Malays. Appl. Biol. (2019) 48(1): 157–162

ELUCIDATING THE DYNAMIC OF DROUGHT TOLERANCE RICE, MR219-4 TO THE *Xanthomonas oryzae* INFECTION

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Accepted 9 January 2019, Published online 20 March 2019

ABSTRACT

Rice (*Oryza sativa*) is a primary source of food for more than half of the world's population. Despite its importance, rice production struggles with various pathogenic attacks particularly by *Xanthomonas oryzae* that causes bacterial blight. Overcoming this issues is of utmost priority especially when the use of drought-tolerant varieties to secure production in erratic climate is on the rise. Thus it is important to comprehend the pathogenicity of this proteobacteria towards rice. Consequently this study was conducted to determine the pathology of *X. oryzae* infection on drought tolerance rice variety namely MR219-4 grown under normal conditions. 16-days old seedlings were infected with the pathogen and subsequently the morphological traits, total oil content and the variance between total oil, biomass (except for day 20) and dry weight of the infected and non infected plants were assessed in three developmental phases; seedlings, vegetative and reproductive. The results showed that plant height, root length, biomass and total oil content did not significantly differ between the infected and the control plants. However, variance analysis shows that bacterial infection caused a deterministic toward stochastic response in total oil/biomass variance over growth stages. Thus indicating that the population dynamic of MR219-4 defence mechanism to the infection is deterministic during the seedling stages and becoming stochastic during the vegetative phase paddy.

Key words: Pathogenic infection, MR219-4, biotic, morphological, total oil, variance

INTRODUCTION

Rice is undoubtedly one of the most commonly grown food crops in both tropical and subtropical region. The annual losses of rice production ranging between 20% and 40% of global agricultural productivity due to bacterial infection (Savary *et al*., 2012). The bacterial infection causes biotic stress that leads to various rice diseases thus limiting the rice yield. Bacterial leaf blight (BLB) is one of rice diseases due to bacterial infection. One of the pathogens that can cause this disease is *Xanthomonas oryzae,* which a bacterium that can infect the rice seed to mature plant through irrigation water and rain (NiñO-Liu *et al*., 2006; Sakthivel *et al*., 2001).

In Asia, rice is grown in four different ecosystems such as irrigated lowlands, rain-fed lowlands, flood-prone environments, and upland systems (Cottyn *et al*., 2001). Irrigation is usually applied as a supplement during the rainy season. MR219-4 is a locally developed aerobic rice variety known to be more drought-tolerant than its predecessor MR219 (Abd Wahid *et al*., 2016). However, MR219-4 response to any infection has never been studied before. Therefore, the objective of this study was to determine the pathology of *X. oryzae* infection on the growth and degree of disease resistance of MR219-4.

MATERIALS AND METHODS

Source of bacteria and seeds

Bacterial *Xanthomonas oryzae* strain used was previously isolated from infected rice (*Oryza sativa*) in a greenhouse at Universiti Malaysia Terengganu (UMT). The isolated rice was purified, characterised, identified and kept in the Microbiology Laboratory

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at UMT. MR219-4 seeds were obtained from MARDI and further proliferated in a greenhouse.

Seedling preparation and treatments

MR219-4 seeds were soaked in distilled water overnight. The seeds were germinated in a petri dish containing distilled water and incubated in the dark at 28°C until the coleoptile emerged. Germinated seeds with 5-10 cm coleoptile were transferred to a culture tube that contained nutrient solution prepared according to Podar (2013).

The experiment was set up in a completely randomised design (CRD) with five replicates and fifteen seedlings each for both the treatment and control. The seedlings were grown in hydroponic solution for 16 days or until three leaves had emerged. A bacterial broth of *X. oryzae* containing 10^1 CFU/ml with the optical density of 0.8 was transferred into the hydroponic culture of 16-days old seedling and incubated for 24 hours. Subsequently, the roots of the seedlings was rinsed with distilled water before being transplanted into the mix-soil (3:2 ratio of topsoil: river sand). The plants were subsequently grown in the greenhouse. Each pot of the infected rice was covered with a plastic curtain to prevent airborne dispersal of bacteria within the confined greenhouse. Fertilizer was applied according to a suggestion by MARDI and watered when required. All the changes in morphological traits from the infected and the non-infected paddy such as plant height, root length and biomass were recorded.

Assessment of total oil content

Five samples (whole plant excluding roots) from each condition were harvested and dried in an oven at 80°C until a constant weight was obtained. Five hundred milligram of the dried samples were digested with 10 ml of HCl in the boiling water for 30 min, cooled for 15 min then added with 25 ml of hexane before being vortexed for 30 min with the final addition of 15 ml of hexane. The upper layer of the mixture was harvested and dried overnight in an oven at 70°C until consistent weight was obtained. The oil was weighed using an analytical balance as described by Cha *et al*. (2011).

Data analysis

The morphological traits such as plant height, root length and biomass weight along with the total oil content of the infected rice were compared with the controlled (non-infected) using two-tailed Student T-test. The variance of total oil, dry weight and biomass was recorded in each phases; seedlings, vegetative and reproductive as to observe the dynamic viability of MR219-4 towards infection. Pearson correlation (r^2) and Regression (R^2) was assessed as to measure the correlation between dry weight and total oil of the infected and the non-infected paddy.

RESULTS AND DISCUSSION

Effects of *Xanthomonas oryzae* infection on morphological traits

Our results showed that infection of pathogenic bacteria *X. oryzae* did not significantly (p-value > 0.05) affected the morphological traits; plant height, roots length and biomass (except for day 20) of drought tolerance rice variety, MR219-4 (Figure 1). This indicates that the *X. oryzae* infection was asymptomatic towards MR219-4 due to the plant defence mechanism. Generally, the plant defence response recognise pathogen-associated molecular patterns (PAMPs) via pattern recognition receptors (PRRs) which trigger the PAMP-triggered immunity (PTI) such as promote resistance proteins which was secreted upon recognition of microbial pathogens (Boller & Felix, 2009; Zipfel, 2009). The plant will induce the plant hormones such as salicylic acid (SA), which acts as signals to trigger the salicylic acid dependent signalling pathway that associated with systemic acquired resistancy (SAR) (Le Thanh *et al*., 2017). This SAR will stimulate the expression of resistance genes which can promote defence enzymes, phytoalexins as well as other gene products which can kill or inhibit pathogen growth (Gális *et al*., 2004; Shadle *et al*., 2003). Although these mechanism never been reported to occur in MR219-4, presumably, similar defence response mechanism may be employed by MR219-4 causing the infection to become asymptomatic (Denancé *et al*., 2013).

Effects of *Xanthomonas oryzae* infection on total oil of the MR219-4

In the event of bacterial infection, plant produced lipid based compound such as Jasmonate (JAs), methyl jasmonate (MeJA) and ethylene as a form of defence mechanism (Browse, 2009). Therefore, the infected plant may physiologically increased the total oil production. Physiological assessment on total oil content showed that no significant differences (p-value > 0.05) the infected and the control plants (Figure 2). This finding indicated that the infection did not affected the total oil due to biotic and abiotic stresses (Chu & Tso, 1968; Mohan & Mahadevan, 2001; Zhu, 2016). The yield of total oil is highly correlated with the dry weight (Figure 3) and biomass (data not shown) of the plants.

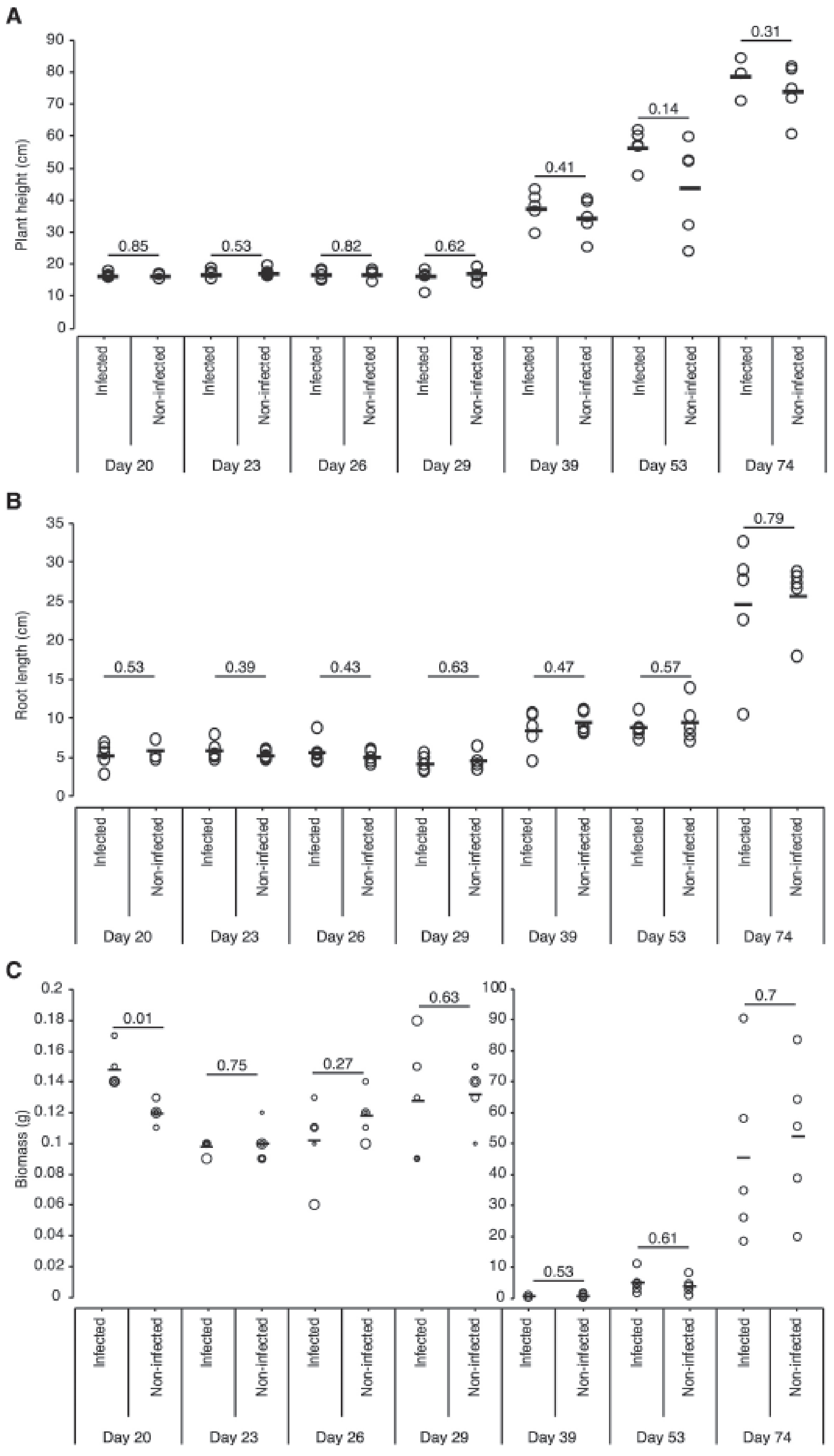

Fig. 1. Comparison of **(A)** plant height, **(B)** root length and **(C)** biomass between the infected and the non-infected paddy in each phases; seedlings, vegetative and reproductive. $P_{value} \geq 0.05$, two-tailed Student's t-test. The line indicates the average of the samples while the circle indicates replicates, n=5.

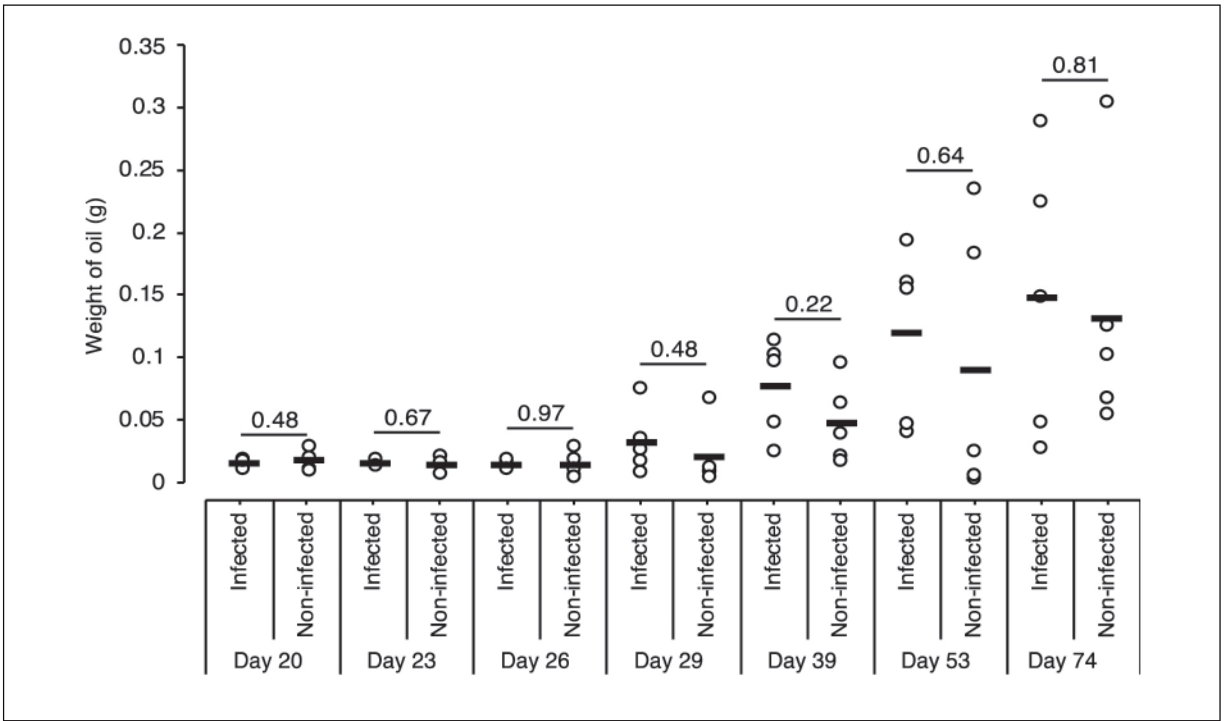

Fig. 2. Comparison the total oil in each phases; seedlings, vegetative and reproductive between the infected and the non-infected paddy. Data are presented as mean ± SE (n=5). Two-tailed Student T-test at P_{value} e" 0.05. The line indicates the average of the samples while the circle indicates replicate of each samples.

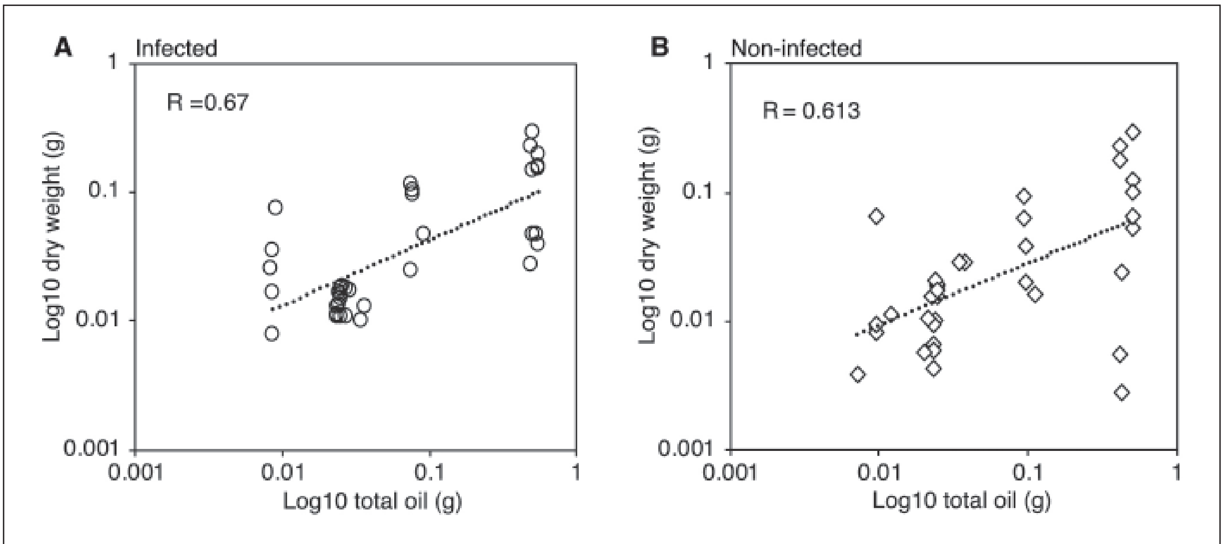

Fig. 3. The correlation between total oil and dry weight of the **(A)** infected and the **(B)** non-infected of MR219-4 paddy in each phases; seedlings, vegetative and reproductive. Clustering of samples is in log 10 transformation for both parameters and regression line is fitted in power model. R shown is Pearson correlation.

Xanthomonas oryzae **infection effecting the growth dynamic of the MR219-4**

Assessment from the dynamic perspective able to elucidate the defence dynamic of MR219-4 overcoming the *X. oryzae* infection. Upon infection the total oil/biomass variance of the infected seedling consistently to be smaller than the non-infected until day 26th entering vegetative phase (Figure 4). This clearly indicates that the infected paddies adopted deterministic respond during early infection. Until later on in the vegetative phase (day 29th and 39th), the infected plants dynamic became more stochastic indicating some of the plants may have overcome the infection and physiologically

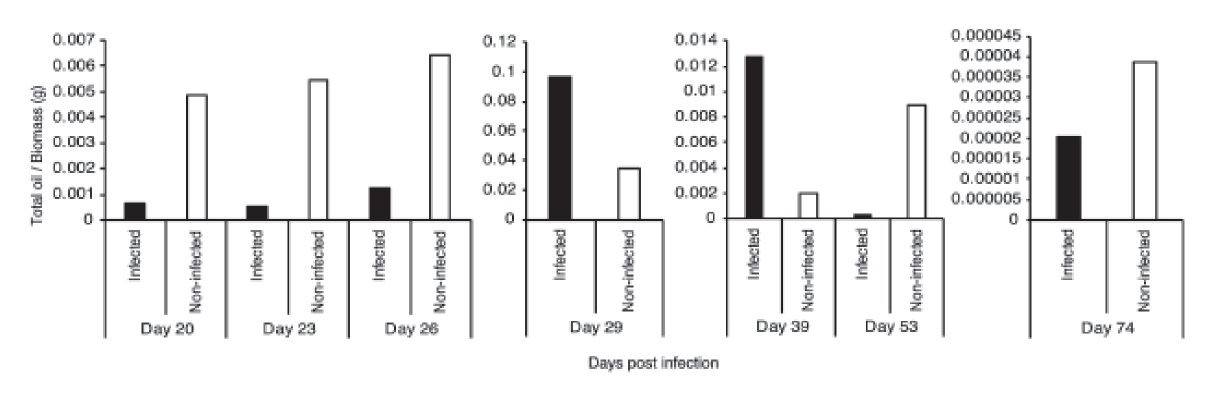

Fig. 4. The variance of total oil per biomass against days between the infected and the non-infected paddy, n=5.

(total oil/biomass) focused on the individual growth as the non-infected during the seedling stage (Figure 5). The variance of the infected clearly showed delayed in the physiological dynamic than the non-infected plants.

CONCLUSION

The *Xanthomonas oryzae* infection did not significantly affect the morphological and the physiological characteristics of MR219-4 as there are no significant differences in plant height, root length and also biomass (except day 20^{th}) as well as the total oil between the infected and the non-infected paddy in each phases. However, *X. oryzae* infection caused the changes in the physiological dynamics of the infected plants. Thus clearly indicating that the infection did affect the physiological dynamics of the plant. However, the locally developed drought tolerant paddy, MR219-4 is resistant to pathogenic bacterial infection under normal conditions.

ACKNOWLEDGEMENT

This project was funded by School of Fundamental Science Research Fund, Universiti Malaysia Terengganu.

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