# Effect of Increased Silicon Content of Paddy Rice on Sheath Blight Development through Carbonized Rice Husk Application

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# Abstract

Silicon (Si) application is considered one means of cultural management having beneficial effects on the control of rice diseases. Carbonized rice husk (CRH)—a cost-effective biochar derived from a by-product of rice production—has been proposed as Si fertilizer as well as for promoting carbon sequestration in soil. This experiment was conducted in an irrigated paddy field in Tsukuba, Japan, to evaluate the potential effects of CRH application on sheath blight among four treatments (two levels of CRH application at 1.5 and 3.0 t ha<sup>-1</sup>, a spray of fungicide with flutolanil, and the control without any application). The results demonstrated that CRH at 3.0 t ha<sup>-1</sup> increased rice Si content by 9% and regulated sheath blight development in the plant community to some extent after inoculation, whereas CRH at 1.5 t ha<sup>-1</sup> showed no clear impacts on rice plants and fungal pathogen development. The fungicide application with flutolanil had the lowest rate of sheath blight development, but did not affect rice Si content. The results suggest that CRH application at 3.0 t ha<sup>-1</sup> or more could be an option for integrated management of sheath blight without negative effects on rice yield.

**Discipline:** Crop Science Additional key words: biochar, silicon, *Oryza sativa*, *Rhizoctonia solani*, yield

# Introduction

Rice sheath blight caused by Rhizoctonia solani J.G. Kühn [Thanatephorus cucumeris (A.B. Frank) Donk], anastomosis group 1 IA (AG-1 IA) is prevalent and an economically important fungal disease in most temperate, subtropical and tropical rice-production areas (Srinivasachary et al. 2011). It has a wide host range, and the causal fungus can survive between crop seasons as sclerotia formed on or near the lesions that lie dormant in soil for a few years under a temperate climate. Initial infections start with a sclerotium or a piece of infected debris floating on the water surface and coming in contact with the leaf sheath near the waterline in paddy fields. Under favorable conditions with low sunlight, high humidity (>95%) and high temperature (28-32°C),

runner hyphae of the fungus spread rapidly to upper plant parts and adjacent plants (Uppala & Zhou 2018). The disease leads to softness of the stem and increases the chance of plant lodging, thereby producing poorly filled grains (Wu et al. 2012). Complete resistance to sheath blight has not been found, although many quantitative trait loci (QTLs) conferring partial resistance were detected on different rice chromosomes (Turaidar et al. 2018). The application of fungicides remains the major option for controlling the disease. Thus, there is a need to explore less expensive and more environmentally friendly practices, including biological and cultural control (Belmar et al. 1987, Rodrigues et al. 2001).

Silicon (Si) is reportedly a beneficial element for rice under various abiotic and biotic stress conditions (Meharg & Meharg 2015). Several studies have reported

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that Si application regulates the development of diseases (Robichaux 2001, Rodrigues & Datnoff 2005, Shirai et al. 1999). Si deposit in plants also increases lodging resistance, water utilization efficiency and salt tolerance, and prevents Al, Fe and Mn toxicities (Robichaux 2001, Ma & Takahashi 2002), thus making Si fertilizer increasingly important for an economic and sustainable rice production system (Alvarez & Datnoff 2001). Carbonized rice husk (CRH), known as "kuntan" in Japanese, is characterized by its high Si content as compared to other kinds of biochar produced through the thermochemical conversion of woody materials in oxygen-limited conditions (Jindo et al. 2014). CRH is also a very cost-effective biochar used in rice-based farming systems (Ogawa & Okimori 2010), as about 148 million tons of rice husk are generated annually as a by-product of global rice production (Pode 2016). The application of CRH has positive impacts on enhancing soil carbon (C) sequestration, mitigating greenhouse gas emissions from paddy fields, and improving rice productivity (Koyama et al. 2015 and 2016). However, the effect of CRH application on regulating the development of diseases has never been well-documented at the field level. Therefore, the present study was conducted to evaluate the effect of CRH application on the sheath blight of paddy rice.

# Materials and methods

## 1. Experimental site and treatments

A paddy field experiment was conducted at the Tsukuba International Center, Japan International Cooperation Agency (JICA), in 2017. The field soil is classified as clay loam with a pH level of 5.65. Treatments consisted of two levels of CRH application at 1.5 and 3.0 t ha<sup>-1</sup> (CRH-1.5 and CRH-3.0, respectively), one level of fungicide application (FLUT), and non-treated control (CONT) laid out in a randomized complete block design with three replications. The size of each plot was  $4 \text{ m} \times 3$ m with a 1-m gap between the plots and the blocks. All the plots were separated by using vinyl chloride resin levees so as to impede soil and roots movement among the treatments. CRH obtained from Otsuka Engei Ltd. in Japan (containing 14.3 g Si kg<sup>-1</sup>) was incorporated into the CRH-treated plots with a rake and a small power tiller (MS67, Mitsubishi Mahindra Agricultural Machinery Co., Ltd.) on May 17, one day before transplantation (DBT). For fungicide treatment, Moncut® (wettable powder containing 25% flutolanil; Nihon Nohyaku Co., Ltd.) diluted 1,000 times with water containing 0.05% of spreader Myrinoh® (Nihon Nohyaku Co., Ltd.) was applied at the rate of 150 mL m<sup>-2</sup> with a hand sprayer on July 21 (18 days after inoculation (DAI)).

# 2. Crop management

The well-matured seeds of rice (cv. IR28: moderately susceptible to sheath blight, International Rice Research Institute 1984) were soaked in a 200-times solution of Benlate-T<sup>®</sup> (containing 20% thiram and 20% benomyl; Hokko Chemical Industry Co., Ltd.) for 24 hours to control seed-borne diseases. The disinfected seeds were dried for one day, and then soaked in 17°C water for seven days as pre-germination treatment. Fifty grams of the pre-germinated seeds were sown in nursery trays (58 cm (L)  $\times$  28 cm (W)  $\times$  2.8 cm (H)) filled with commercial soil "Kanuma A" (granulated culture soil composed of zeolites containing 0.4, 1.1 and 0.5 g L<sup>-1</sup> soil of N, P<sub>2</sub>O<sub>5</sub>, and K<sub>2</sub>O, respectively; Kanuma Sangyo Co., Ltd.) on April 17. The commercial soil was disinfected with Tachigaren Ace® M (containing 4% hydroxyisoxazole and 0.25% metalaxyl M; Mitsui Chemical Agro Co., Ltd.) at the rate of 1.6 g  $L^{-1}$  soil before use. The temperature in the nursery was maintained in the range of 13-35°C. Prior to transplanting, the seedlings were treated with V-Get Admire<sup>®</sup> (granules containing 2% imidacloprid and 12% tiadinil; Nihon Nohyaku Co., Ltd.) at the rate of 50 g per nursery tray to control blast and insect pests. Basal fertilizers were applied to the main field at 60, 100, and 80 kg ha<sup>-1</sup> of N, P<sub>2</sub>O<sub>5</sub>, and K<sub>2</sub>O, respectively, on May 15 (3 DBT). Three seedlings per hill were manually transplanted at a spacing of 30 cm × 15 cm on May 18 (31 days after sowing). A herbicide - granular Hokuto® (containing 0.6% cyhalofop butyl, 1.5% pretilachlor, 0.2% dimethametryne and 0.07% pyrazosulfuron-ethyl; Syngenta Japan K. K.) - was applied at the rate of 30 kg ha<sup>-1</sup> five days after transplanting (DAT) and manual weeding was conducted as required during cultivation. Topdressing was applied once at 20 kg ha<sup>-1</sup> of N and K<sub>2</sub>O at 46 DAT. Flooded fields were maintained except for the five-day summer drainage period in mid-July and for ten days prior to harvest.

#### 3. Inoculation and assessment of rice sheath blight

A virulent isolate of *Rhizoctonia solani* (AG-1 IA) provided from the Japan Plant Protection Association was cultured at 28°C for seven days on rice husk-wheat bran medium (dry rice husk 10 g, wheat bran 10 g, and 20 mL of 0.5% polypepton solution per 300 ml flask). The medium colonized by mycelium of the pathogen was mixed with dry rice husks at a 1:3 ratio on a volume basis, and then scattered by hand to the rice plant base in each plot at the rate of 47 mL m<sup>-2</sup> at 46 DAT. Disease assessments were conducted for 50 hills randomly selected in each plot at 27, 40, and 55 days after inoculation (DAI). Hills with more than one lesion were regarded as diseased, and the percentage of diseased hills

(PDH) was determined. The percentage of infected tillers (PIT) and relative height of the uppermost lesions to plant height (RHUL) were calculated to examine horizontal and vertical development of the disease, respectively. The RHUL was determined according to Hashiba (1984) using the following equation:

RHUL = Height of the uppermost lesions (cm) / Plant height (cm) × 100

Degree of disease severity (DDS) was defined according to Yoshimura (1954) as follows:

 $DDS = (3n_1 + 2n_2 + 1n_3 + 0n_4) / 3N \times 100$ 

where,  $n_1$  denotes the number of tillers with all four uppermost leaf sheaths infected,  $n_2$  the number of tillers with the four uppermost leaf sheaths except the top sheath infected,  $n_3$  the number of tillers with the four uppermost leaf sheaths except the top two sheaths infected,  $n_4$  the number of tillers with all four uppermost leaf sheaths healthy, and N the total number of stems investigated  $(N = n_1 + n_2 + n_3 + n_4)$ .

#### 4. Rice plant growth, yield, and Si analysis

Data on the plant height, number of tillers, and leaf color chart were collected weekly from six hills in each plot. At the ripening stage on September 11 (116 DAT), 24 hills in the center of each plot were harvested to determine the yield components. The harvested rice plants were airdried, and four representative hills of each plot were selected based on the average plant height and panicle number of the plot. Saline solution with a specific gravity at 1.11 was used to separate the matured and unfilled grains. The ripening ratio was calculated with the number of matured grains divided by the total number of grains. The moisture content of the matured grains was measured by the grain moisture meter (CD-6, Shizuoka Seiki Co., Ltd.). Grain weight was adjusted to a moisture content of 14% for obtaining the paddy yield. A few culms (stems and leaves) from every representative hill were randomly sampled, dried in an oven at 80°C for 48 hours, and then ground to a fine powder ( $\leq 2 \text{ mm}$ ) for chemical analysis.

Si concentration in the rice samples and the CRH used were determined using gravimetric analysis after wet ashing as described by Saito et al. (2005). Data on temperatures and rainfall during rice cultivation were collected from a meteorological station of the Japan Metrological Agency located within the same city as the experimental site.

# 5. Statistical analysis

Collected data were analyzed with StatView 5.0 for Windows (SAS Institute Inc.). The values related to rice growth, yield, Si content, and sheath blight assessments were averaged for each plot and subjected to statistical analysis by analysis of variance (ANOVA). Tukey's multiple range test was used for comparisons after ANOVA at the 5% significance level.

# Results

#### 1. Rice growth, yield, and Si uptake

The plant height and leaf color did not differ statistically among the treatments throughout rice cultivation (data not shown). Similarly, the number of tillers did not indicate any significant differences among the treatments throughout rice cultivation, except at 87 DAT when CRH-3.0 had a statistically higher number of tillers (16.6) than CONT (14.5). As a result, the yield and yield components were not significantly different among treatments (Table 1). Conversely, the clear effects of CRH application were observed in rice Si concentration. CRH-1.5 and CRH-3.0 increased Si concentrations by 4% and 9%, respectively, as compared to CONT (Table 2). The higher rate of CRH application resulted in the higher Si concentration.

#### 2. Sheath blight assessment

The weather conditions during the experiment were favorable for disease development. During 55 days from inoculation to final assessment, the average daily

Treatment	No. of panicles $(m^{-2})$	No. of spikelets (panicle <sup>-1</sup> )	1000 grain weight (g)	Ripening ratio (%)	Paddy yield (kg m <sup>-2</sup> )
CONT	313	112	28.7	80.7	809
FLUT	320	111	28.0	87.7	872
CRH-1.5	318	106	28.5	85.5	825
CRH-3.0	330	102	28.5	81.7	786

Table 1. Yield and yield components of rice in respective treatments

CONT: Non-treated control, FLUT: Fungicide application, CRH-1.5 and CRH-3.0: Carbonized rice husk application at the rate of 1.5 t  $ha^{-1}$  and 3.0 t  $ha^{-1}$ , respectively. 1000 grain weight and paddy yield were adjusted to a moisture content of 14%. There was no significant difference among the treatments in each column according to the ANOVA at 5%.

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temperature ranged 22-28°C and precipitation was 201 mm with 19 rainy days (Fig. 1). After inoculation, PDH gradually increased in all the treatments, although there was no significant difference among the treatments on the three assessment dates (Fig. 2). The DDS of FLUT was significantly lower than those of CONT in the  $2^{nd}$  and  $3^{rd}$  assessments, while the DDS of CRH-3.0 recorded

Table 2.	Silicon concentration of rice plants in
	respective treatments

Treatment	Si (%)
CONT	6.70 b
FLUT	6.76 ab
CRH-1.5	7.00 ab
CRH-3.0	7.33 a

Mean values followed by the same letters are not significantly different by Tukey's multiple range test ( $P \le 0.05$ ).





Inoculation was conducted on July 3. Disease assessments were made on July 30, August 12, and August 27, respectively.



Fig. 2. Change in percentage of diseased hills (PDH), degree of disease severity (DDS), percentage of infected tillers (PIT), and relative height of uppermost lesions to plant height (RHUL) after inoculation

Fungicide was applied at 18 days after inoculation. The same letters within the same assessment date indicate no significant difference by Tukey's multiple range test (P < 0.05).

intermediate values between CONT and FLUT. Similarly, the PIT of FLUT was significantly lower than those of CONT in the 2<sup>nd</sup> and 3<sup>rd</sup> assessments, while the PIT of CRH-3.0 recorded intermediate values between CONT and FLUT. The RHUL of FLUT was also significantly lower than those of CONT in the 1<sup>st</sup> and 2<sup>nd</sup> assessments, and the RHUL of CRH-3.0 recorded intermediate values between CONT and FLUT in the 1<sup>st</sup> assessment.

# Discussion

Constant rainfall after inoculation (Fig. 1) induced high humidity within the rice canopy and probably enhanced the development of sheath blight (Takeda & Ogawa 1986). Even under such favorable conditions, onetime spraying with flutolanil (FLUT) could maintain PDH, DDS, PIT, and RHUL values lower than those of the other treatments (Fig. 2) due to the long-lasting curative and protective properties of the fungicide (Mochizuki et al. 1987). Although CRH application did not demonstrate significant effects on the investigated parameters as compared to CONT, the RHUL of CRH-3.0 in the 1st assessment and the DDS and PIT of CRH-3.0 in the 2<sup>nd</sup> and 3<sup>rd</sup> assessments were not significantly different from those of FLUT (Fig. 2). This suggested that CRH application at 3.0 t ha<sup>-1</sup> retarded vertical and horizontal development of rice sheath blight in the plant community. To examine the relationship between the amount of CRH and sheath blight, regression analysis was conducted between the applied amount of CRH (CONT, CRH-1.5, and CRH-3.0) and the disease parameters (PDH, DDS, PIT, and RHUL). All the correlation coefficients were negative values ranging from -0.573 (P = 0.612) to -0.999 (P = 0.026), and a significant negative correlation was detected only between carbonized rice husk application and PDH at 27 DAI (Fig. 3), indicating that a larger amount of carbonized rice husk application could delay the spread of diseased



Fig. 3. Correlation between application amount of carbonized rice husk (CRH) in CONT, CRH-1.5 and CRH-3.0 and percentage of diseased hills (PDH), degree of disease severity (DDS), percentage of infected tillers (PIT), and relative height of uppermost lesions to plant height (RHUL) at different days after inoculation (DAI) no and \* indicate not significant and significant at P < 0.05, respectively.

ns and \* indicate not significant and significant at  $P \le 0.05$ , respectively.

hills after inoculation. As Robichaux (2001) described that Si concentration in rice plant tissues can be used as an indicator of sheath severity, the reduction of sheath blight development could be largely attributed to the increase in rice Si concentration (Table 2). The proportional increase in rice Si concentration through CRH application (4% at 1.5 t  $ha^{-1}$ , and 9% at 3.0 t  $ha^{-1}$ ) was similar to the findings of Koyama & Hayashi (2017). However, no substantial improvement in rice growth and yield was observed after CRH application in the present experiment, even though Si deposit in rice plants reportedly promotes effective photosynthesis (Ma & Takahashi 2002). According to Imaizumi & Yoshida (1958), the effect of Si fertilizer on rice yield would be significant when applied to soil where growing rice plants contain less than 5.1% Si. In our result, CONT already had a higher Si concentration (6.70%) than this critical concentration; therefore, the impact of additional Si supplied from CRH application might be obscured. The effect of Si fertilizer on rice Si concentration varies depending on soil properties such as Si availability, Si adsorption capacity, and pH under a flooded condition (Makabe-Sasaki et al. 2014). Si fertilizer may offer a viable option for sheath blight control in the tropics and subtropics where plant-available Si is extensively low or limited due to heavy desilication-aluminization arising from high temperature and rainfall (Abe et al. 2016, Raven 2003).

The results demonstrated for the first time that CRH application at 3.0 t ha<sup>-1</sup> delayed the spread of sheath blight to adjacent tillers and upper plant parts without negative effects on rice yield as compared to the control treatment. This suggests that CRH application can be used as a part of integrated disease management, as the efficacy level is elevated cumulatively with an increase in the number of management components (Zadoks & Schein 1979). The results were derived from a single season field experiment. Thus, the effects of carbonized rice husk on sheath blight development must be confirmed in further experiments under different climatic conditions and soil types, in order to promote sustainable rice farming systems in the world.

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