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ORIGINAL ARTICLE

Potential of *Bacillus subtilis* inoculation in Biorichar^{TM} amended soil for suppression of Fusarium wilt of banana (*Musa acuminata* cv. Berangan) under water stress condition

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ABSTRACT

The present research was conducted to evaluate the ability of *Bacillus subtilis* to supress Fusarium wilt disease of banana in Biorichar^{TM} amended soil under different soil moisture regimes. Banana plants were inoculated with different volumes of *Bacillus subtilis* (0, 20, 40 and 60 mL) given at concentration 10^8 CFU mL^{-1} and subjected to three water stress levels based on field capacity (FC) *viz.* well watered (100% FC), mild stress (75% FC), and severe stress (50% FC). Banana plantlets were inoculated with *Fusarium oxysporum* one week after *Bacillus subtilis* were applied. The results showed that, minimum percentage of disease incidence in banana plants was recorded at high *Bacillus subtilis* rate (40 mL and 60 mL) at 50% FC. However, at 75% FC and 100% FC conditions, disease incidence increased from 35.28% to 45.09% following the time. Proline content showed 0.33% high under 75% FC compared to 50% FC at 45 DAT and similar trend was observed at 90 DAT. Malondialdehyde (MDA) content in banana plants was high in control treatment than those inoculated with *Bacillus subtilis*. 100% FC condition gave significantly higher net photosynthesis (14.95%), stomatal conductance (60.47%), transpirations rate (54.58%) and vapor pressure deficit (14.14%) compared to 50% FC at 45 DAT. However, values of net photosynthesis at 90 DAT were 30.07% and 20.79% lower at 50% FC and 100% FC, respectively in comparison to the values recorded at 45 DAT as pathological process progressed. Inoculation of *Bacillus subtilis* @ 60 mL increased photosynthesis rate by 9.07% as compared to non-inoculated plantlets at 100% FC at 45 DAT. However, no significant difference observed when the plants were inoculated by *Bacillus subtilis* @ 40 mL and @ 60 mL under 75% FC condition. Therefore, inoculation of *Bacillus subtilis* @ 60 mL could be a promising biological control agent that can trigger resistance against Fusarium wilt in susceptible Berangan banana under water stress condition.

Keywords: PGPR, photosynthesis, proline content, water stress, disease incidence

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1 Introduction

Fusarium wilt of banana caused by *Fusarium oxysporum* is one of the serious fungal diseases that affect banana production worldwide (Lin et al., 2008). A variety of control approaches have been conducted to fight against this disease such as the use of fungicide, biological control, and transgenic plantlets (Ghag et al., 2015). However, all have limitations. The use of chemical approaches strongly correlated with environmental contamination and has adverse effects on human health, which has also raised significant concerns (Khan et al., 2017). Development of resistance transgenic plant require time and not permanent because pathogen mutate or recombine to overcome host plant resistance (Khan et al., 2017). As an alternative sustainable agriculture approaches, the antagonistic rhizobacteria against Fusarium wilt were studied. Some important and most commonly used rhizobacteria include *Bacillus* spp. and *Pseudomonas* spp. (Haas and Défago, 2005). *Bacillus subtilis* which is also considered as plant growth promoting rhizobacteria was found able to reduce soil borne pathogen through multiple mechanisms (Ongena and Jacques, 2008).

However, it was found that direct inoculation of rhizobacteria into the soil may lead to poor activity of this microbe (Alabouvette et al., 2006). In order to overcome this problem, application of organic amendments were attempted to achieve better results (Saravanan et al., 2003). Supplementation of media with organic amendment can provide nutrients and increase survival of some microbes such as *Actinobacter* spp, *Rhizobium* spp, *Bradyrhizobium* and *Mesorhizobium* in the soil (Ahemad and Kibret, 2014). Noble and Coventry (2005) stated that biochar amendment can effectively suppress disease caused by soil-borne pathogen. Amending biochar based compost also can improve the quality of agricultural land in impoverished and low fertility regions (Manickam et al., 2015). Biorichar^{TM} is an organic biochar fertilizer developed through a composting process using a combination of rice husk and empty fruit bunch biochar with selected high nutrient substrates and enhanced with effective microbes, zeolite and plant enzymes. Biorichar^{TM} having similar characteristics as biochar and usually used as fertilizer based. Combination of various nutrients in Biorichar^{TM} intended to increase effectiveness of this product to shorter maturity period, increase yield production and maintain good quality of fruits (Manickam et al., 2016). In recent years, many studies had been conducted on biochar amendment as a tool for an effective control of plant diseases (Graber et al., 2014). However, there is little information about the interactive effect of rhizobacteria with biochar and/or compost, particularly under water stress conditions.

Besides diseases, water stress in banana is one of the main problems which require serious attention. In Malaysia, limited water source and growing competition for unpolluted water reduced growth performance of banana. Many crop experienced deficit water stress which negatively affect the plant's production. Water stress results in root injury, blockage to the xylem, ameliorate normal stomata function, and damage to the cuticle (Ayres, 1978). It is difficult to separate the direct effects of an excess or water deficit in the field on susceptibility to disease from the indirect effects caused by change in the soil water status (Kramer, 1983). However, It is generally believed that water stressed plants are more susceptible than unstressed plants to attacks by pathogens since the nature defense mechanism in stressed plant reduced when major plant's mechanism become restricted (Ghaemi et al., 2011). Several studies in *Arabidopsis*, bean, grapevine have shown that water stress increase the vulnerability of plant to fungal infection (McElrone et al., 2001; Mayek-PÉrez et al., 2002; Mohr and Cahill, 2003; Prasch and Sonnewald, 2013). According to Ghaemi et al. (2011) stated that disease incidence of tomato caused by *F. oxysporum* increased as water stress level increased. Yadeta and Thomma (2013) discussed that *F. oxysporum* infect the plants through roots. First infection occur through xylem vessel, caused necrosis on the corm, restricted the water movement in the vascular bundles and eventually resulted in lethal wilting of the infected plant. Conversely, Ramegowda et al. (2013) and Hatmi et al. (2014) reported that water stress enhances the defense response of plants against pathogen. In view of the current problem therefore, it is important to understand the impact of combined water stress and the cognate defense strategies by using rhizobacteria inoculated in Biorichar^{TM} amended soil by banana plants to circumvent the concurrent onslaught of water stress and FOC infection.

2 Materials and Methods

2.1 Experimental treatment and design

This study was conducted under rain shelter at Field 2, Faculty of Agriculture, Universiti Putra Malaysia (UPM), Serdang, Selangor. One month old banana *cv.* Berangan plantlets were transplanted in the polybag of 40 cm $\times$ 40 cm size contained Bungor series soil which had been mixed thoroughly with Biorichar^{TM} at the rate of 4.5 t ha^{-1}. The recommended amount of Biorichar^{TM} was applied based on plant density in the field of 2500 plants per ha with distance 2 $\times$ 2 m^2. The treatments were two factorial (water stress and *Bacillus subtilis*) arranged by split plot design with three replications. The plants were subjected to

different level of water stress given based on field capacity *viz.* well watered (100% FC), mild stress (75% FC), and severe stress (50% FC), and inoculated with different volume of *Bacillus subtilis* (0, 20, 40, and 60 mL) at concentrations of 10^8 CFU mL^{-1}. The physicochemical properties of studied soil were presented in Table 1.

2.2 Inoculum preparation

Pure isolates of *Bacillus subtilis* and *F. oxysporum* were obtained from the culture collection of Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia. The preparation of inoculum was made using modified method Fishal et al. (2010). In preparing aqueous antagonist suspensions, isolates were grown on nutrient agar (NA) (Biolab) at 27 °C for 24 h. A loop of each culture was then transferred to a 250 mL conical flask containing 50 mL of Tryptic soy (TSA, Difco Laboratories, Detroit, USA), and incubated on an orbital shaker (125 rpm) for 48 h at 25 °C. Cultures were centrifuged for 15 min at 750 g using a Labofuge GL. The pellets finally adjusted to 10^8 CFU mL^{-1} using hemocytometer. The bacteria suspension was diluted with 1:10 of distilled water and applied about 100 mL per plant as soil drenching. Meanwhile, *F. oxysporum* was cultured on potato dextrose agar medium at 25 °C in incubator for 7 d. Then, 8 mm disc was excised from the cultured, suspended in distilled water and quantified using a hemocytometer. The inoculation of plantlets with *F. oxysporum* was made by application of 40 mL inoculum at concentration 10^8 CFU mL^{-1} as soil drenching.

2.3 Disease incidence assessment

Disease incidence (DI) was made followed Cachinero et al. (2002) and observed at weekly basis. Severity symptoms on individual plants were rated on a scale from 0 to 4, according to the percentage of foliage with chlorosis or necrosis in acropetal progression: 0 = no browning (necrotic) symptom on leaf pieces, score 1 = necrotic symptom less than 25%, score 2 = necrotic symptom between 25–50%, score 3 = necrotic symptom 50–75%, and score 4 = necrotic symptom >75%, leaf pieces have completely rotten. Observation for internal symptom was made using scale 0–4: 0 = healthy; 1 = 5–10% discoloration of vascular area; 2 = 10–20% discoloration of vascular area; 3 = 30–50% discoloration of vascular area, and 4 $\geq$ 50% discoloration of vascular area. DI for both external and internal symptom was calculated using the equation by Cachinero et al. (2002) as follow:

$$\mathrm{DI} = \frac{n_i \times s_i}{N \times S} \times 100 \quad (1)$$

where, DI = disesede incidence (%), n_i = number of banana plants with ith score of symptoms, s_i = the value of the ith score of symptoms, N = total number of tested banana plants, and S = the highest value of score of symptoms (Cachinero et al., 2002).

The infected banana plantlets were identified followed Yusnita (2004) as immune (I_m) if DI = 0%; resistance (R_s) = if 0%<DI<5%; moderately resistance (M_r) = if 5%<DI<10%; moderately susceptible (M_s) = if 10%<DI<25%; susceptible (S_c) = if 25%<DI<50%; very susceptible (V_s) = if DI>50%.

2.4 Biochemical assay

Proline content in the leaves was determined following method of Bates et al. (1973) by using fresh leaf sample and expressed as proline per gram fresh weight. On the other hand, the level of lipid peroxidation was measured in terms of malondialdehyde (MDA) content, a product of lipid peroxidation following method of Heath and Packer (1968) and the results were expressed as μmol MDA g^{-1}FW.

2.5 Determination of leaf gas exchange

Leaf gas exchange measurements which includes photosynthetic rate (μmol CO_2 $m^{-2}s^{-1}$), stomata conductance (mol $m^{-2}s^{-1}$), transpiration rate (mmol $m^{-2}s^{-1}$) and vapour pressure deficit (VPD) (mol H_2O $m^{-2}s^{-1}$) were performed in fully expanded leaves using an infrared gas analyzer (IRGA) (LI-6400XT Portable Photosynthesis System, LI-COR, Lincoln, NE, USA) according to Dias et al. (2017).

2.6 Statistical analysis

The collected data were subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS 9.4) and the entire means were evaluated by using least significant difference (LSD) test at significant difference, P<0.05. Correlation analysis was performed to establish relationship between vapour pressure deficit versus stomatal conductance and stomatal conductance versus transpiration.

3 Results and Discussion

3.1 Effect of treatments on disease incidence

Table 2 indicates that disease incidence of *Fusarium oxysporum* as influenced by water stress and *Bacillus subtilis* treatment. There were no significance interaction between water stress and *B. subtilis* application at 45 DAT on percentage of leaf damage but significant at 90 DAT. Analysis of variance at main plot revealed, significance difference for disease incidence at 90 DAT. Disease incidence were found increased from 6.85% at 45 DAT to 42.79% at 90 DAT under well watered (100% FC) conditions. An increased up to 57.40% was

Table 1. Selected physicochemical properties of studied soil (Bungor series) and Biorichar^{TM}

Properties	Soil	Biorichar^{TM}
Sand (%)	44	–
Silt (%)	9.91	–
Clay (%)	46.08	–
pH (1:2.5 water)	5.2	7.23
Cation exchange capacity (1 M NH_4OAc, pH 7) cmol(+) kg^{-1}	4.12	12.92
Organic C (Dry Combustion) (%)	1.85	12.39
N content (Dry Combustion) (%)	0.18	1.32
Exchangeable K (1 M NH_4OAc, pH 7) (mg kg^{-1})	22.2	151.6
Exchangeable Ca (1 M NH_4OAc, pH 7) (mg kg^{-1})	8.14	10.04
Exchangeable Mg (1 M NH_4OAc, pH 7) (mg kg^{-1})	1.51	12.21

Table 2. Percentage of leaf damage, disease incidence and resistance of banana cv. Berangan at 45 and 90 DAT

	Leaf Damage (%)		Disease Incidence (%)		Resistance†	
	45 DAT	90 DAT	45 DAT	90 DAT	45 DAT	90 DAT
Main plot (water stress)						
Severe stress: 50% FC	10.73	15.2	10.73	18.23b	M_s	M_s
Mild stress: 75% FC	4.89	27.89	4.89	46.46a	R_s	S_c
Well watered: 100% FC	6.85	25.26	6.85	42.79a	M_r	S_c
$LSD_{0.05}$	NS	NS	NS	21.37*		
Sub plot (*B. subtillis*)						
B_0: 0 mL	9.58	30.16a	9.58	48.73	M_r	S_c
B_{20}: 20 mL	6.68	23.78b	6.68	37.25	M_r	S_c
B_{40}: 40 mL	7.51	20.32bc	7.51	30.58	M_r	S_c
B_{60}: 60 mL	6.2	16.87c	6.2	26.76	M_r	S_c
$LSD_{0.05}$	NS	5.88**	NS	NS		
Significance interaction	NS	*	NS	NS		

Means followed by the same letters within a column is not significantly difference at P = 0.05 by least significant difference (LSD) with n=36. * and ** significantly difference at P = 0.05 and P = 0.01, respectively, and NS= not significant. † R_s = Resistance, M_r = Moderately resistance, M_s = Moderately susceptible, S_c = Susceptible.

observed under well watered condition compared to severe stress condition at 90 DAT. These results suggested that the severity of disease increased when the soil is moist. The plants were observed to have uneven growth with associated leaf yellowing that caused stunted of plant growth. According to Ghaemi et al. (2011) *F. oxysporum* and possibly Verticillium wilts are apparently favored by wet soils but illustrate some special problems in interpretation. However, Linford (1928) and Ryker (1935) reported that Fusarium wilt of peas and celery were shown to be more severe in wet than in dry soil. These reports apparently contradict earlier ones by Gilman (1916) and Humbert (1918) for Fusarium wilt of tomato, who observed severe disease following hot dry weather and little or non during cool moist weather. However, among the sub plot means significance difference was observed for percentage of leaf damage at 90 DAT. Non-inoculated *B. subtilis* (control) was 44.06% increase in leaf damage as compared to inoculation at 60 mL. Inoculation of *B. subtilis* at high rate apparently decreased leaf damage and disease incidence. Based on the results, it was found that the resistance of disease is high at 45 DAT as there was no obvious infection symptoms observed but as time progress and at 90 DAT the resistance reduced significantly. The banana plantlets at 90 DAT were marked susceptible to the disease as the disease intensity were 25%<DI<50% followed Yusnita (2004). Meanwhile, the plants status at 45 DAT marked moderately resistance at different *B. subtilis* application. The increase resistance against *F. oxysporum* at higher *B. subtilis* possibly because the higher number of spore produced. The spore may increase root exudate and volatile compound against *F. oxysporum*.

The disease incidence and development for every week interval was presented in Fig. 1. There was a gradual increased in disease incidence with time.

Table 3. Proline and malondialdehyde (MDA) content in foliar tissue at 45 and 90 DAT as affected by water stress and *Bacillus subtilis* application

	Proline content (μmol g-1g^{-1})		MDA content (μmol g^{-1})	
	45 DAT	90 DAT	45 DAT	90 DAT
Main plot (water stress)				
Severe stress: 50% FC	21.04c	21.12	1.41a	1.11a
Mild stress: 75% FC	21.12a	21.16	1.25b	0.88c
Well watered: 100% FC	21.05b	21.13	0.91c	1.02b
$LSD_{0.05}$	0.0084***	NS	0.133**	0.0463***
Sub plot (*B. subtillis*)				
B_0: 0 mL	21.08b	21.34	1.50a	1.27a
B_{20}: 20 mL	21.09a	21.08	1.06b	0.98b
B_{40}: 40 mL	21.07c	21.06	1.09b	0.97b
B_{60}: 60 mL	21.05d	21.07	1.11b	0.82c
$LSD_{0.05}$	0.0007***	0.137**	0.095***	0.040***
Significance interaction	***	NS	***	***

Means followed by the same letters within a column is not significantly difference at P = 0.05 by least significant difference (LSD) with n=36. ** and *** are significantly difference at P = 0.01 and P = 0.001, respectively, and NS= not significant.

Table 4. Photosynthesis, stomatal conductance, transpiration rate and vapour pressure deficit (VPD) as affected by water stress and *Bacillus subtilis* application at 45 and 90 DAT

	Photosynthesis		Stomata conduc.		Transpiration rate		VPD	
	(μmol CO_2 m^{-2} s^{-1})		(mmol m^{-2} s^{-1})		(mmol H_2O m^{-2} s^{-1})		(mol H_2O m^{-2} s^{-1})	
	45 DAT	90 DAT	45 DAT	90 DAT	45 DAT	90 DAT	45 DAT	90 DAT
Main plot (water stress)								
Severe stress: 50% FC	19.29b	13.49b	0.1	0.21b	1.01b	3.84b	1.01a	1.90a
Mild stress: 75% FC	22.14a	16.16ab	0.25	0.44a	2.19a	5.55a	1.04a	1.48b
Well watered: 100% FC	22.68a	17.90a	0.25	0.43a	2.23a	6.19a	1.17a	1.62ab
$LSD_{0.05}$	2.85***	2.69***	0.35***	0.18***	0.68***	1.13***	0.96***	0.28***
Sub plot (*B. subtillis*)								
B_0: 0 mL	20.50b	14.75b	0.18	0.30b	1.52b	4.61b	1.01	1.73
B_{20}: 20 mL	21.26a	15.78a	0.20	0.36a	1.81a	5.16ab	1.08	1.67
B_{40}: 40 mL	21.90a	16.48a	0.21	0.39a	1.96a	5.48a	1.14	1.64
B_{60}: 60 mL	21.82a	16.47a	0.21	0.39a	1.94a	5.53a	1.07	1.63
$LSD_{0.05}$	0.74**	0.88***	NS	0.04***	0.26**	0.64*	NS	NS
Significance interaction	NS	*	NS	NS	NS	NS	NS	NS

Means followed by the same letters within a column is not significantly difference at P = 0.05 by Least Significant Difference (LSD) with n=36. *, ** and *** significantly difference at P = 0.05, P = 0.01 and P = 0.001, respectively, and NS= not significant.

Banana plantlets showed first symptom of disease in week 3 after challenged inoculated with *F. oxysporum*. The highest percentage of first disease incidence was recorded for treatment 75% FC + 0 mL *B. subtilis* (36%) followed by treatment 75% FC + 20 mL *B. subtilis* (27%). Banana plantlets started showing foliar symptom of Fusarium wilt with occurance of yellowish streak and necrosis started from the edge of the leaves and progress inwards then spreading inwards gradually. The banana plantlets without *B. subtilis* inoculation under mild stress (75% FC) condition showed fastest and highest disease incidence as compared to other treatment. In contrast, banana plantlets with highest *B. subtilis* inoculation of 40 mL and 60 mL under severe stress condition (50% FC) showed lowest disease incidence. Cao et al. (2011) observed a reduction in disease incidence by 49–61% from *F. oxysporum* infection in cucumber plant after inoculated with *B. subtilis* SQR 9. They also observed increased in shoot height from 67.4 to 99.3 cm and two fold increased in plant biomass by inoculation of *B. subtilis* SQR 9.

3.2 Effect of treatments on biochemical changes

Accumulation of proline and MDA in plants is a main strategy against oxidative stress and disease infection. Table 3 shows proline content and MDA content in foliar tissue at 45 and 90 DAT as affected by water stress and *B. subtilis* application. Analysis of variance at main plot showed significance difference between water stress level on proline content and MDA content at 45 DAT and 90 DAT. Severe stress (50% FC) showed 12.69% and 7.58% high in MDA content at 45 and 90 DAT respectively as compared to well watered condition. However, proline content showed 0.33% high under mild stress (75% FC) compared to severe stress (50% FC) at 45 DAT. Proline content also was 0.57% high under well watered (100% FC) at 90 DAT compared to severe stress (50% FC).

Proline content did not show apparent significant variation at both water stress and *B. subtilis* inoculation although statistical analysis showed significant differences between the treatments. However, MDA content in non-inoculated *B. subtilis* was found higher at both 45 and 90 DAT as compared to plant inoculated with *B. subtilis*. The increased result could be because of plant's reaction to reduce deleterious effect of water stress and protect membrane injury from pathogen effect. When the plant exposed to pathogen infection, the plant concomitantly induce programmed cell death and produced reactive oxygen species to counteract the infection (Apel and Hirt, 2004).

Recently, it was reported that *F. oxysporum* infection resulted in increased activities of antioxidant enzymes together with increased levels of reactive oxygen species and lipid peroxidation in cucumber roots (Ye et al., 2006). The incorporation of water stress and *F. oxysporum* infection was believed to increase accumulation of MDA content as a defense mechanism process. Accumulation of MDA content in stress plant and plant experienced pathogen infection is regarded as an indicator of cellular damage (Dallagnol et al., 2011). Many reports showed the deleterious effects of reactive oxygen species, whose production is stimulated under water stress (Blokhina, 2003). Reactive oxygen species cause lipid peroxidation, and consequently membrane injuries, protein degradation and enzyme inactivation (Sairam et al., 2005).

3.3 Effect of treatments on leaf gas exchange parameter

According to Table 4, results shows that there were no significance interaction between difference levels of water stress and *B. subtilis* on net photosynthesis, stomatal conductance, transpiration rate and VPD at 45 DAT. Comparison of water stress at main plot means revealed well watered (100% FC) significantly higher in net photosynthesis by 14.95%, stomatal conductance by 60.47%, transpiration rate by 54.58% and VPD by 14.14% compared to severe stress (50% FC). Among the sub-plot treatments, plants inoculated with *B. subtilis* have higher net photosynthesis and transpiration rate compared to non-inoculated. Whereas no significance different recorded for stomatal conductance and VPD. These results were in accordance with Zhang et al. (2008) which found an increased in photosynthesis of *Arabidopsis* plant as a result of *B. subtilis* inoculation. The modulation of endogenous abscisic acid signaling and the production of other phytohormone were believed to affect physiological changes in the plant. The decreased in photosynthesis and transpiration rate in non-inoculated *B. subtilis*, could be triggered by *F. oxysporum* infection and water stress effect. Dong et al. (2002) found a reduction of photosynthesis accompanied with increased in stomata closure which restricted water loss through transpiration in infected banana by *F. oxysporum*.

The infected plant usually developed secondary effect through disturbance function of chloroplast. At 90 DAT there were significance interactions between different levels of water stress and *B. subtilis* on net photosynthesis but no significance interaction for stomatal conductance, transpiration rate and VPD. However, comparison at different levels of water stress showed that well watered (100% FC) were 24.91% and 37.95% high in value for net photosynthesis and transpiration rate compared to severe stress (50% FC). The data of net photosynthesis at 90 DAT also reduced progressively compared to 45 DAT. The decreased trend in net photosynthesis continued as the *F. oxysporum* infection process progressed. The

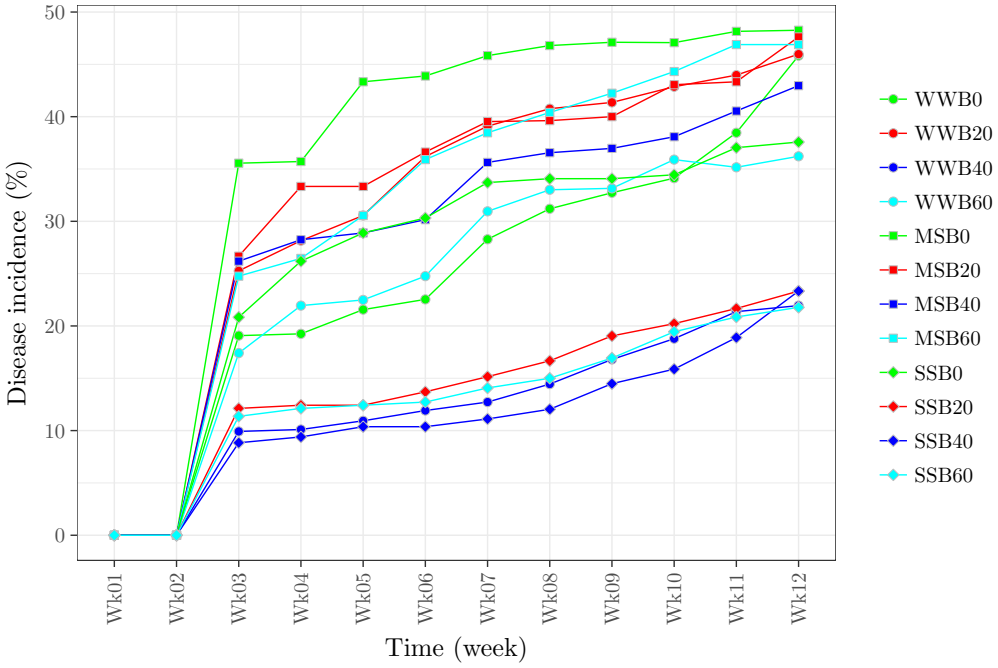

Figure 1. Disease incidence of Berangan plantlets pre-inoculated with different rates of *Bacillus subtilis* at different water stress level on weekly basis observation after challenged inoculated with *Fusarium oxysporum*. WWB0= well watered + 0 mL; WWB20= well watered + 20 mL; WWB40= well watered + 40 mL; WWB60= well-watered + 60 mL; MSB0= mild stress + 0 mL; MSB20= mild stress +20 mL; MSB40= mild stress + 40 mL; MSB60= mild stress + 60 mL; SSB0= severe stress + 0 mL; SSB20= severe stress + 20 mL; SSB40= severe stress + 40 mL; SSB60= severe stress + 60 mL.

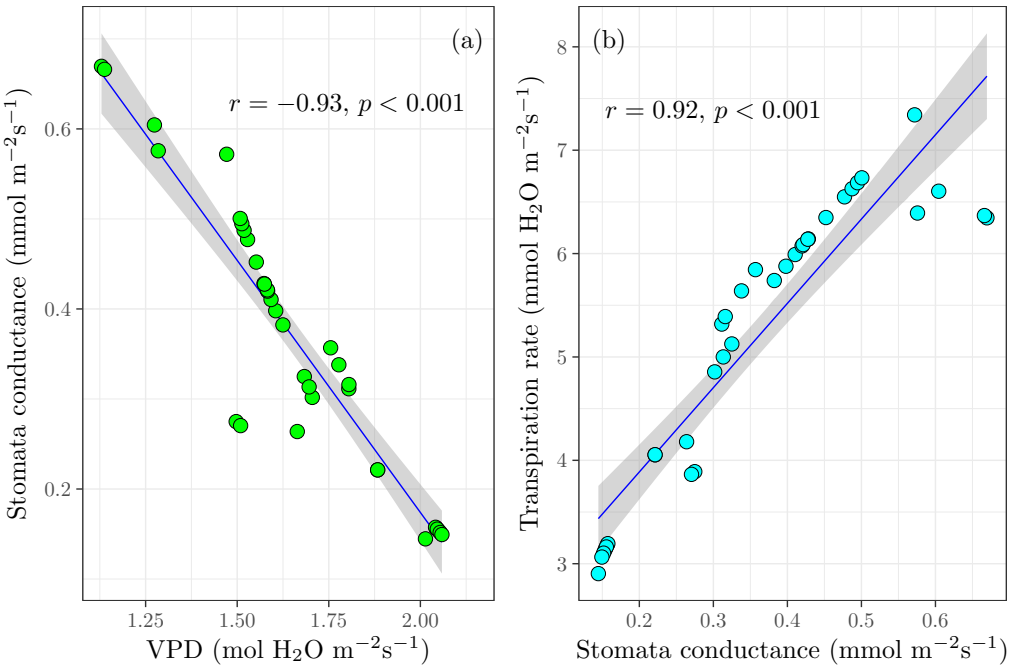

Figure 2. The correlation analysis between (a) vapour pressure deficit (VPD) and stomata conductance, and (b) stomata conductance and transpiration rate.

value of photosynthesis at 90 DAT was 30.07% and 20.79% lower at severe stress (50% FC) and well watered (100% FC) in comparison to 45 DAT. Decreased in photosynthesis simultaneously decreased transpiration rate. The effect of *F. oxysporum* in suppresses photosynthesis had been studied in tomato and water melon plant but focused on external hypha penetration (Lepoivre, 2003). However, the immediate causal in the changes of pigment-protein complexes and structural damage to photosynthetic membrane remain unclear. It is observed that vapor pressure deficit (VPD) was high in severe stress (50% FC) compared to well watered and mild stress at 90 DAT. Increased in VPD simultaneously decreased stomatal conductance and transpiration rate. Bunce (1997) also reported an increase VPD concomitantly decreased stomata conductance.

3.4 Correlation between vapor pressure deficit and stomata conductance

Fig. 2(a) shows a significant negative correlation between VPD and stomatal conductance. Stomatal conductance reduced consistently as VPD increased. Under severe stress (50% FC), non-inoculated *B. subtilis* treatment showed lowest stomata conductance as in comparison with well watered (100% FC). Farquhar (1978) earlier stated that stomatal conductance decreased directly when vapor pressure deficit increased as a result of stomata closure in limited water condition.

However, Grassi and Magnani (2005) reported the same results of reduced in stomata conductance with respect to increase in vapor pressure deficit in the leaf of oak tree under water stress. The possible causal of this trend could be explained by alteration in the rate of abscisic acid (ABA) delivery to the guard cell which subsequently caused changes in the transpiration surrounding area of stomata (Bunce, 1997). Previously, Timmusk et al. (2014) stated that rhizobacteria like *B. subtilis* potentially stimulate synthesizing of phytohormone such as abscisic acid (ABA), gibberellic acid, cytokinins, and indole-3-acetic acid (IAA) which increased drought tolerance in the plant.

The result for correlation analysis between transpiration rate and stomatal conductance was shown in Fig. 2(b). Transpiration becomes increased as the stomatal conductance increased but decreased when stomatal conductance reached threshold value. Transpiration rate started to increase from 2.90 to 7.34 mmol H_2O $m^{-2}s^{-1}$ as the stomatal conductance increased from 0.14 to 0.67 mmol $m^{-2}s^{-1}$. However, it is observed that, transpiration rate decreased from 7.0 to 6.0 mmol H_2O $m^{-2}s^{-1}$ when the stomatal conductance value reached 0.60 mmol $m^{-2}s^{-1}$. Inoculation with *B. subtilis* could be the reason for this relationship since *B. subtilis* induce production of phytohormone such as cytokinin. A study by Farber et al. (2016) found the relationship between cytokinin application on transpiration and stomata on tomato plant. Application of cytokinin promotes transpiration indirectly and increase stomata density of that plant. Increase cytokinin applications directly enhance transpiration by increase in stomata density, thus slow down leaf senescence. Such response by the plant was mainly to minimize water loss. Ilgin and Caglar (2009) also suggested that disturbances on the stomata were directly followed by a decreased in transpiration rate.

4 Conclusions

Based on this study, we found that Biorichar^{TM} amended soil enhanced with *Bacillus subtilis* incorporation of *F. oxysporum* on banana cv. Berangan under water stress condition significantly reduce disease incidence, alters biochemical changes and leaf gas exchange. Inoculation of *B. subtilis* at a high rate (60 mL) potentially reduced disease incidence up to 35.28% and 35.16% at both 45 and 90 DAT. However, it was found that disease susceptibility increased under well watered condition as compared to severe stress condition potentially because of *F. oxysporum* more favored under wet soils. Increased in MDA content in non-inoculated *B. subtilis* was found as a respond to reduce deleterious effect of water stress. Decreased in net photosynthesis and stomata conductance was observed as pathological process progressed. Despite of that, the integration of compost (Biorichar^{TM}) and application of *B. subtilis* could be a promising biological control agent that can trigger resistance against Fusarium wilt in susceptible Berangan banana under water stress condition.

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