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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

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