Effect of Foliar and Root Application of Silicon Against Rice Blast Fungus in MR219 Rice Variety

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Rice blast disease caused by Magnaporthe grisea (Hebert) Barr [teleomorph] is one of the most devastating diseases in rice plantation areas. Silicon is considered as a useful element for a large variety of plants. Rice variety MR219 was grown in the glasshouse to investigate the function of silicon in conferring resistance against blast. Silica gel was applied to soil while sodium silicate was used as foliar spray at the rates of 0, 60, 120, 180 g/5 kg soil and 0, 1, 2, 3 ml/l respectively. The treatments were arranged in a completely randomized design. Disease severity and silicon content of leaves were compared between the non-amended controls and rice plants receiving the different rates and sources of silicon. Silicon at all rates of application significantly (α = 0.05) reduced the severity of disease with highest reduction (75%) recorded in treatments receiving 120 g of silica gel. SEM/EDX observations demonstrated a significant difference in weight concentration of silicon in silica cells on the leaf epidermis between silicon treated (25.79%) and non treated plants (7.87%) indicating that Si-fertilization resulted in higher deposition of Si in silica cells in comparison with non-treated plants. Application of silicon also led to a significant increase in Si contents of leaves. Contrast procedures indicated higher efficiency of silica gel in comparison to sodium silicate in almost all parameters assessed. The results suggest that mitigated levels of disease were associated with silicification and fortification of leaf epidermal cells through silicon fertilization.

**Keywords**: blast, Magnaporthe grisea, rice, silicon

Rice (Oryza sativa L.) is among the most important crops all over the world since it is consumed as a staple as well as primary source of energy and protein (Zhang et al., 2008). In Malaysia rice is one of the major crops grown by the private and public sectors. Rice blast, caused by the fungus Magnaporthe oryzae (Couch and Kohn, 2002) (anamorph, Pyricularia oryzae Cavara), is among the most significant diseases affecting rice cultivation, since it is prevalent in most rice growing regions and causes serious yield losses (Hayasaka et al., 2008). The disease is widely distributed (85 countries) (Hajano et al., 2011) and under favorable environmental conditions it can be very disastrous (Scardaci et al., 1997). Yield and harvest losses estimated from other areas of the world have ranged from 10–30% (Tongen et al., 2006). Applying fungicide to control the disease is neither economical nor environmentally friendly. Furthermore, rice blast spreads very fast and the use of resistant varieties is considered as a short term remedy. Silicon is the second element which is found in abundance in the earth's crust (Datnoff et al., 1997; Epstein, 1994; Marschner, 1995) and is a main component of plant tissues as well as cell walls, despite the fact that it is not considered as an essential nutrient for terrestrial plants (Epstein, 1994). This element can be absorbed from soil in significant amount and it is taken up by plants in the form of monosilicic acid (Mitani et al., 2005). Supply of soluble silicon in plants provides stronger, tougher cell walls which serve as a mechanical barrier against sucking and piercing insects. Also silicon can be deposited by the plants at the infection site thus inhibiting the penetration of cell walls by the attacking fungus. Rice is known as a Si-accumulator (Mitani et al., 2005) and accumulation of Si in leaves and tissues in addition to conferring resistance against fungal diseases and insect pests, can improve erectness of leaves, increase yield and alleviate water stress, salinity stress and nutrient deficiency or toxicity stresses as well. Silicon is also considered as an environmentally-friendly element in relation to soils, fertilizers and plant nutrition (Ma and Takahashi, 2002). Based on the aforementioned it is concluded that silicon deficiency can be considered as a limiting factor for crop production. There have been plenty of reports on profitable effects of silicon in rice plantations (Ando et al., 2002;
Yoshida et al., 1962) especially under biotic and abiotic stresses. Si application is considered as a suppression factor for fungal diseases such as rice blast, brown spot, leaf scald, sheath blight and stem rot (Datnoff et al., 1991, 1992, 1997; Savant et al., 1996; Seibold, 1998; Winslow, 1992). Datnoff et al. (1991, 1992) reported decrease in blast severity ranging from 17% to 30% in rice planting regions on Colombian Histosols. According to their findings disease severity tended to be reduced with increasing Si concentration in tissues.

The objective of this study was to determine the role of foliar applied sodium silicate and root applied silica gel as silicon sources in controlling rice blast.

Materials and Methods

Plant materials and cultivation. Rice seeds of MR219 variety were used as source of plant material in this experiment. The soil used was an Ultisol collected from a paddy field located in Field 10, at the University Agriculture Park in UPM (Universiti Putra Malaysia) with the following characteristics: pH 4.84; P = 26.00 mg/g; K = 443.00 mg/g; Ca, Mg, Na, Fe = 1335.00, 339.40, 4.07, 170.00 mg/g respectively. Seeds were soaked in water at around 25–30°C for three days to hasten germination. Germinated seeds were planted into plastic seedling boxes (22.7 × 18.6 × 6.9 cm). Six g of N-P-K (15-15-15) fertilizer (CCM Fertilizers Sdn Bhd, Malaysia) was mixed with 300 g of soil. The emerged seedlings at the second-leaf stage, were transferred into pots (29 cm dia, 29.5 cm deep) containing five kg of the paddy soil and placed in the glasshouse. The pots containing the rice plants were flooded with water and plants were inoculated at the fourth-leaf growth stage.

Silicon application. Granular silica gel (Classic Chemicals Sdn Bhd, Malaysia) as well as liquid sodium silicate (Kooksong Co., Korea) were used as silicon sources in this study. Method of application and concentrations of supplemental silicon treatments were as follow:

Granular silica gel with a minimum SiO$_2$ content of 95% and particle size ranging from 3–6 mm was applied to the soil prior to planting at the rate of 60, 120 and 180 g per 5 kg soil. Liquid sodium silicate with SiO$_2$ content of 12% (w/v) was applied as foliar spray at the rate of 1, 2 and 3 ml/l. Tween 20 (0.02%, v/v) was added as a surfactant (Hayasaka et al., 2008).

Plant inoculation and disease severity assessment. A highly virulent strain of Mannaporthe grisea was used for inoculation. The fungus was obtained from Malaysian Agricultural Research and Development Institute (MARDI) and was cultured on PDA culture medium at 26–28°C for two weeks until the whole surface of the plate was covered with mycelium. Mycelia mats were gently scraped by spatula and plates were placed under wet cheese cloth for 2 days with continued light to induce sporulation and finally produce spores. These spores were used as inoculum source. Rice plants in their fourth leaf growth stage were sprayed with spore suspension containing $3 \times 10^6$ conidia ml$^{-1}$ (15 ml/pot). Non inoculated plants were sprayed with distilled water containing same amount of Tween20 (0.02%). After spraying, both inoculated and non inoculated plants were kept in a moist chamber with 98–100% relative humidity for 24 hours. Seven days after inoculation, leaf blast severity was evaluated based on visual assessment of lesions caused by Mannaporthe oryzae on a nine grade scale according to IRRI standards (2002). Disease severity for each replication was calculated according to the equation below (Cai et al., 2008):

$$\text{Disease severity} (\%) = \frac{\sum (r \times n_i)/(9 \times N)}{9 \times N} \times 100$$

where $r$ indicates rating value (0–9), $n_i$ indicates the number of infected leaves with a rating of $r$, $N_i$ indicates the total number of leaves tested for each replication.

Experimental design. The experiment was laid out in a completely randomized design with three replications. Each replication corresponds to eight rice plants per experimental unit. The experiment was conducted to evaluate effect of silica gel and sodium silicate on blast severity with seven treatments: (1) inoculation with $M.$ oryzae and no silicon application, as control; (2) inoculation with $M.$ oryzae and addition of 60 g silica gel; (3) inoculation with $M.$ oryzae and addition of 120 g silica gel; (4) inoculation with $M.$ oryzae and addition of 180 g silica gel; (5) inoculation with $M.$ oryzae and addition of 1 ml sodium silicate; (6) inoculation with $M.$ oryzae and addition of 2 ml sodium silicate; (7) inoculation with $M.$ oryzae and addition of 3 ml sodium silicate.

Microscopy observation. Scanning Electron Microscope (JEOL, JSM-6400, JAPAN) at an accelerating voltage of 15 keV and magnification of ×1000 was used for morphological observation of silica cells in leaf epidermis. The percentage of silica deposition was analyzed with an energy-dispersive X-ray spectrometer (EDS) combined with the microscope at magnification of x1000. Prior to the X-ray analysis of Si, the topmost completely developed rice leaves were dehydrated in desiccators. Thereafter leaf segments (1 × 1 cm) were mounted on aluminum stubs previously covered with double-sided adhesive carbon tape and coated with gold in a sputter coater (Baltec SC 030). Finally stubs
were loaded on to the scanning electron microscope (JEOL, JSM-6400, JAPAN).

**Plant assessment.**

**Plant height:** Plant height was used to evaluate the growth rate of rice plants in relation to disease and fertilizer application. Plant height was measured 10 days after inoculation from ground level to the tip of the tallest leaf.

**Dry weight measurement.** Plants were harvested for the shoots and roots by uprooting the plants and cutting the shoots at soil level. The shoots and roots were dried in an oven (Memmert, Western Germany) at 70°C for about 3 days to constant weight and dry weight of shoots and roots was measured on a weighing scale.

**Determination of silicon content of leaves.** Autoclaved induced digestion (AID) method described by Elliott and Snyder (1991) was used to prepare leaf samples for the colorimetric determination of silicon content. Samples comprising of 0.1 g of dried leaves were placed in 50 ml polyethylene screw cap tubes. Two ml of 30% H₂O₂ and 4.5 g of 50% (w/w) NaOH were added to each tube at ambient temperature and tubes were gently shaken and placed in an autoclave at 138 Kpa pressure for 1 h. After autoclaving, the volume of each tube was diluted to 50 ml with DI water. One ml of sample solution was transferred into 50 ml polyethylene tubes. Thirty ml of 20% acetic acid and 10 ml of ammonium molybdate solution (54 g/l, pH 7.0) was added to each tube. After five minutes 5 ml of 20% tartaric acid and 1ml of reducing agent was added and the volume was brought to 50 ml with 20% acetic acid. Reducing agent was prepared with 25 g of sodium bisulfite (NaHSO₃) dissolved in 200 ml distilled water and this solution was added to another solution containing 2 g of sodium sulfite (Na₂SO₃), 0.4 g of 1-amino-2-naphtol-4-sulfonic acid in 25 ml of distilled water. The final volume was brought to 250 ml with distilled water (Elliott and Snyder, 1991). After 60 minutes the absorbance was measured with a spectrophotometer (UV-Visible; Varian, Australia) calibrated at 650 nm. Silicon standard solutions (Nacalai tesque, Inc. Kyoto, Japan) at rates of 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 ml (100 mg/l) were used to establish the standard curve.

**Data analysis.** All data were expressed as means ± standard error and analyzed using the ANOVA procedure in the SAS Statistical software package (version 9.2 for windows). Differences among treatments were determined using the least significant difference (LSD) test at the 0.05 probability level. Comparison among silica gel treated plants and sodium silicate treated ones was made by the contrast procedure using SAS software.

**Results**

**Disease severity.** All rates of applied silicon led to a significant reduction ($\alpha = 0.05$) in disease severity and incidence compared to non-treated plants regardless of product type (Table 1).

Silica gel application at the rate of 120 g, consistently reduced disease severity by as much as 75% compared to the control. In both silica gel and sodium silicate treatments, greater reduction in disease severity was observed with increasing rates of silicon up to 120 g and 2 ml/l. No significant reduction in disease severity was recorded between applications of 120 g and 180 g of silica gel and between applications of 2 ml/l and 3 ml/l of sodium silicate. Contrast analysis indicated that disease severity reduction was higher with silica gel application than sodium silicate treatment at all rates compared to the control treatment (Table 2).

**Table 1. Disease severity and incidence in rice plants treated with silica gel and sodium silicate**

| Treatment                | Disease incidence (%) | Disease severity (%) |
|--------------------------|-----------------------|----------------------|
| Control (non-treated)    | 57.14 ± 2.74 a        | 38.88 ± 0.64 a       |
| Silica gel (60 g/5 kg soil) | 30.15 ± 4.20 c      | 19.71 ± 0.55 c       |
| Silica gel (120 g/5 kg soil) | 14.28 ± 2.74 d     | 9.50 ± 1.32 d        |
| Silica gel (180 g/5 kg soil) | 17.45 ± 1.58 d     | 10.40 ± 0.89 d       |
| Sodium silicate (1 ml/l) | 41.26 ± 4.19 b       | 26.16 ± 0.63 b       |
| Sodium silicate (2 ml/l) | 26.98 ± 1.59 c       | 17.03 ± 2.42 c       |
| Sodium silicate (3 ml/l) | 30.08 ± 1.51 c       | 17.69 ± 0.64 c       |

Values are means ± standard error. Different letters in the same column denotes statistical difference at $P < 0.05$ according to Fisher’s protected least significant difference (LSD).

**Table 2. Comparison between treated and non-treated plants and between silica gel treated group with sodium silicate treated group using the contrast procedure**

| Contrast                     | Mean Square | F Value | Pr > F |
|------------------------------|-------------|---------|--------|
| Control vs. silica gel treatment | 1483.405  | 346.87  | <.0001 |
| Control vs. sodium silicate treatment | 777.108  | 181.72  | <.0001 |
| Silica gel vs. sodium silicate treatment | 226.348  | 52.93  | <.0001 |

The relationship between disease severity with amount of silica gel and sodium silicate applied to the plants are shown in Fig. 1 and Fig. 2 respectively. The relationship between the rates of silicon fertilizers and disease severity could be explained by the exponential decay curve.
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SEM Observations. With regards to SEM/EDX observations and X-ray spectra of adaxial surfaces of the youngest rice leaves in each treatment two types of silicified cells were observed: Dumbbell-shaped or ladder-like silica cells and small scattered silica cells (Fig. 3).

The corresponding EDX spectra compared with the SEM images demonstrated a significant difference in silicon content between silicon treated (25.79%) and non-treated plants (7.87%). Si-treated plants contained more silicon in comparison with non-treated ones. Evidently the Si peak and weight (%) of the rice plants treated with 120 g of silica gel was the highest (25.79%) among silica gel treated plants and the Si peak and weight (%) in plants treated with 2 ml/l sodium silicate was higher (17.93%) than with the other rates (Table 3).

Table 3. Effect of silicon treatment on silica deposition and enhancement of dumbbell-shaped silica cells

| Treatment                        | Weight Conc. of Si (%) | Increment (%) |
|----------------------------------|------------------------|---------------|
| Control (non-treated)            | 7.87 ± 0.28 e          | –             |
| Silica gel (60 g/5 kg soil)      | 15.77 ± 0.32 c         | 7.9           |
| Silica gel (120 g/5 kg soil)     | 25.79 ± 0.70 a         | 17.92         |
| Silica gel (180 g/5 kg soil)     | 24.59 ± 0.85 a         | 16.72         |
| Sodium silicate (1 ml/l)         | 11.41 ± 0.17 d         | 3.54          |
| Sodium silicate (2 ml/l)         | 17.93 ± 0.07 b         | 10.06         |
| Sodium silicate (3 ml/l)         | 17.59 ± 0.73 b         | 9.72          |

Values are means ± standard error from three replication. Different letters in the same column denotes statistical difference at $P < 0.05$ according to Fisher’s protected least significant difference (LSD).

Table 4. Comparison of weight conc. of Si in dumbbell-shaped silica cells in treated and non-treated plants as well as in silica gel and sodium silicate treated plants using the contrast procedure

| Contrast                        | Mean Square | $F$ Value | Pr > $F$ |
|---------------------------------|-------------|-----------|----------|
| Control vs. silica gel treatment| 451.845     | 528.84    | <0.0001  |
| Control vs. sodium silicate treatment | 135.761    | 158.89    | <0.0001  |
| Silica gel vs. sodium silicate treatment | 184.512    | 215.95    | <0.0001  |

The results of contrast analysis performed using the SAS software showed that the Si deposition in leaf epidermal cells in silica gel treated group was higher than sodium silicate treated ones for both kinds of silica cells (Table 4).

Fig. 4 (A to C) shows silica cells in the leaf blade of rice with and without silicon treatments. Quantitative analysis of the X-ray spectra of leaf surface are presented in Fig. 4 (D to F) corresponding to Fig. 4 (A to C) respectively to provide an integral view. As can be seen in both figures silicification of silica cells is more intensive in silicon-treated plants.

Si content of leaves. The results showed that application of silicon either as silica gel or sodium silicate significantly increased silicon content of rice leaves. The highest content...
of silicon (25.03 g/kg) was observed in plants treated with silica gel at the rate of 120 g (Table 5).

The contrast procedure indicated that silica gel treated plants had higher content of silicon in their leaves compared with the Si-deprived plants (A). (D to F): X ray spectra of A to C respectively.

Table 5. Effect of silicon treatments on Si content in rice leaves

| Treatment            | Si content of leaf (g/kg) |
|----------------------|---------------------------|
| Control              | 6.28 ± 0.53 e             |
| Silica gel 60 g/5 kg | 12.44 ± 0.28 c            |
| Silica gel 120 g/5 kg| 25.03 ± 1.06 a            |
| Silica gel 180 g/5 kg| 24.04 ± 1.02 a            |
| Sodium silicate 1 ml/l| 8.46 ± 0.28 d            |
| Sodium silicate 2 ml/l| 17.26 ± 0.62 b          |
| Sodium silicate 3 ml/l| 17.16 ± 0.64 b          |

Values are means ± standard error from three replications. Different letters in the same column denotes statistical difference at $P < 0.05$ according to Fisher's protected least significant difference (LSD).

Table 6. Comparison of Si content in leaves of control and treated groups and silica gel and sodium silicate treated groups using the contrast procedure

| Contrast                         | Mean Square | $F$ Value | $Pr > F$ |
|----------------------------------|-------------|-----------|----------|
| Control vs. silica gel treatment  | 455.253     | 308.81    | <.0001   |
| Control vs. sodium silicate treatment | 144.400     | 97.95    | <.0001   |
| Silica gel vs. sodium silicate treatment | 173.724     | 117.84   | <.0001   |
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The above results were consistent with the results based on SEM observations as well as disease severity in relation to the silicon treatments.

**Plant height and weight.** The height of inoculated plants after silicon treatments (regardless of source) did not differ significantly from plants that did not receive any silicon treatment (Table 7).

However, the shoot and root dry weights of the plants were significantly heavier in treatments receiving silica gel at the rate of 120 g (Table 7). The apparent difference in weight of treated plants was related to silicon deposition.

### Discussion

It has been reported in previous studies that application of silicon materials and silicon-based fertilizers such as potassium silicate, sodium silicate and silica gel improved growth and yield of rice plants as well as enhanced plant resistance against biotic and abiotic stresses (Belanger et al., 1995; Datnoff et al., 1997; Seebold et al., 2001). In the present study the beneficial effects conferred by two different sources of silicon were evaluated. The results of this study showed that application of silica gel and sodium silicate effectively reduced the severity and incidence of rice leaf blast. These results further confirm previous reports (Cai et al., 2008; Datnoff et al., 1997; Rodrigues and Datnoff, 2005). The results also revealed that plants treated with silica gel suffered lower levels of disease severity as compared with those treated with sodium silicate. The mechanism of enhanced resistance to disease via Si application can be associated with accumulation of silicon in leaf epidermal cells which acts as a mechanical barrier against fungal infestation (Bowen et al., 1992; Cai et al., 2008). Seebold et al. (2001) showed that Si can alleviate disease severity through blocking of fungus ingress. The documentation on silicon conferring physical resistance has also been substantiated by Kim et al. (2002) through their study of X-ray microanalysis, which demonstrated that improved levels of resistance to blast was closely related to silicon-enhanced cell wall fortification. Hayasaka et al. (2008) reported the Si-enhanced density of silicon layer in the leaf epidermis and the role of this layer as a physical barrier against fungus penetration. The results of SEM/EDX analysis in this study indicated that silicon application, regardless of product type led to a significant increase in concentration of accumulated silicon in silica cells, especially dumbbell-shaped ones which are believed to affect the mechanical properties (Yamanaka et al., 2009) of the rice leaf epidermis and resulted in the formation of Si layer with higher density as compared with the control group, and consequently increased resistance to rice blast. In the present study weight concentration of Si in plants receiving 120 g silica gel was approximately three times greater than the controls. Also plants receiving silica gel had higher accumulation of silicon as compared to sodium silicate treated ones which is consistent with the results on disease severity assessment confirming higher efficiency of silica gel in comparison to sodium silicate. Datnoff et al. (2007) reported that silicon taken up by roots was more effective in improving rice resistance to pathogens. However enhanced disease resistance cannot be explained solely by physical barrier mechanism. It has been reported that Si-induced plant resistance can be also related to increased activity of defense related enzymes such as POD and PPO as well as higher accumulation of antifungal compounds such as phytoalexins (Borel et al., 2005; Fawe et al., 1998; Rodrigues et al., 2004). Therefore, further studies are needed to clarify this phenomenon. In this investigation no significant difference was observed between silicon treated and deprived plants in terms of plant height, confirming reports by Yoshida et al. (1962). Likewise Ma and Takahashi (2002) reported positive effects of silicon supplementation on improvement in stem rigidity and increased resistance to lodging with improved plant architecture. Increased plant growth under normal and stress conditions (biotic and abiotic) via silicon application has been demonstrated in other studies (Ma, 2004; Rodrigues

| Treatment               | Height (cm)       | Shoot dry weight (g) | Root dry weight (g) |
|-------------------------|-------------------|----------------------|---------------------|
| control                 | 36.93 ± 4.21 a    | 0.90 ± 0.02 d        | 0.306 ± 0.003 d     |
| Silica gel 60 g/5 kg    | 38.43 ± 2.98 a    | 1.14 ± 0.02 c        | 0.332 ± 0.002 b     |
| Silica gel 120 g/5 kg   | 39.10 ± 1.80 a    | 1.50 ± 0.01 a        | 0.368 ± 0.005 a     |
| Silica gel 180 g/5 kg   | 40.50 ± 2.46 a    | 1.41 ± 0.01 a        | 0.364 ± 0.003 a     |
| Sodium silicate 1 ml/l  | 38.33 ± 3.03 a    | 0.98 ± 0.04 d        | 0.300 ± 0.000 d     |
| Sodium silicate 2 ml/l  | 36.86 ± 3.53 a    | 1.29 ± 0.04 b        | 0.307 ± 0.004 d     |
| Sodium silicate 3 ml/l  | 37.10 ± 3.90 a    | 1.26 ± 0.05 b        | 0.320 ± 0.002 c     |

Values are means ± standard error from three replication. Different letters in the same column denotes statistical difference at P < 0.05 according to Fisher’s protected least significant difference (LSD)
et al., 2003). In the present study shoot dry weight of rice plants was increased with Si addition. These results are in agreement with those reported by Zanao Júnior et al. (2010) where silicon application increased dry matter of rice plants and improved plant architecture. Sodium silicate treated plants were not different from the control group in terms of root dry weight, but in silica gel treated plants root dry weight was higher than in the control group, although the difference was minor. The results showed that application of silicon increased silicon content of leaves in silicon-supplied plants either with silica gel or sodium silicate. But silica gel treated plants performed better with regards to silicon content of leaves compared to sodium silicate treated plants. The positive effects of silicon on plants in almost all assessed parameters were more pronounced with the silica gel treatment rather than sodium silicate. In order to exhibit positive effects silicon should firstly be absorbed by plants (Epstein, 1994). When silicon is deposited in plant tissues it cannot be re-translocated since it is immobile. Therefore to have a promising effect on disease control, silicon must be constantly available for root uptake since the resistance of new leaves will be lost in the case of foliar applications. Liang et al. (2005) showed that foliar application of silicon reduced powdery mildew in cucumber plants through a physical barrier on leaf epidermis, and the activity of enzymes associated with host resistance against pathogen attack were induced via root application of silicon. Generally there is not a significant body of literature confirming efficient absorption of Si in plant tissues following foliar sprays. The results of the present study suggest that decreased disease severity through foliar application of Si is the result of a direct effect on the pathogen rather than one alleviated by the plant.

In conclusion rice blast disease is a problem in almost all rice cultivating areas of the world. Special attention is needed to find the appropriate method of control which in addition to being efficient and cost-effective does not cause any harm to the environment. Silicon application is a method that is consistent with environmental friendly strategies for rice blast management. Finding more efficient silicon sources is importance. Extension of knowledge on silicon to farmers and rice growers will help the agriculture industry in the management and control of rice diseases, especially blast with respect to producing safe food and environmental protection.

The findings of the present study in conjunction with earlier reports on other pathosystems (Guevel et al., 2007; Rezende et al., 2009) indicated that although both foliar and root application of Si is effective in decreasing the intensity of blast, the root application was more effective compared to the foliar application.

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