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The mechanisms underlying the anthracnose disease reduction by rice hull as a silicon source in capsicum (*Capsicum annuum* L.) grown in simplified hydroponics

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Abstract

Silicon has proven to be effective in controlling many diseases in plants and could be used as an alternative strategy against chemical control of diseases. Rice hull is an environmental friendly natural source of silicon. This study was conducted to investigate the effect of rice hull as a Si source on anthracnose disease and also on fruit cuticle thickness (CT), total soluble and cell wall-bound phenolic compounds (TSP and CWBP) in fruits and formation of fungal appressoria on fruits as possible mechanisms of disease reduction in capsicum (*Capsicum annuum* L.). In this study a simplified hydroponics system (SHS) with rice hull as an inert media and nutrients supplied with either NF (New Formula) or Albert's solution was used. A liquid hydroponic system (LHS) was also maintained with same nutrient solutions as controls. Disease development was assessed by challenge inoculation with *Colletotrichum gloeosporioides* on fruits. CT was measured using stage and ocular micrometer. TSP and CWBP in fruits were analysed by Folin-Ciocalteu method during first 5 days after inoculation (DAI). Appressoria formation by fungal conidia on fruit peels at inoculated spots was observed through micrometer daily after inoculation. More than 83% disease reduction was observed in fruits harvested from SHS compared to that of LHS supplied with both nutrient solutions. There were significantly higher values of CT and CWBP (about 45% and 30% respectively) in fruits from SHS compared to that of LHS (Si-free). However, TSP was not significantly affected by Si treatment. A higher percentage of appressoria was prevailed on fruits harvested from SHS thus the disease initiation was delayed compared to that of LHS. There may be a possibility that germination of appressoria was hindered by thicker cuticle or biochemical reaction involved with induced CWBP in fruits from Si treated plants.

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Keywords: Silicon; *Capsicum annuum* L.; *Colletotrichum gloeosporioides*; Rice hull

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1. Introduction

Alternative methods for disease management have an increasing concern since fungicides have negative impact on environment and human health. Numerous promising results were achieved by silicon (Si) for the controlling of many fungal diseases in many crops. our previous study revealed that silicon suppressed the anthracnose disease occurrance in capsicum (Jayawardana *et al*., 2014). The mechanism involved in disease resistance mediated by Si in plants is not yet fully understood. It has been reported that Si deposited beneath the cuticle acted as a physical barrier to impede penetration by fungal appressoria, thereby disrupting the process of infection while some strong evidences indicate that Si enhances the natural defense systems of the plant through the production of phenolic compounds and phytoalexins *etc.*

The use of natural sources of silicon such as rice hull as alternative to the chemicals (*eg.* potassium silicate) would be a promising method since it is environmentally friendly and cost effective. Simplified hydroponic system (SHS) is an aggregate hydroponic system having rice hull in the inert media. About 50 ppm of soluble silicon leached by rice hull in the simplified hydroponics system (H. A. R. K. Jayawardana, unpublished). Therefore, the current study was conducted to investigate the effect of rice hull as a Si source in the SHS on anthracnose disease and also on fruit cuticle thickness (CT), total soluble and cell wall-bound phenolic compounds (TSP and CWBP) in fruits and formation of fungal appressoria on fruits as possible mechanisms of disease reduction by Si in capsicum (*Capsicum annuum* L.) hybrid variety 'Muria F1'.

2. Methodology

Healthy, six weeks old capsicum seedlings were transferred to simplified hydroponic and non circulating liquid hydroponic systems (LHS) in a plant house (28-30ºC temperature and 80-85% RH). The SHS included with inert media consists with rice hull and sand (3:2 v/v). Four treatments were conducted; SHS supplied with NF solution (SHNF), Albert's solution (SHAL), LHS supplied with NF solution (NF) and Albert's solution (AL). Treatments were arranged in Completely Randomized Design (CRD) with three replicates each with four plants. The nutrients were supplied with NF nutrient solution (Saparamadu, 2008) and Albert's solution (Unipower (pvt) Ltd.) separately. SHS was supplied with 200 ml of nutrient solution once in two days. Nutrient solutions of LHS were renewed once a week.

C. gloeosporioides, isolated from anthracnose lesions on diseased capsicum fruit, were cultured on potato dextrose agar (PDA). Harvested capsicum fruits were challenge-inoculated with *Colletotrichum gloeosporioides* by placing a 20 µl drop of conidial suspension ($5x10^5$ conidia per ml) at three different spots on the surface sterilized fruit. Twenty-four fruits per treatment were inoculated. The inoculated fruits were incubated in a moist chamber (20-30°C and 95-100% RH). The lesion areas were measured using a transparent graph paper each day until 10 days after inoculation. Three cross-sections (0.1 mm thick) of each fresh fruit were mounted on a glass slide and the CT was measured using a stage and ocular micrometer at 400×. Tissue samples were taken from each inoculated spots at 2, 3, 4 and 5 d after inoculation (DAI) to determine TSP and CWBP using Folin-Ciocalteu reagent (Ascensao and Dubery, 2003). A thin peel of the fruits taken from the inoculated spots and appressoria formation by conidia was observed through the microscope at 2, 3, 4, 5 days after inoculation. The total number of conidia and the number of appressoria were counted in five fields of vision under 400×. Then the percentage of appressoria formation by conidia per vision was calculated. Data were analyzed using one-way ANOVA and mean separation was done by Duncan's Multiple Range Test in SPSS 16.0.

3. Results, Discussion and conclusion

3.1. Lesion area development of capsicum fruits

The lesion area observed at 10 days after inoculation was 17.5 mm^2 and 12.8 mm^2 in SHNF and SHAL treatments whereas that was 103.8 mm^2 and 99.2 mm^2 in NF and AL treatments respectively. The lesion area observed in fruits from SHNF treatment was 83% lesser than NF treatment while that was 87% lesser in SHAL treatment than AL treatment. Further, it was observed that the lesion initiation was delayed by 2 days and the rate of lesion development was slow (average 3.7 mm^2/ day) during 10 DAI in the fruits of capsicum plants grown in SHS compared to that of LHS (average 14.5 mm^2/ day). There was no significant difference in lesion areas of SHNF and SHAL treatments indicating that the disease suppression was not affected by the nutrient solution applied for the treatments.

3.2. CT, TSP, CWBP and percentage of appressoria formation

Although cuticle thickness is a varietal character**,** the mean cuticle thickness was significantly greater in capsicum fruits from SHS (average 36.1 μm) than fruits from LHS (20.6 μm) (as determined by one-way ANOVA at $P \leq 0.05$). It has been reported that the fruits of a *C. gloeosporioides* resistant capsicum variety had thicker cuticle than that of a susceptible capsicum variety revealing that thick cuticle of pepper possibly acts as a pre existing structural barrier to fungal penetration. It has also shown that there is a negative correlation between cuticle thickness and disease incidence caused by *C. gloeosporioides* in pepper fruits (Oh *et al*., 1999).

There was no significant difference in TSP between fruits of silicon treated and control plants. Nevertheless, significantly higher cell wall bound phenol content was detected in Si treated fruits compared to the control fruits at 3, 4 and 5 DAI (Table 1.). It has been reported that Si induces phenolic compounds as a resistance mechanism against diseases (Cherif and Belanger, 1992)

There was no significant difference in the percentage of appressoria formation among the treatments. However, the percentage of appressoria formation seemed to be related to the date of disease initiation. Although the disease initiation on capsicum fruits from SHS treatments was reported in 6 DAI of the pathogen, there was more than 24% of appressoria formation from 4 DAI - 6 DAI. In contrast, there was a sudden increase in the % of appressoria formation in the fruits from LHS on the date of disease initiation (4 DAI) (Table 1.). Therefore, prevalence of a higher percentage of appressoria and delay in disease initiation which was observed in the fruits from SHS might be due to a barrier present in the fruits of Si treated plants. Hayasaka *et al*., (2008), confirmed that Si in the leaf epidermis may confer resistance against appressoria penetration.

It is reported that *C. gloeosporioides* is a pathogen which directly penetrates the host and its penetration and further infection process can be inhibited by preformed or induced chemical inhibitors in plant cells. Phenolic polymers act as barriers in cell walls, rendering cell walls highly resistance to mechanical and enzymatic disruption by pathogens (Prusky, 1998).

Table 1. Total and cell wall bound phenolic compounds and appressoria formation on inoculated fruits

DAI	TSP mg g^{-1} of fresh weight		CWBP mg g^{-1} of fresh weight		Percentage of Appressoria formation	
	LHS	SHS	LHS	SHS	LHS	SHS
2	356.0 ^{a}	375.9 ^{a}	101.7 ^{a}	110.8 ^{a}	0 ^{a}	0 ^{a}
3	500.8 ^{a}	530.7 ^{a}	135.3 ^{a}	346.2 ^{b}	3.5 ^{a}	5 ^{a}
4	517.1 ^{a}	544.4 ^{a}	132.3 ^{a}	184.5 ^{b}	30* ^{a}	24 ^{a}
5	576.5 ^{a}	562.5 ^{a}	122.1 ^{a}	176.6 ^{b}	**	28
6	-	-	-	-	**	30*
	NS				NS	

Mean Values followed by the same letter for each parameter at each DAI are not significantly different at $P \leq 0.05$ as determined by one way ANOVA.

Ns – non significant

*Disease initiation

**It was difficult to observe the samples due to sporulation on fruit lesions.

Therefore, it could be suggested that the reduction of disease development by Si treatment may possibly be due to the thicker cuticle formed in fruits from silicon treated plants or induced resistance by increasing the concentrations of cell wall-bound phenolic compounds or both factors may equally contribute towards the disease resistance of fruits from silicon treated plants.

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