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Effects of Silicon, Copper and Zinc Applications on Sheath Blight Disease Severity on Rice

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Abstract Rice (Oryza sativa L.) sheath blight disease is one of the most important diseases globally which makes its management very difficult. Severe soil micronutrients deficiency and high dosage of nitrogen fertilizer leading to the development of sheath blight disease has become a constraint in rice production in Malaysia. The study was conducted to compare between MR219 and MR253 rice varieties to sheath blight inoculations and micronutrient applications and to determine the impact of disease on rice yield. Sheath blight epidemics in pots were initiated by inoculation at maximum tillering growth stage under glasshouse conditions in April 2013. Silica gel, copper sulphate and zinc sulphate were applied to the soil prior to planting at the rates of 360g, 0.30 g, 0.45g per 15 kg soil respectively. Inoculation significantly (P=0.05) increased sheath blight severity and incidence which caused yield losses of 11% in moderately resistant variety MR219 and 50% in moderately susceptible variety MR253. Micronutrients application reduced sheath blight incidence and severity regardless of varieties. Fertilization with Si was significantly more effective than Cu and Zn treatments in minimizing yield loss due to sheath blight in both varieties.

Keywords: *silicon, copper, zinc, sheath blight disease, grain yield*

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1. Introduction

Sheath blight disease caused by *Rhizoctonia solani* Kuhn (Teleomorph: *Thanatephorus cucumeris*), anastomosis group 1 IA (AG1-IA) is an economically important rice disease in all rice growing areas of the world, especially under intensive production [1]. The disease is soil-borne causing up to 25% of yield losses and is a major disease of rice affecting 50% of all global rice production countries [2,3,4]. Symptoms of sheath blight include lesions on the base of the leaf sheath near the water line which are elliptical or oval-shaped and greenish-gray with yellow margins [5,6]. Under favorable conditions, sheath blight caused up to a 50 % decrease in yield losses each year worldwide [7]. Unfortunately, at present, there is no known rice varieties with complete resistance to sheath blight [8] and other suitable economic disease management measures are not available [9].So far control of the disease has relied mainly on the use of fungicides when affordable by farmers [10]. Applying fungicide to control the disease is neither economical nor environmentally friendly and there is a potential risk for emergence of pathogen populations that are resistant to fungicides.

In Malaysia, rice is planted as upland rice or wet paddy conditions with farmers practicing double cropping of high-yielding varieties such as MR219 or MR220 [11]. Various nutritional disorders may be encountered in irrigated rice ecosystem. Rice production in Malaysia is focused in these irrigated areas to achieve the target self-sufficiency level of100% by 2015 [12]. Long-term intensive cropping with high-yielding varieties on the same land has altered soil micronutrients availability [13]. Severe soil micronutrients deficiency was reported to be associated with rice production in the main granary areas in Malaysia [14]. Balanced nutrition does not only help to achieve better yield in crop production but also allows plants to protect themselves from disease infections [15,16]. While Malaysian farmers apply nitrogen (N), phosphorus(P) and potassium(K) fertilizers widely, it is found that the applications of micronutrients such as Zn, Cu, Mn and B are not the usual practice [17]. Micronutrient deficiencies are markedly increased due to intensive cropping, loss of fertile top soils and nutrients through leaching and surface runoff [18]. Therefore, the disease management approach emphasized here is to manipulate host plant resistance cultural practices, and application of micronutrients for disease management [19]. Efficacy of micronutrients against sheath blight has been reported [20,21,22].

Silicon (Si) is the second most abundant mineral element in soil and has important role in alleviating various environmental stresses and enhancing plant resistance against pathogen [23].Application of complete Si fertilizer not only increases rice yield but also decreases the incidence of rice fungal diseases [24,25]. Effects of silicon on yield are related to the deposition of the element under the leaf epidermis contributing to a physical mechanism of defense; reduce lodging, increases photosynthesis capacity and decreases transpiration losses [26]. Zinc (Zn) is essential for several biochemical processes in the rice plant, such as cytochrome and nucleotide synthesis, auxin metabolism, chlorophyll production, enzyme activation, and membrane integrity [27]. Numerous researches [28,29,30,31] were conducted on Zn applications in rice crop contributing to increase in grain yield. A balanced Zn application was found to increase the phenol contents of plant and reducing the severity of rice sheath blight [32]. Copper (Cu) fertilization had decreased the severity of a wide range of fungal and bacterial diseases associated by cell wall stability and lignification in plants [33]. Copper plays vital role in the formation of chlorophyll and the increment is positively related with the increase in yield and fruits produced [34]. Studies on improving yield of rice affected by sheath blight disease in Malaysia have not been done. Therefore, the objective of this study was to determine the effects of silicon, copper and zinc applications on sheath blight severity to increase yield and the effects between MR219 and MR253 rice varieties were compared.

2. Materials and Methods

2.1. Plant Materials and Cultivation

Varieties of rice (*Oryza sativa* L.) viz MR 219 and MR253 released by Malaysian Agriculture Research and Development Institute MARDI were chosen in this experiment because they are the most widely cultivated and high yielding rice varieties in Malaysia. Experiments were conducted under glasshouse conditions from February to June of 2013 at the research complex, University Putra Malaysia. The soil used was collected from a rice field located in Tanjong Karang, Selangor with the following characteristics: Al 0.17 cmolckg^{-1} ; Cu 0.15 mg kg^{-1}, Zn 1.06 mg kg^{-1}, Fe 43.18 mg kg^{-1} and Mn 3.94 mg kg^{-1} ; Si 4.34 mg kg^{-1}. The soil type was silty clay soil classified as fine textured by USDA Soil Taxonomy System (pH 4.87, clay 49.19%, silt 43.44%, sand 7.36%, cation exchanged capacity [CEC] 1.54 mol lit^{-1}). Rice seeds were soaked in water for 24 h and dried for another 24 h at room temperature to hasten germination. Germinated seeds were planted into plastic seedling boxes (22.7cm x 18.6cm x 6.9 cm). Seedlings of 12 days old were uprooted carefully from the nursery tray and transplanted to the well prepared experimental pots (35 cm diameter, 38 cm depth) containing 15 kg of the paddy soil and placed in the glasshouse. No modifications were made to the pots to allow for drainage, and plants were kept under flooded conditions until the end of the experiment. All the pots were applied nitrogen (N), phosphorus (P) and potassium (K) fertilizers according to the recommended rate. Applications of nitrogen fertilizers (150 kg N ha^{-1}) as urea split into 3 times at 15 days after planting (DAP) (25%), 35 DAP (30%) and 55 DAP (45%). Phosphorus (90 kg P_2O_5 ha^{-1}) as rock phosphate and potassium (150 kg K_2O ha^{-1}) as muriate of potash applied at basal and at panicle initiation stage at 55DAP.

2.2. Silicon, Copper and Zinc Application

Granular silica gel (Classic Chemicals Sdn Bhs, Malaysia) with a minimum SiO_2 content of 95% and particle size ranging from (0.6-2 mm), Cu source as copper sulphate ($CuSO_4.5H_2O$) with 99% purity and Zn source as zinc sulphate ($ZnSO_4.7\ H_2O$) were used in this study. Silica gel, copper sulphate and zinc sulphate were applied to the soil prior to planting at the rates of 360g, 0.30 g, 0.45g per 15 kg soil respectively, as compared to the inoculated and un-inoculated (control) treatment without incorporation of these supplements.

2.3. Experimental Design

Treatments were replicated four times in a randomized complete block design with a factorial arrangement of two rice cultivars and five sheath blight pathogen inoculations and fertilizer combinations. Each replication corresponds to three rice plants per experimental unit. Sheath blight inoculation and fertilizer treatment combinations included the following: non-inoculated and untreated (control), inoculated and untreated (control), inoculated and silicon application, inoculated and copper application and inoculated and zinc application.

2.4. Plant Inoculation

A virulent isolate of *R. solani* (AG-1 IA), collected from symptomatic rice plants was used for inoculation. Inoculum was obtained as follows: the isolate of *R. solani* was grown on potato dextrose agar (PDA) and incubated for five days at room temperature to produce mycelia. At maximum tillering stage (60DAP), the plants were inoculated with five- day- old mycelia plug of *R. solani* placed on the stem on one cm below the axil of the fully mature leaf and wrapped with paraffin. Immediately after inoculation, all plants were transferred to a moist chamber with 90-96% relative humidity for one week. The temperature and relative humidity were measured using a traceable relative humidity/temperature meter (Fisher Scientific, Atlanta, GA) and recorded three times per day.

2.5. Disease Assessment and Yield Determination

Pots were evaluated for sheath blight development approximately 1 week before maturity and the disease intensity was determined by Highest Relative Lesion Length (HRLH) using the scale of Standard Evaluation System for rice by International Rice Research Institute [35]. At the same time, disease incidence was determined by examining all stems per pot and calculating percentage of infected stems. At maturity stage, grain yield was determined at 14% moisture content.

$$\text{Highest Relative lesion height }(\%) = \frac{\textit{Vertical height of uppermost lesion on stem or leaf or sheath}}{\textit{Plant height}} \times 100$$

2.6. Lignin Content

Leaf samples were randomly collected from each pot at 75 DAP and dried in the oven at 60˚C for 72 hrs. Samples were grinded and subjected for 2 steps extraction process for the determination of acid detergent fiber (ADF) and acid detergent lignin (ADL) as reported by [36].

2.7. Data Analysis

All data were analyzed using the ANOVA procedure by SAS Statistical software package (version 9.2 for windows). Mean comparisons were conducted using the least significant difference (LSD) test at 0.05 probability level and standard errors of the difference were computed. Arcsine transformation was used in the analysis of the percentage of infected tillers.

3. Results

In experimental pots inoculated with *R. solani*, initial symptoms was developed on 10 to 15% of the stems within 7 to 10 days of inoculation, and these infection levels were within sheath blight treatment thresholds for the varieties in the study. Severe sheath blight developed occurred in the inoculated/untreated (control), averaging 40 to 46% infected stems with 30 to 44% of the lesion length to the height of the rice plant. During the growing season, light infestations of sheath blight observed in the non- inoculated experimental pots but did not reached treatment threshold. The main effect of variety and sheath blight inoculation-fertilizer treatment combination had highly significant effects ($P<0.01$) on sheath blight incidence; sheath blight severity and rice grain yield (Table 1). The interaction effect between varieties and sheath blight inoculation-fertilizer treatment combination was also significant ($P <0.05$) on severity of sheath blight and rice grain yield.

The susceptible variety MR253 showed greater disease incidence and sheath blight severity than the moderately resistant variety MR219 (Table 2). The grain yield of MR219 was significantly ($P<0.001$) higher than MR253 as shown in Table 2. Disease incidence and severity rating were greater for inoculated/untreated (control) compared with non-inoculated and untreated (control) across the varieties (Table 3). No significant reduction was recorded among Si, Cu and Zn applications but the least sheath blight incidence and sheath blight severity occurred in Si treatment. Sheath blight incidence was reduced by 23.69% for Si, 18.16 % for Cu and 13.49% for Zn compared to the inoculated control (Table 3). When Si was applied, sheath blight severity was reduced by 17.16% for MR219 and 29.04% for MR253 variety compared to the respective control treatments (Figure 1).

The response of grain yield was significantly different among the varieties and also among the sheath blight inoculation and fertilizer treatment combination (Table 4). Although treatment with Si produced the highest grain yield of 56.20gpot^{-1} and 27.54gpot^{-1} on MR219 and MR253 respectively, the yield response for Zn was less than for both varieties. The lowest grain yield 30.09gpot^{-1} for MR219 and 16.11gpot^{-1} for MR253 were observed in the inoculated and untreated (control) (Table 4). Compared with the inoculated/ untreated (control) check, application of Cu increased yield for both varieties.

3.1. Lignin Content

Lignin content was significantly (P=0.05) different between MR219 and MR253 rice varieties with silicon treatment. Silicon treatment showed significantly higher lignin content of 6.62% in variety MR219, and 5.09% in MR253 compared to the two controls of MR219 (3.60%) and MR253 (3.33%). These were also significantly higher compared to Cu and Zn treatments (Fig 2). The highest lignin content was followed by the rice plant treated with Zn (5.0%) for MR219 and (3.72%) in MR253. The lowest lignin content was recorded in plants treated with Cu (3.37%) for MR219. For MR253, the lignin content of Cu and Zn treatments were not statistically significant and the minimum lignin content was observed in the control.

4. Discussion

With inoculation, incidence of sheath blight disease developed and caused significant grain yield losses for the susceptible rice cultivar (MR253) but not for moderately resistant cultivar (MR219). The difference between non-inoculated and the inoculated, untreated controls showed that sheath blight can cause 11% grain yield loss in MR219 and 50 % grain yield loss in MR253. Similarly, the yield loss were reported to be 8% in moderately resistant variety and up to 30% in very susceptible variety by artificial inoculations [37]. Yield loss occurred in the inoculated / control treatment which are typical for heavily infested commercial rice fields. Apparently, MR219 variety is less susceptible and more tolerant than MR253 variety possibly due to their ability to resist lodging when heavily diseased, which is associated with the fungus inability to penetrate the culm [37]. Since genotypes vary in disease resistance, the relationship between Si content among genotypes and disease resistance need to be investigated. According to the reports on rice cultivars grown with highest Si rates [38] sheath blight intensities were greatly reduced compared with cultivars grown in pots not amended with Si.

The grain yield was increased significantly by Si treatment compared to other treatments. The highest grain yield of 56.2 gpot^{-1} and 27.54gpot^{-1} on MR219 and MR253 varieties respectively was recorded with silicon application. These differences appear to be responsible for the significant-inoculation and fertilizer treatment interaction for this variable. Increased in grain yield was attributed to an increase in the number of grains per panicle [39] and spikelet fertility has been associated with Si concentration in rice [40] (data not sown).When Si was applied, sheath blight severity was reduced by 17.16% for MR219 and 29.04% for MR253 variety compared to the respective control treatments. These findings concur with others by [24,25] for disease reduction. Enhanced resistance to disease via Si application can be associated with accumulation of Si in leaf epidermal cells which acts as a mechanical barrier against fungal infestation [23].

Silicon treatment showed significantly higher lignin content of 6.62% in variety MR219, and 5.09% in MR253 compared to the two controls of MR219 (3.60%) and MR253 (3.33%). These were also significantly higher compared to Cu and Zn treatments. This finding was in line with other study conducted [41] where Si application had increased lignin content and enhanced activities of the enzymes peroxidase, polyphenol oxidase, and phenylalanine ammonialyase (PAL) in rice leaves infected by rice sheath blight disease. Increased lignification in rice cells was accompanied by silicic acid triggered transcription of genes related to lignin metabolisms [42].

5. Conclusions

This study indicated the potential contribution of silicon application in reducing sheath blight disease severity and increasing rice yield. Copper and zinc applications were observed to reduce disease severity and increase yield compared to control. Silicon fertilization could be incorporated as a sustainable and environmentally friendly practice for management of rice sheath blight disease.

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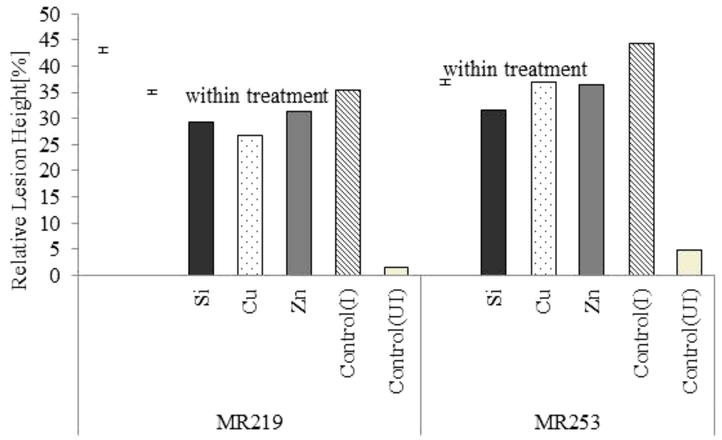

Figure 1. Disease severity in rice plants treated with Si, Cu and Zn after inoculation with *Rhizoctonia solani*

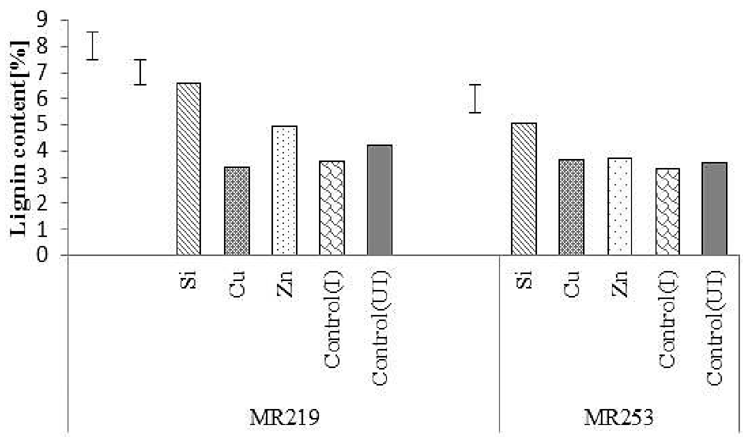

Figure 2. Percentage of acid detergent lignin (ADL) in rice epidermal cells treated with Si, Cu and Zn at 75DAP

Table 1. Significance (P value) of the main effects of variety and sheath blight inoculation-fertilizer treatment combination (treatment) and interactions among the main effects

Effect(df)	Incidence	HRLH	Grain yield
Variety(1)	0.0005	<0.01	<0.0001
Treatment(4)	<0.0001	<0.0001	<0.01
Variety x treatment(4)	0.3104	<0.05	<0.01

Table 2. Effect of varieties on sheath blight incidence, severity and whole rice grain yield [a]

Variety	Sheath blight Susceptibility [b]	Incidence [c] (%)	HRLH[d] (%)	Grain Yield(gpot^{-1})
MR219	MR	33.37 b	24.95 b	40.33 a
MR253	MS	40.85 a	30.99 a	24.77b
LSD(0.05)		5.12	2.32	3.97

[a] Means followed by same letter for each parameter are not significantly different at *P* =0.05.
[b] Sheath blight susceptibility levels included moderately resistant (MR) and moderately susceptible(MS).
[c] Percentage of stems infected with sheath blight at approximately one week before harvest.
[d] Highest relative lesion height one week before harvest

Table 3. Effect of sheath blight inoculation-micronutrient fertilizer treatment combination on sheath blight incidence, highest relative lesion height (HRLH) and whole rice grain yield

Inoculation	Treatment	Incidence%	HRLH%	Grain yield
Non-inoculated	non-treated	8.33 c	3.18 c	32.91 ab
Inoculated	non-treated	51.42 a	40.00 a	22.21 c
Inoculated	Si	39.24 b	30.49 b	39.49 a
Inoculated	Cu	42.08 b	32.27 b	31.87 b
Inoculated	Zn	44.48 ab	33.92 b	31.14 b

Table 4. Effect of cultivars and sheath blight inoculation-micronutrient application combination on rice grain yield

Variety	Susceptibility [y]	Yield (ghill^{-1})[x]				
		Non-inoculated/ non treatment	Inoculated/ non-treatment	Inoculated/ silicon [z]	Inoculated Copper [z]	Inoculated zinc [z]
MR 219	MR	33.72 cd	30.09 cde	56.20 a	44.46 b	37.17 bc
MR253	MS	32.07 cd	16.11 f	27.54 de	22.50 fe	22.11 fe

[x] Means followed the same letters are not significantly different at P= 0.05.

[y] Sheath blight susceptibility level included moderately resistant (MR) and moderately susceptible (MS).

[z] Silicon at (360g/15kg soil), copper at (0.30g/15kg soil) and zinc at (0.45g/15kg soil) applied in soil before planting.

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