microbiology Laboratory. The pathogenicity test was performed inside a controlled room where the temperature and humidity can be closely monitored. The growing of the rice cultivars was done in a mini chamber following a set up commonly used for gardening of indoor plants using artificial light [15].

Collection of infected leaves of rice (*Oryza sativa* L.)

Infected rice leaves of rice cultivar PSB Rc82 showing bacterial leaf blight (BLB) symptoms were obtained from rice paddies located in Santo Tomas, La Union, Philippines. Leaves showing pale-to-green and yellowish-white wavy margins were removed or cut from the plant and were placed in plastic containers and brought into the laboratory. The collected leaves were rinsed with distilled water and blotted dry using a clean dry paper.

Isolation and culturing of the microorganisms

The procedure for the isolation and culturing of microorganism was based from [12] with some modifications described by [16]. Approximately 0.16 mm^2 leaf tissues were excised from the lesion margins using sterile scalpel. The leaf surface were sterilized with 1% sodium hypochlorite for three minutes and washed with sterile distilled water. Instead of swab method on the diseased portion of collected leaves, samples were blotted dry and were directly overlaid on the surface of nutrient agar medium (Hi-Media Laboratories, India) on petri plates and incubated at 28°C for 24 hours. The colonies or bacterial growth which oozed along the periphery of the excised tissue served as the source of the next inoculum for successive streaking for isolation of desired microorganism. Yellow, mucoid, xanthomonad-like colonies were transferred using sterile inoculating loop and sub-cultured in a freshly prepared nutrient agar (NA) in 150 mm x 15 mm culture media plates. The growth of the bacteria were initially compared to the usual growth behavior and appearance of *Xanthomonas oryzae* pv. *oryzae* based on the study of [9] and [17]. Some of the microorganisms were also grown in a nutrient broth (NB) to limit the frequent sub culturing procedure common in NA plates.

Pathogenicity testing

Pathogenicity test was conducted in a normal environmental condition with daytime temperature of 28-32°C and night temperature of 20-25°C with relative humidity ranging from 40-65 atm. The plants were planted in sterilized field soil [18]. The inoculum prepared was adjusted to

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optical density of 0.5 McFarland standard (5×10^8 cfu/ml) using distilled water as the diluting agent to adjust the absorbance. To facilitate the easy penetration of the microorganisms and successful virulence, clip method was employed and inoculated rice were sprayed (3 times full spray mist) with distilled water every three hours. The spraying of distilled water is a technique to establish a growth environment conducive for BLB initiation and development. After sometime (i.e. 3 or 4 days to some), the appearance of patches of pale green to yellowy discoloration on the leaves confirmed the pathogenicity of the isolated microorganisms. Moreover, the infected leaves with BLB symptoms were again sampled and the microorganisms were isolated and grown for verification of their identity using the conventional method of isolating *Xanthomonas oryzae* pv. *oryzae* [9], [17].

Inoculation and incubation

Rice seeds used in the experiment were obtained from the Municipal Agriculturist Office of Malasiqui, Pangasinan. The seeds were identified and certified variety commonly supplied by the government as aid for farmers or stock. Upon confirmation of the identity of the variety of rice seeds, the researchers immediately proceed to the next step in the study. The seeds were initially sown in tray containing sterilized field soil (i.e. do not received fertilizer treatment for at least 10 years). Sterilization of the soil was based from the protocol described by [18]. Soil dug up 20cm deep from the surface was sterilized by heating the soil for 4 hours with constant stirring at 100°C. An 820 g of the sterilized soil was prepared using digital electronic weighing scale (ACS System, 3208) and were transferred into each propagation plastics with 17.5 cm height in maximum and a diameter of 13 cm.

Twenty seeds each of PSB Rc82 (Peñaranda) and NSIC Rc160 (Tubigan 14) were initially sterilized by washing them with 70% methanol and immediately rinsed with distilled water for 5 minutes and sown. To limit contamination from the water source, plants were watered using distilled water. Fifteen-day old rice seedlings of the PSB Rc82 and NSIC Rc160 cultivars were clipped inoculated and initially placed under improvised indoor greenhouse lighting [16] for the initiation of *Xanthomonas* sp. infection under 12h dark and 12h light period.

A standardized inoculum suspension equivalent to method applied by [19] was initially prepared. Inoculation using of the microorganism in the plants was done by opening a wound into the tip part of the plant leaf. A 4mm incision into the lamina at the apex and symmetrically 1cm to each side of the apex was employed using a scissors pre-dipped with the standardized inoculum. After this, the plants were sprayed with fine mist using distilled water once every 3 h to help maintain a high humidity until the appearance of symptoms suggesting BLB disease. At the same time, the control set up will not be subjected to clipped method while equally sprayed with fine mist of distilled water.

After 48 h, the 10 inoculated seedlings each of PSB Rc82 and NSIC Rc160 cultivars and control were arranged separately in the improvised growth chambers with varying temperatures namely, 34°C, and 36°C. We take note that the temperature considered for this study were higher than the usual field temperature during March and April (summer time) which ranges from 28-32°C. Plant cultivars were placed individually in a propagation plastic containing sterilized 820 g of soil and were watered with equal amounts of 300 ml every 6 hours. Also, as part of limiting the sources of error in pot experiments like this, weeds are not allowed to grow in all set ups. No fertilizer or herbicides were applied during the experiment and phytophagous insects were driven away or hindered from interacting with the rice seedlings. Moreover, as another part of this study, disease index and disease severity was rated in all the plants subjected under two different set-ups. Disease reactions were rated following the scale 1-9 suggested by the International Rice Research Institute [1]. The following tables below shows the scale rating used to evaluate the disease index and disease severity observed in this study. Assessments were conducted at the 0, 5^{th}, 10^{th}, and 15^{th} day after inoculation (DAI) in which the uninfected/non-inoculated samples were all rated zero in the first observation for disease index and disease severity.

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Table 1. The scale rating and evaluation of disease severity for BLB Assessment (Green House Test)

Scale	Lesion area
0	
1	No disease observed
2	Less than 1%
3	1-3%
4	4-5%
5	11-15%
6	16-25%
7	26-50%
8	51-75%
9	76-100%

Disease index

The disease index was calculated for each treatment. Disease index represent both disease incidence and symptom severity, and can be used as an indicator for virus or bacterial resistance in greenhouse test. Disease index can be calculated as:

$$\mathrm{DI} = \frac{\mathrm{n}(3) + \mathrm{n}(5) + \mathrm{n}(7) + \mathrm{n}(9)}{\mathrm{tn}}$$

Where: n(3), n(5), n(7), and n(9) = number of plants showing a reaction in a scale (3), (5), (7), and (9) respectively. tn= total number of plants scored. The resulting DI may classify as resistant or tolerant, Moderate or susceptible. A DI from 0-3 indicates that the rice plant is resistant. A DI of 4-6 indicates that rice plant is moderately resistant and a DI with 7-9 indicates that the rice plant is susceptible.

Disease severity

Disease severity was also computed. Disease severity is the percentage of relevant host tissues or organ covered by symptom or lesion or damaged by the disease. Severity results from the size and number of lesion. It generally indicates the damaged caused by the disease. Disease severity will be computed using the following equation.

$$\mathrm{DS} = \frac{\text{Sum of all disease ratings}}{(\text{Total no. of rating})\ (\text{max. disease grade})} \text{ x } 100$$

RESULTS AND DISCUSSION

Collection, isolation, and inoculation

The initial characterization of the 21 isolates through Gram staining and bacterial shape evaluation only revealed seven potential candidates of *Xanthomonas oryzae* . The seven rod-shaped isolates were then subjected to further conventional characterization suggested by [9] and [17] for the isolation and characterization of *Xanthomonas oryzae* pv. *oryzae*. Specifically, the authors considered the similar result that would indicate the highest probability that the isolate belongs to the family Xanthomonadaceae and under the genus *Xanthomonas.* From the successful seven isolates with similar features of *Xanthomonas oryzae* pv. *oryzae* based on the conventional method, isolate number 2 (*X-2*) was utilized in the study (see Table 2).

The *Xanthomonas* species are regarded as Gram negative bacteria with rod-shaped cells (Jonit et al. 2016), KOH test positive [20], [21], [9], catalase positive [21], [22], [9], oxidase negative [21], [9], [17], and negative for egg yolk hydrolysis [23], [17]. However, the study of [9] revealed that under similar result for all tests in table 2, except for *X-2*, the XOR and Xoo-9 isolates having positive result in starch hydrolysis were more *Micrococcus aloeverae* with 100% and *Xanthomonas sacchari* with 99% of similarity respectively using PCR and constructed phylogenetic tree by NCBI Blast Tree Method. It was seen that imperfect match may be due the nature and evolution of interactions of the microorganisms with their hosts and with the environment. Meanwhile, negative result for starch hydrolysis was described by [17], [23], and [24] for some *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) isolates. Thus, in this study the *X-2* isolate (*Xanthomonas* sp.) was utilized in the Kirby-Bauer Assay.

Table 2. Morphological and Biochemical Characterization of Various Isolates of *Xanthomonas oryzae* from PSB Rc82 (Peñaranda).

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Isolate	Gram stain	3% KOH	Catalase	Oxidase	Hydrolysis	
					Egg yolk	Starch
X-1	+	+	+	-	-	+
X-2	+	+	+	-	-	-
X-3	+	+	+	-	-	+
X-4	+	+	+	-	-	+
X-5	+	+	+	-	-	+
X-6	+	+	+	-	-	+
X-7	+	+	+	-	-	+

Legend: X-1 – Xanthomonas isolate 1

Response pattern-foliage of PSB Rc82 and NSIC Rc160

Environmental factors are known to influence the plant-pathogen interaction which is oftentimes observed as either enhancing or limiting the disease severity in the infected plant. Also, disease resistance of plants species is affected by these environmental factors [25], [26]. It can also be seen that the influence exerted by environmental factors like temperature is governed by the synergistic contribution of host-pathogen established relationship [27]. To be more specific, a favorable condition is needed for a pathogen to effectively and continuously cause the disease on its host and on one hand, for the host to respond to the pathogen attack to the best of its genetic potential. Thus, temperature is a critical factor in the initiation, development and spread of many plant diseases.

The present study evaluated the resistance pattern (foliage) of rice cultivars PSB Rc82 and NSIC Rc160 under 34ºC in terms of mean width and length of leaf. The table below shows the mean values at 0, 5, 10, and 15 DAI and the corresponding LSD values and p-values at 0.05 level of significance (IBM SPSS v. 23).

Table 3. Mean values of the effect on resistance pattern-foliage of rice cultivars PSB Rc82 and NSIC Rc160 in terms of width and length of leaves at 34°C.

DAI		PSB Rc82			NSIC Rc160		
		+	-	LSD	+	-	LSD
0	W	3.70	3.10	**0.60***	4.30	4.10	**0.20**
	L	5.87	9.71	**-3.84***	13.26	12.99	**0.27**
5	W	3.90	3.50	**0.40**	4.20	4.30	**-0.10**
	L	5.98	10.51	**-4.53***	13.40	13.12	**0.28**
10	W	3.80	4.30	**0.20**	3.60	4.40	**-0.10**
	L	5.92	10.59	**-4.67***	13.40	13.22	**0.18**
15	W	3.00	4.00	**-1.00**	3.60	4.70	**-1.10**
	L	4.45	10.69	**-6.24***	10.88	13.43	**-2.55**

*Legend: DAI = days after inoculation; W (mm) = width; L (mm) = length; + =X-2 inoculated; - = X-2 not inoculated; LSD = Least Significant Difference; * = significant at p = 0.05*

In terms of mean leaf width, infected PSB Rc82 was observed to have significantly wider result only at the start of observation (0 DAI). Meanwhile, data for NSIC Rc160 showed no difference ($p < 0.05$) in the leaf width from 0-15 DAI for both infected and uninfected rice plants. On one hand, mean length of leaves was found to be significantly lower ($p < 0.05$) at 0 -15 DAI for infected PSB Rc82 against the uninfected PSB Rc82. Interestingly, this behavior was not observed in the NSIC Rc160 variety.

To evaluate the resistance pattern-foliage of the two cultivars under different temperature conditions, another set up under higher temperature, 36ºC, was also conducted and plants were subjected for evaluation. The following table reflects the similar parameters and the computed p-values from 0 DAI to 15 DAI.

Table 4. Mean values of the effect on resistance pattern-foliage of rice cultivars PSB Rc82 and NSIC Rc160 in terms of width and length of leaves at 36°C.

DAI		PSB Rc82			NSIC Rc160		

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		+	-	LSD	+	-	LSD
0	W	3.90	3.80	**0.10**	3.90	4.10	**-0.20**
	L	7.22	10.37	**-3.15**	11.94	11.19	**0.75**
5	W	3.70	3.90	**-0.20**	4.00	4.10	**-0.10**
	L	7.14	10.51	**-3.37***	11.85	11.41	**0.44**
10	W	3.70	4.60	**-0.90**	4.00	4.40	**-0.40**
	L	6.86	10.79	**-3.93***	11.75	11.61	**0.14**
15	W	3.30	5.00	**-1.70***	3.90	4.80	**-0.90**
	L	6.58	10.97	**-4.39***	10.72	11.79	**-1.07**

*Legend: DAI = days after inoculation; W (mm) = width; L (mm) = length; + =X-2 inoculated; - = X-2 not inoculated; LSD = Least Significant Difference; * = significant at p = 0.05*

Statistical analysis (IBM SPSS v. 23, $p < 0.05$) revealed that at 36ºC both the infected and uninfected plants of PSB Rc82 and NSIC Rc160 showed that the width generally remained similar. In terms of mean leaf length, the PSB Rc82 infected plants starting from 5 DAI to 15 DAI showed a significantly shorter leaf length while NSIC Rc160 infected and uninfected plants showed no significant difference in leaf length from 0 DAI to 15 DAI. Based from the statistical analyses presented, it appears that the PSB Rc82 (Peñaranda) is more susceptible to the adverse effect of the phytopathogenic bacteria mainly decreasing the leaf length of plants under both temperature conditions. On one hand, NSIC Rc160 (Tubigan 14) was observed to generally remain constant in terms of all the parameters for both infected and uninfected samples under the two different growth temperature conditions.

The variety of rice plant can be a factor in the resistance from pathogen attack. . There are several varieties of rice which are engineered to solve problems on their establishment on Philippine fields and augment the negative effects of El- Niño and La- Niña. According to the report by [28] and [29], Philippine Rice Research Institute (PhilRice) introduced new drought-tolerant variety like NSIC Rc160 also referred to as Tubigan 14. It was developed as an early-maturing variety at 107 days resulting to a maximum yield of 8.2t/ha. In addition, NSIC Rc160 (Tubigan 14) is characterized to have a longer grain size, fast maturation, and has a higher milling recovery than PSB Rc82 (Peñaranda). Based from the actual observation in the present research, the maturity rate appears to be really faster in NSIC Rc160 than the PSB Rc82 where mean width and length are higher in NSIC Rc160 rice plant samples.

According to [30], PSB Rc82 (Peñaranda) has a maximum yield of 12t/ha and matures at 110 days after seeding. It was described that both the PSB Rc82 (Peñaranda) and NSIC Rc160 (Tubigan 14) has an intermediate reaction to bacterial leaf blight (BLB) [31]. Analysis also revealed that PSB Rc82 exhibited a more susceptible condition to pathogen adverse impact at 5-15 DAI specifically for leaf length parameter for both temperatures compared to a more stable or resistant response of NSIC Rc160. One possible reason for such result is the established plant-pathogen interaction between the plant variety and the isolated microorganism. One reason causing effective attack of phytopathogens is the long established plant-pathogen interaction [26] which is also similar to the report by [27]. One of the possible reasons for the pathogenicity of *Xanthomonas* is its evolved genetic makeup for it to cause infection. It means that such genus of bacteria is able to adapt better, to rice plant PSB Rc82 (Peñaranda) than on NSIC Rc160 (Tubigan 14).We have to take note that in this study, the isolated *Xanthomonas* sp. was originally obtained from previously BLB infected PSB Rc82 rice plants. Thus, the infection is likely to be expected to be well manifested in the PSB Rc82.

On molecular point of view, genome sequences from seventeen strains represented by *Xanthomonas* suggested that *tal* genes were found to be the reason of *Xanthomonas* pathogenicity. *Trancription activator-like* (*Tal*) effectors are bacterial type III effectors which are able to promote infection or resistance by inducing expression of their host's gene. It means that for them, these *Tal* genes are for initiation of their adverse effect on the plant body or a defense mechanism they can use against the host plant [12], [32].

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Effect of temperature on disease index and disease severity (%)

Species of *Xanthomonas oryzae* are undeniably the notorious cause of severe plant disease commonly called bacterial leaf blight (BLB). These disease-causing microorganisms are also able to tolerate the conditions set by the environment in order for them to establish pathogenicity. Environmental conditions that promotes *X. oryzae* infection is characterized by warm temperatures (25 to 30°C), high humidity (77 to 86°F), rain, and deep water [33]. Bacterial leaf blight which is the most serious disease of rice worldwide is caused by the species of *Xanthomonas oryzae*. A study stated that there are at least 350 different plant diseases caused by *Xanthomonas* spp. The microorganism is a plant pathogenic gram-negative bacterium that has systemic attack on its common host which is *Oryza sativa* (rice). It is often the cause of Kresek syndrome (wilt syndrome) in seedlings and described as bacterial leaf blight (BLB) in mature rice plants [34].

The Kresek syndrome is known to be the most destructive symptom of the disease caused by *X. oryzae*. Leaves of the rice plants starts to roll up and turn to grayish green and eventually turning to yellow. Symptom usually starts from the formation of somewhat water-soaked stripes on the leaf blades of rice then its increase on width and length coverage and eventually turning into yellow and white that may occupy the whole leaf blade with high severity. It was described to attack the xylem of the rice plant and often starts infection through entrance at the hydathodes regions at the leaf tip and margins, stomata, and through wounding [34]. It multiplies in the intercellular spaces of the underlying epithelial cells and may eventually spread in the mesophyll [33].

Another essential part of the present research is the evaluation of the pathogenicity of the isolated *Xanthomonas* sp. to the two different rice cultivars grown under two different temperature conditions in terms of percent disease index (DI) and disease severity (DS). The following Table 5 shows the result for plant varieties under 34ºC.

Table 5. Mean values of the effect on resistance pattern-foliage of rice cultivars PSB Rc82 and NSIC Rc160 at 34°C in terms of disease severity and disease index.

DAI		PSB Rc82			NSIC Rc160		
		+	-	LSD	+	-	LSD
0	DI	1.90	0.00	**1.90***	2.40	0.00	**2.40***
	DS	21.11	0.00	**21.11***	26.67	0.00	**26.67***
5	DI	3.50	1.30	**2.20***	4.10	1.10	**3.00***
	DS	38.89	14.44	**24.44***	45.56	12.22	**33.33***
10	DI	4.20	1.60	**2.60***	4.50	1.10	**3.40***
	DS	46.67	17.78	**28.89***	50.00	12.22	**37.78***
15	DI	5.00	1.70	**3.30***	4.90	1.10	**3.80***
	DS	55.56	18.89	**36.67***	54.44	12.22	**42.22***

*Legend: DAI = days after inoculation; W (mm) = width; L (mm) = length; + =X-2 inoculated; - = X-2 not inoculated; LSD = Least Significant Difference; * = significant at p = 0.05*

Under growth temperature condition of 34ºC and from 0 DAI to 15 DAI, both the PSB Rc82 (Peñaranda) and NSIC Rc160 (Tubigan 14), showed statistically significant ($p<0.05$) difference in the exhibited disease index (DI) and percent disease severity (DS) of the infected and uninfected plants. All the varieties follow the same response wherein the uninfected plant has a significantly lower DI and DS in an increasing manner from 0 DAI to 15 DAI. On one hand, a similar evaluation was conducted to evaluate the DI and DS for plants under 36ºC. The following table shows the summary of the result of the statistical analysis of the gathered data.

Table 6. Mean values of the effect on resistance pattern-foliage of rice cultivars PSB Rc82 and NSIC Rc160 at 36°C in terms of disease severity and disease index.

DAI		PSB Rc82			NSIC Rc160		
		+	-	LSD	+	-	LSD

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0	DI	1.80	0.00	**1.80***	2.10	0.00	**2.10***
	DS	20.00	0.00	**20.00***	23.33	0.00	**23.33***
5	DI	4.40	1.20	**3.20***	3.80	1.30	**2.50***
	DS	48.89	13.33	**35.56***	42.22	14.44	**27.78***
1 0	DI	5.20	1.30	**3.90***	4.10	1.30	**2.80***
	DS	57.78	14.44	**43.33***	45.56	14.44	**31.11***
1 5	DI	5.40	1.30	**4.10***	4.20	1.30	**2.90***
	DS	60.00	14.44	**45.56***	46.67	14.44	**32.22***

*Legend: DAI = days after inoculation; W (mm) = width; L (mm) = length; + =X-2 inoculated; - = X-2 not inoculated; LSD = Least Significant Difference; * = significant at p = 0.05*

Similarly, under growth temperature condition of 36ºC and from 0 DAI to 15 DAI, both PSB Rc82 (Peñaranda) and NSIC Rc160 (Tubigan 14) revealed a significant ($p<0.05$) difference in the exhibited disease index (DI) and percent disease severity (DS) of the infected and uninfected plants. All the varieties follow the same trend wherein the uninfected plant has a significantly lower DI and DS in an increasing manner from 0 DAI to 15 DAI. It can be infer from the mean DI and percent DS values from Table 5 and Table 6 that pathogenicity are usually higher in plants grown under 34ºC specifically, the infected plants often found to have greater computed DI and percent DS. This phenomenon is seen as a factor of temperature as well as dependent on the optimum condition of the *Xanthomonas* sp. isolate for its pathogenicity.

There are certain diseases which are promoted in the warm areas of the globe while the other plant diseases commonly appear in temperate regions. Several studies showed that temperature can affect the pathogenicity of many microorganisms. A study by [35] revealed that bacterial spot caused *by Xanthomonas perforans* and *X. gardneri* in tomato is affected by temperature. It was found that 20°C promotes the severity of disease in tomato plants caused by *X. gardneri*. However, the higher temperature at 30°C supports the further development of bacterial spot primarily caused by *X. perforans*. Similarly, it was emphasized that common bacterial blight is a warm weather disease. At the range of 28ºC to 32ºC, *Xanthomonas* can cause greatest damage. In the study by [12] focusing on *Phaseolus vulgaris* or common bean, common bacterial blight caused by *Xanthomonas axonopodis* pv. *phaseoli* and *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscan* was found to be more severe in the lower temperature of 28°C than on higher temperatures of 30, 32, and 34°C. Moreover, the higher temperature of 34°C was found to be lowest for both strains of *Xanthomonas*.

Although the bacterial strains were reported to survive at temperature range of 25-35°C, the result was found to be affected by the optimum temperature for the growth of common bacterial blight disease-causing microorganisms which is within the range of 28-32°C. Other reports suggested that *Xanthomonas* can be infective at temperature ranges from 28ºC to 32ºC [36] and 25ºC to 35ºC [37], [38]. In the present investigation, 34ºC falls under these several ranges that would support the optimum growth of *Xanthomonas* spp. while 36ºC falls outside this range. Temperature was also shown to be a driving force for the initiation and development of wheat streak mosaic disease caused by a virus (WSMV). It was shown that lower temperatures at 10 and 15°C impaired proliferation of WSMV in Tomahawk plants while, higher temperatures promotes the severity of the disease [25]. In is interpreted that in this study, that temperature is a limiting factor in the pathogenicity of the disease-causing microorganism for BLB. The higher temperature at 36ºC was observed to impede the severity of the disease by lessening the pathogenicity of the isolated *Xanthomonas* sp. since it is outside the range of its optimum condition. Meanwhile, 34ºC can be a better condition for the pathogenicity of the isolated *Xanthomonas* sp. causing the BLB.

In connection to a higher disease severity in the lower temperature, the virulence is an essential characteristic of the bacterial pathogen. The major factors utilized and regulated by bacterial pathogens include type III secretion system (T3SS), polysaccharides, extracellular enzymes, toxins and plant hormones. Many Gram-negative bacterial pathogens are known to use T3SS which is encoded by the hypersensitive

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response and pathogenicity (*hrp*) gene, for their pathogenicity. The *hrp* gene is found to occur in at least 20 genes in a cluster. Pathogens use this to export bacterial effectors mostly under AvrBs3/PthA family into their host with the aid of chaperon proteins like XopJ and XopF1 needing HpaC (*hrp*-associated protein). In *Xanthomonas* sp., the expression of this gene is controlled by regulators HrpG and HrpX. The AraC-type HrpX protein controls the expression of genes in *hrp* and other effectors. Once effectors are delivered into the host, these can activate the expression of certain genes that will disrupt the normal processes of the host plant. Another way that promotes *Xanthomonas* sp. pathogenicity is thru the use of polysaccharides. The lipolysaccharides (LPS), lipooligosaccharide (LOS), and extracellular polysaccharide (EPS) present in Gram-negative bacteria like *Xanthomonas* sp. is utilized for their pathogenicity. In fact, the O-polysaccharide (O-chain) which the main hydrophilic component of the LPS is needed for the transport of the bacterial effectors into the host cells. The use of extracellular enzymes (EPS) is also a characteristic of *Xanthomonas* sp. particularly it has been observed in *Xanthomonas oryzae* pv. *oryzae (Xoo). Xanthomonas* is seen to use the extracellular enzyme as virulence factor by overexpression of protein that codes for an enzyme xylanase that effectively degrades xylan which is a component of the xylem vessels [34].

In another more recent study, virulence of *Xanthomonas oryzae* pv. *oryzae* was described to utilized histone-like nucleoid-structuring (H-NS) proteins to regulate its virulence during rice infection. The H-NS family of protein is known to be conserved in the Gram-negative bacteria. The *xrvC* gene of the H-NS protein family was found to regulate virulence of the bacteria *Xathomonas oryzae* pv. *oryzae* (*Xoo*) Philippines strain $PXO99^{A}$ [39]. In the study by [32], it was found that *Xanthomonas* pathogenicity can be the result of adaptation of *Xanthomonas* to common bean *Phaseolus vulgaris*. Genome sequences from seventeen strains represented by *Xanthomonas* suggested that *tal* genes were found to be the reason of *Xanthomonas* pathogenicity. *Trancription activator-like* (*Tal*) effectors are bacterial type III effectors which are able to promote infection or resistance by inducing expression of their host's gene. It means that for them these *Tal* genes are for initiation of their adverse effect on the plant body or a defense mechanism they can use against the host plant.

Resistance mechanism of plants infected by *Xanthomonas* spp.

There are 29 *R* genes that confer rice resistance to *Xoo* and among the 29, six were cloned namely *Xa1, xa5, xa13, Xa21, Xa26 and Xa27*. Meanwhile, the *avr* genes of Xoo which confers the pathogenicity of Xoo were also first cloned in the Philippines namely, *avrXa7, avrXa10, and avrXa27*. Pattern-triggered immunity (PTI) and effector-triggered immunity (ETI) was observed to play a role on the pathogen responses. Using *Arabidopis thaliana* as a model plant, they found that ETI signaling is activated at relatively low temperature (10-23ºC) while they shift to PTI signaling at moderate elevated temperatures (23-32ºC) [34]. Mutants grew in elevated temperatures shows features of enhanced PTI but reduced ETI responses. Thus, immune responses by plants are also affected by environmental factors [13]. To [40] the optimum temperature for rice growth is within the range of 25-32°C). In their study, a set up greenhouse for rice cultivars (shirudi, fajr, local tarom, hybrid, and line 843) growth at normal temperature was maintained at 32°C and at humidity between 80-85%. It was found that colder temperature at 13°C showed a significant adverse effect at 1% level on all treatments wherein affecting the number of panicles, length of panicles, the number of full, empty, total grains, and yield. The present study utilized two growth chambers that exhibit temperature conditions higher than the suggested optimum growth temperature of *Oryza sativa* L. as reported by [40]. It was found that at higher case scenario, temperature above optimum level of the susceptible plant or its variety is not a key factor in the pathogenicity. In fact, both PSB Rc82 (Peñaranda) and NSIC Rc160 (Tubigan 14) were previously known to exhibit an intermediate reaction to bacterial leaf blight (BLB) while PSB Rc82 is resistant to blast disease. Moreover, PSB Rc82 known as the progeny of IR64 belongs to the inbred lines of PSB rice varieties in 2004 known to have

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resistant genes that could control bacterial leaf blight [31]. However, the result of the this study does not support the resistance of PSB Rc82 against the *Xanthomonas* sp. isolate instead, the present investigation showed that at higher temperature the infecting-microorganism will not be favored to establish an enhanced pathogenicity.

CONCLUSION

The result of the experiment indicated that the initiation and development of bacterial leaf blight (BLB) disease at low or high temperature caused by the *Xanthomonas* sp. on the two rice cultivars (PSB Rc82 and NSIC Rc160) can be affected by previously established specific plant-pathogen interaction. Evaluation of pattern-foliage response of plants inoculated with *Xanthomonas* sp. isolate from PSB Rc82 revealed that PSB Rc82 is more susceptible than NSIC Rc160 under both temperature growth conditions. The higher temperature at 36ºC was observed to impede the severity of the disease by lessening the pathogenicity of the isolated *Xanthomonas* sp. since it is outside the range of its optimum condition. Meanwhile, 34ºC can be a better condition for the pathogenicity of the isolated *Xanthomonas* sp. causing the BLB.

REFERENCES

[1] International Rice Research Institute (IRRI)a. (2013). *Standard Evaluation System (SES) for Rice, Fifth Edn*. Manila, Philippines.

[2] International Rice Research Institute (IRRI)b. (2013). Ricepedia: IRRI's rice almanac.

[3] Ou, S.H. (1985). *Rice Diseases*. 2nd ed. Common Wealth. Mycological Institute. Kew, Surrey. England. pp. 61-96.

[4] Jackson, G. (2014). Bacterial leaf blight of rice. *Cabi Africa Soil Health Consortium.*

[5] Kiran, R. & Singh, P.P. (2015). Effect of rain simulation on *Xanthomonas oryzae* pv. *oryzae* population density in guttation fluid and on the spread of bacterial blight in rice. *Journal of Applied and Natural Science. 7* (1), pp. 72 – 76.

[6] Gnanamanickam, S.S., Priyadarshini, V.B., Narayanan, N.N., Vasudevan, P., & Kavitha, S. (1999). An overview of bacterial blight disease of rice and strategies for its management. *Current Science*, *77*, pp. 1435- 1443.

[7] Tan, S.Y., Gu, Y., Yang, C.L., Dong, Y., Mei, X.L., Shen, Q.R. & Xu, Y.C. (2016) *Bacillus amyloliquefaciens* T-5 may prevent *Ralstonia solanacearum* infection through competitive exclusion. *Biology and Fertility of Soils, 52*, pp. 341–351.

[8] Mew, T.W., Alvarez, A.M., Leach, J.E., Swings, J. (1993). Focus on bacterial blight of rice. *Plant Disease*, *77*(1), pp. 5-12.

[9] Jonit, N.Q., Low, Y.C., & Tan, G.H. (2016).*Xanthomonas oryzae* pv. *oryzae,* Biochemical Tests, Rice (*Oryza sativa*), Bacterial Leaf Blight (BLB) Disease, Sekinchan. *Journal of Applied & Environmental Microbiology, 4* (3), pp. 63-69.

[10] Kala, A., Soosairaj, S., Mathiyazhagan, S. & Raja, P. (2015). Isolation and Identification of *Xanthomonas oryzae* pv. *oryzae* the causal agent of rice bacterial leaf blight and its activities against of six medicinal plants .*Asian Journal of Plant Science and Research, 5(*6*)*, pp. 80-83.

[11] Ahmed, W. & Singh, R. (1975). Disease development and yield losses in rice varieties by bacterial leaf blight. *Ind. Phytopathology*, *28*, pp. 502–507.

[12] Hailu, N., Fininsa, C., Tana, T., & Mamo, G. (2017). Effects of temperature and moisture on growth of common bean and its resistance reaction against common bacterial blight (*Xanthomonas axonopodis* pv. *phaseoli* strains). *Journal of Plant Pathology and Microbiology. 8*(9), 1000419.

[13] Cheng, C., Gao, X., Feng, B., Sheen, J., Shan, L. & He, P. (2013). Plant immune response to pathogens differs with changing temperatures. *Nature Communications*, *4*, 2530.

[14] IPCC (Inter-governmental Panel on

Climate Change) (2007). *Climate change: Mitigation, contribution of working group III to the fourth assessment report of the intergovernmental panel on climate change*. IPCC, Cambridge University, New York, USA. p. 987.

[15] Torres, J.R.D. & Malaya, V.N. (2016). Biological properties of different extracts derived from body wall and cuvierian tubules of *Pearsonothuria graeffei* Semper (Black-spotted Sea Cucumber). Unpublished Thesis. Don Mariano Marcos Memorial State University.

[16] Torres, J.R.D., Billote, R.A., Villena, C.D.B., Jugo, R.J.T., & Osillos, P.L. (2019). Bacteriostatic property of *Holothuria ocellata* crude methanol extracts against isolated *Xanthomonas* sp. from a rice cultivar. Unpublished Technical Report.

[17] Jabeen, R., Iftikhar, T., & Batool, H. (2012). Isolation, characterization and pathogenicity of *Xanthomonas oryzae* pv. *oryzae* causing BLB disease in rice. *Pakistan Journal of Botany, 44*(1), pp. 261-265.

[18] Osillos, P.L. & Nagpala, A.L. (2014). The effects of arbuscular mycorrhizal fungi (AMF) as biofertilizer on the growth, yield and nutrient uptake of tomato (*Lycopersicon esculentum* Mill.). *International Journal of Scientific Engineering and Research, 3* (11), pp. 49-65.

[19] Torres, J.R.D. (2017). Antibacterial and antifungal property of extracts derived from the body wall and cuvierian tubules of *Pearsonothuria graeffei* Semper (Black-spotted Sea cucumber). *Frontiers in Medicinal Chemistry and Drug Discovery. 2*(1), pp. 010-018.

[20] Meena, C., Kadam, J.J., & Safna, M. (2018). Physiological and biochemical characterization *of Xanthomonas oryzae* pv. *oryzae* inciting bacterial leaf blight of rice and proving its pathogenicity. ` *International Journal of Chemical Studies*, *6*(1), pp. 165-167.

[21] Shankara, K., Patil, M.B., Pramesh, D., Sunkad, G., Yenjerappa, S.T., Ibrahim, M., Rajesh, N.L., & Chikkannswamy. (2017). Characterization of *Xanthomonas oryzae* pv. *oryzae* isolates from rice growing regions of Southern India. *Int. J. Pure App. Biosci., 5*(4), pp. 452-461. doi: http://dx.doi.org/10.18782/2320-7051.2436

[22] Patil, B., Karegowda, C., Narayanaswamy, H., & Wasimfiroz, M. (2017). Biochemical variation among isolates of *Xanthomonas oryzae* pv. *oryzae* causing bacterial leaf blight in rice. *International Journal of Chemical Studies, 5*(6), pp. 1265-1268.

[23] Rafi, A., Hameed, A., Ahktar, M.A., Farhatullah, ?., Sohail, K., Shahid, M., & Fahim, M. (2013). Identification and characterization of *Xanthomonas oryzae* pv. *oryzae* in north-west of Pakistan. *Sarhad Journal of Agricultural, 29*(3), pp. 423-443.

[24] Breed, R.S., Murray, E.G.D., & Smith, N.R. (Eds.) (1957). Bergey's manual of determine bacteriology, 7th Ed., William & Wilkins, Baltimore, pp. 152-183.

[25] Wosula, E.N., Tatineni, S., Wegulo, S.N., & Hein, G.L. (2017). Effect of temperature of wheat streak mosaic disease development in winter wheat. *Plant Disease, 101*, pp. 324-330. http://dx.doi.org/10.1094/PDIS-07-16-1053-RE

[26] Agrios, G.N. (2012). *Plant Pathology*. Burlington, USA: Academic Press. ISBN: 978-81-312-0639-3.

[27] Wang, Y., Bao, Z., Zhu, Y., & Hua, J. (2009). Analysis of temperature modulation of plant defense against biotrophicmicrobes. *Mol. Plant-Microbe Interact, 22*, pp. 498-506.

[28] Nidoy, M.G. (2015). PhilRice recommends El Niño-ready varieties, technologies. [Article on-line] Retrieved from http://www.philrice.gov.ph/philrice-recommends-el-nino-ready-varieties-technologies/

[29] Jose L. (2015). *Philippine Rice brings out drought-resistant rice varieties*.Retrieved April 17, 2018 from http://e

extension.gov.ph/elearning/mod/forum/discuss.php?d=996

[30] Philippine Rice Research Institute (PhilRice).(n.d.). Pinoy Rice Knowledge Bank: Philippine Seedboard (PSB)/NSIC Rice Varieties. Philippine Rice Research Institute. Retrieved April 16, 2018 from http://www.pinoyrice.com/rice-varieties/

[31] Abalos, M.C., Tabien, D., Alfonso, R.E., & Dela Cruz, A.A. (2014). Genetic analysis of selected PSB rice varieties for resistance to bacterial blight (*Xanthomonas oryzae* pv. *oryzae ex Ishiyama*). *Philippine Journal of Crop Science*. Retrieved from http://agris.fao.org/agrissearch/search.do?recordID=PH2005000479

[32] Ruh, M., Briand, M., Bonnean, S., Jacques, M-A. & Chen, N.W.G. (2017). *Xanthomonas* adaptation to common bean is associated with horizontal transfer of genes encoding TAL effectors. *BMC Genomics*, *18*, p. 670. doi: 10.1186/s12864-017-4087-6.

[33] Sullivan, M., Daniells, E., & Southwick, C. (2011) CPHST Pest Datasheet for *Xanthomonas oryzae* pv. *oryzae*. USDA-APHIS-PPQ-CPHST.

[34] Wang, L. (2007). Understanding the virulence mechanisms of bacterial phytophatogens: from *Pseudomonas* to *Xanthomonas. Retrospective Theses and Dissertations*. 15915. Retrieved from http://lib.dr.iastate.edu/rtd/15915

[35] Araújo, E., Café-Filho, A.C., & Moita, A.W. (2011). Effect of temperature on pathogenicity components of tomato bacterial spot and competition between *Xanthomonas perforans* and *X. gardneri*. *Acta Horticulturae, 914*. doi: 10.17660/ActaHortic.2011.914.3

[36] Belete, T. & Bastas, K.K. (2017) Common Bacterial Blight (*Xanthomonas axonopodis* pv. *phaseoli*) of Beans with Special Focus on Ethiopian Condition. *J Plant Pathol Microbiol. 8,* 403. doi: 10.4172/2157-7471.1000403

[37] Nunes, W.M.C., Corazza, M.J., Souza, C.D., Tsai, S.M.& Kuramae, E.E. (2008). Characterization of *Xanthomonas axonopodis* pv. *phaseoli* isolates. *Summa Phytopathology*, *34*, pp. 228-231.

[38] Gent, D.H., Lang, J.M., & Schawartz, H.F. (2005). Epiphytic survival of *Xanthomonas axonopodis* pv. *allii* and *Xanthomonas axonopodis* pv. *phaseoli* on leguminous hosts and onion. *Plant Disease, 89*, pp. 558-564.

[39] Liu, Y., Long, J., Shen, D., & Song, C. (2016*). Xanthomonas oryzae* pv. *oryzae* requires H-NS-family protein *XrvC* to regulate virulence during rice infection. *FEMS Microbiology Letters, 363* (10), pp. 1-6. doi: 10.1093/femsle/fnw067

[40] Ghadirnezhad, R. & Fallah, A. (2014). Temperature effect on yield and yield components of different rice cultivars in flowering stage. *International Journal of Agronomy*, *2014*, Article ID 846707, 4 pages. doi:10.1155/2014/846707