# USE OF SILICON IN CONTAINERIZED SYSTEMS AND THE MOLECULAR BASIS OF SILICON-INDUCED DISEASE RESISTANCE

By

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To Mireille Ernestine Marcelle Brunings-Stolz
In Memory of
Raoul Ernesto Brunings (16 November 1972 - 21 August 1991)
and
Ernie Adolf Brunings (20 November 1944 - 20 June 2007)

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# USE OF SILICON IN CONTAINERIZED SYSTEMS AND THE MOLECULAR BASIS OF SILICON-INDUCED DISEASE RESISTANCE

By

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Silicon benefits many monocotyledonous and some dicotyledonous plants, by increasing their resistance to fungal pathogens. For example, silicon increases the resistance of rice (*Oryza sativa* L.) to the rice blast pathogen *Magnaporthe grisea*. This study sought to add to the list of host-pathogen systems for which silicon is beneficial, and described the relationship between silicon and *M. grisea* in terms of whole-genome gene expression.

Three ornamental dicotyledonous ornamental plants, and a monocotyledonous ornamental, were evaluated for silicon uptake. The plants were grown in containerized systems with soilless medium and supplied with silicon as medium-incorporated calcium silicate, or as drench-applied potassium silicate. *Begonia* sp. and *Tagetes* sp. did not take up silicon in a rate-dependent manner, while there was weak support for a rate-dependent silicon uptake of *Impatiens* sp. The ornamental monocotyledonous plant tigergrass (*Thysanolaena maxima*) accumulated silicon in a rate-dependent manner with an estimated maximum of 1.71 % (cg/gm dry weight) silicon at an amendment level of 2.30 kg elemental silicon/$m^3$.

The fungal plant pathogen *Exserohilum rostratum* was identified as the causal agent of tigergrass leaf spot. Spray-inoculation of *E. rostratum* on tigergrass resulted in symptoms as

early as 12 hours after inoculation. Silicon amendment increased resistance of tigergrass to *E. rostratum* inoculation. The onset of disease was delayed up to two days, and the area under disease progress curve was 46 and 86 % lower in two separate experiments, even though the final disease severity did not significantly change.

By assessing gene expression patterns in the rice cultivar Monko-to using microarray technology, the physiological basis for silicon-induced resistance was investigated. Silicon amendment resulted the differential regulation of 221 genes in rice without being challenged with the pathogen. This means that silicon had an observable effect on rice metabolism, as opposed to playing a simple passive role in the resistance response of rice. Compared to control plants, silicon-amended rice differentially regulated 60% less genes, implying that silicon affects the rice response to rice blast infection at a transcriptional level.

# CHAPTER 1
# LITERATURE REVIEW

Silicon is not an essential element according to the criteria proposed by Arnon and Stout (1939) who stated that an element is not considered essential unless:

Deficiency makes it impossible for the plant to complete the vegetative or reproductive stage of its life cycle.

The deficiency is specific to the element and can only be corrected by applying the element in question.

The element is directly involved in the nutrition of the plant, apart from its possible effects in correcting some unfavorable microbiological or chemical condition of the soil or other culture medium.

However, Epstein (1999) emphasized that it is virtually impossible to exclude silicon, the second most abundant element in the Earth's crust, from field experiments; therefore silicon deficiencies were not apparent until culture in artificial nutrient solutions became common. Even then, enough silicon occurs in water, or becomes available from glass containers to make complete exclusion of silicon from the culture of crops difficult if extraordinary measures are not taken to avoid silicon "contamination." For example, Raleigh (1939) studied the role of silicon for growth of beets in asphalt-painted iron containers, and potassium silicate was purified and collected in platinum ware. Silicon can also be present as an impurity of other nutrients supplied. Even so, in practice, most plants grow normally in nutrient medium to which silicon has not been added, but those conditions are also experimental artifacts (Epstein 1999), and do not faithfully mimic the natural environment. Epstein (2001) argues that if silicon, an element that accumulates to levels as high as, or even higher than the levels of other macronutrients in plants, is not considered essential, the definition of what is an essential element is inadequate.

## Silicon in Soils

Silicon (Si; atom number 14, atomic weight 28 gm) makes up approximately 28% of the Earth's crust, second only after oxygen, which makes up about 46% (CRC handbook of Chemistry and Physics. 85th Ed. CRC Press, Boca Raton, Florida, Section 14, 2005). Consequently, silicon is a major constituent of soils. The twelve officially recognized soil orders (see Figure 1-1) contain varying amounts of Si, depending on their degree of weathering. During the weathering process many minerals newly derived from the parent material leach out of the soil profile (Jenny 1941). However, the absolute content of a particular compound is not very useful when studying leaching, because in addition to leaching out of minerals, organic material is often added to the soil. To assess leaching, the ratios of minerals are more informative.

An example is the $SiO_3/Al_2O_3$ ratio. When soil is assessed some years apart, a smaller ratio during the second assessment would indicate a relative enrichment of the soil for $Al_2O_3$, and therefore, leaching of $SiO_2$ (Brady and Weil 2002; Jenny 1941). The parent soil always has relatively more silicon than the weathered products, indicative of silicon leaching. Orders with the most highly weathered soils are usually associated with warmer and wetter climates (Brady and Weil 2002). Ultisols and Oxisols are the most weathered soils, low in plant available silicon, and mostly found in warm and wet climates (Brady and Weil 2002; Datnoff and Rodrigues 2005).

Weathering of rocks involves hydrolysis of minerals. For example, the weathering of microcline, a potassium-containing feldspar, results in the release of monosilicic acid into the soil solution and occurs in two steps.

$$KAlSi_3O_8 + H_2O \Leftrightarrow HAlSi_3O_8 + K^+ + OH^-$$

$$2HAlSi_3O_8 + 11H_2O \Leftrightarrow Al_2O_3 + 6H_4SiO_4$$

Similarly, hydrolysis of muscovite solid, results in the release of $H_4SiO_4$ into the soil solution, while hydrolysis of olivine solid, releases $SiO_2$ into solution. $SiO_2$ in turn, can react with water to form monosilicic acid, which enters the soil solution (Epstein 2001).

$SiO_2+2H_2O \Leftrightarrow H_4SiO_4$

This disassociates as follows:

$Si(OH)_4 + H_2O \Leftrightarrow (OH)_3SiO^{-1} + H_30^+$

Most soil-solutions contain 0.1-0.6 mM silicon (Epstein 1994). The effective concentration of monosilicic acid in soils depends on the presence of quartz and amorphous silica. Contrary to monosilicic acid, quartz ($SiO_2$) is highly insoluble (Epstein 2001). In the presence of quartz, the chemical reaction moves to the left, and the monosilicic acid concentration in the soil solution is about 0.1 mM. In the presence of amorphous silica (when the soil is supersaturated with silica), the concentration of monosilicic acid in the soil solution is about 1.8 mM (Epstein 2001). In soil solutions monomeric silicon is in equilibrium with polymeric silicon (Knight and Kinrade 2001). Monosilicic acid can react with aluminum, iron, manganese, and heavy metals such as cadmium, lead, zinc, and magnesium. It dissociates into $SiO_3^-$, which can replace phosphate ions adsorbed onto soil particles, thereby releasing phosphate into the soil solution. At high pH, $H_4SiO_4$ adsorption is greater. At concentrations exceeding 65 mg/L, monosilicic acid polymerizes into polysilicic acid, which is chemically inert (Daroub and Snyder 2007). Although Kinrade et al. (1999) showed that silicon readily formed complexes with sugar-like molecules in aqueous solutions, no such organosilicon compounds were known to form and remain thermodynamically stable under biologically relevant pH levels and silicon concentration until two years later (Kinrade et al. 2001). Kinrade et al. (2001) suggested that such compounds could sequester silicon in groundwater and biological fluids.

## The Silicon Cycle

The silicon cycle involves weathering of silicate rocks, which releases silicates into the soil solution. Soluble silicon can then be bound into aluminum silicates, precipitate as amorphous silica on mineral surfaces, or be taken up by the plant (Sommer 2006). Soluble monosilicic acid taken up by plants is deposited as solid amorphous silica ($SiO_2 \cdot nH_2O$) in the cell wall matrix, cell lumen, and extracellular spaces of shoots, leaf, culm, and root tissues, and in the inflorescences of grasses (Sangster et al. 2001). Neumann and De Figueiredo (2002) reported the presence of silicon in the cytoplasm of certain heavy-metal tolerant plants. Phytogenic silicon (silicon in plants) is returned to the soil by decaying plant material. Silicon can move between soil horizons, leach out (desilication), end up in waterways via runoff, and eventually ends up in the oceans, where it may be taken up by diatoms. Sedimentation returns silicon to the Earth's crust (Epstein 2001). Little is known about how much biogenic silicon contributes to silicon redistribution, or the rates, processes and driving forces of the silicon cycle (Sommer 2006).

## Silicon Uptake by the Plant

### Silicon uptake

Silicon is taken up by plants from the growing medium in the form of silicic acid ($H_4SiO_4$), an uncharged molecule (Ma and Yamaji 2006), mainly by the lateral roots (Ma et al. 2001a). Active transport was theoretically necessary since the silicon concentration of plants could not be explained by passive uptake of silicon from the soil solution (Raven 2001), and was shown to occur both passively and actively in rice (*Oryza sativa* L.), maize (*Zea mays* L.), sunflower (*Helianthus annuus* L.), wax gourd (*Benincase hispida* (Thunb.) Cogn., and cucumber (*Cucumis sativus* L.; Liang et al. 2006).

**Silicon transporters**

Ma et al. (2004) characterized the silicon uptake system of rice, and concluded that silicic acid is transported against a concentration gradient into the cortical cells, with a $K_m$ value of 0.15 mM.

A rice mutant (*lsi1*) deficient in silicon uptake, was identified with the silicon analog germanium by Ma et al. (2002). Plant roots do not discriminate between germanium and silicon, but germanium is toxic to plants. This property of germanium was used to isolate germanium-insensitive mutant rice plants. The *lsi1* gene was identified as a member of the aquaporin family (Luu and Maurel 2005), was constitutively expressed in roots, and controlled silicon accumulation in rice. Further characterization showed that an Lsi1-GFP fusion protein driven by the *lsi1* promoter localized to the plasma membrane at the distal side of the endodermis and exodermis of roots.

Further screening of the mutant rice population, yielded a second silicon transporter gene (*lsi2*). This gene encodes a putative anion transporter. A GFP gene driven by the Lsi2 promoter resulted in GFP-fusion protein localization to the proximal side of the endodermis and exodermis (Ma et al. 2007). The localization of Lsi1 and Lsi2 on opposite sides of the same cells allows silicon to get past the casparian strips which prevent indiscriminate entry of compounds into the vascular bundle (Ma et al. 2007; Ma and Yamaji 2006).

Yet another transporter gene, *lsi6*, was discovered by sequence similarity search with *lsi1* (Ma et al. 2004), but is not exclusively expressed in root tissue like *lsi1* and *lsi2*, but also in the apical meristem and elongation zone of the root tip, leaf sheaths and leaf blades (Yamaji et al. 2008). Lsi6 appears to transport silicon out of the xylem, and to be responsible for silicon distribution in the leaf.

**Silicon transport and deposition**

While silicon flows upward through the xylem at a supersaturated level, polymerization may be prevented by complexes with organic substances (Sangster and Hodson 1986). Mitani and Ma (2005) identified the form of silicon in xylem sap as monosilicic acid, and the silicon concentration in xylem sap reached as much as 18 mM. This concentration fell back to 2.6 mM after mixing water into the nutrient solution. No complexes of silicon with organic substances were identified, as had been proposed by Sangster and Hodson (1986). However, Kinrade et al. (2001; 2004) showed that it is possible for silicon to form complexes with organic compounds under conditions similar to those occurring in the soil. Silicic acid started polymerizing once the silicon concentration reached 2-2.3 mM at room temperature (Mitani and Ma 2005). The authors explained this discrepancy by proposing that the high silicon concentration was transient in the xylem, and that silicon was deposited in the apoplast once the concentration increases further due to water loss.

Deposition of silicon has been documented in the cell wall of the epidermis by X-ray analysis (Kim et al. 2002) and NMR-spectroscopy (Park et al. 2006). There is also a report of silicon presence in the cytoplasm of a number of heavy-metal tolerant plants, and *Arabidopsis thaliana* (L.) Heynh. (Neumann and De Figueiredo 2002). Kim et al. (2002) measured increasing concentrations towards the outer edge of the epidermal cell wall.

## Benefits of Silicon

Silicon has been shown to provide benefits to a variety of crops, including monocotyledons such as barley (*Hordeum vulgare* subsp. *vulgare* L.), sugarcane (*Saccharum officinarum* L.), turfgrasses, and wheat (*Triticum aestivum* L.), and dicotyledons such as cucumber (*Cucumis sativus* L.), *Arabidopsis thaliana* (L.) Heynh., grape (*Vitis vinifera* L.), strawberry (*Fragaria* x *ananassa* Duchesne), and sunflower (*Helianthus annuus* L.) (Adatia and Besford 1986; Bowen et

al. 1992; Menzies et al. 1991; Datnoff et al. 2001; Kamenidou et al. 2008; Kanto et al. 2007; Liang et al. 2005). Silicon has been demonstrated to increase yields (Datnoff et al. 1992; Seebold et al. 2000), make plants more resistant to lodging (Comhaire 1966), and increase resistance to drought (Epstein 1999). Silicon also alleviates manganese toxicity (Williams and Vlamis 1957) and aluminum toxicity (Britez 2002; Epstein 1999). In rice, it was shown that plants were more erect when there was sufficient silicon, and that the distribution of light within the canopy of the rice field was improved (Ma et al. 1989; Savant et al. 1997).

In sunflower, horticultural traits, such as stem diameter, flower number, and height were improved by silicon amendment, depending on the source of silicon and the concentration (Kamenidou et al. 2008). However, amendment with soluble silicon as potassium silicate drenches, resulted in stunted growth and deformed flowers.

Silicon decreases disease severity in many plant pathogen systems. Silicon increases rice resistance to rice blast infection (*Magnaporthe grisea* (T.T. Hebert) M.E. Barr; Volk et al. 1958). Calcium silicate reduced blast and brown spot (*Bipolaris oryzae)* on rice (Datnoff et al. 1991; 1992), suppressed gray leaf spot (*M. grisea*) on St. Augustinegrass (*Stenotaphrum secundatum* (Walter) Kuntze.; Brecht et al. 2004, 2007a), decreased gray leaf spot on perennial ryegrass turf ((*Lolium perenne* L; Nanayakkara et al. 2008), reduced downy mildew caused by *Sclerospora graminicola* on pearl millet (*Pennisetum glaucum* (L.) R.Br.; Deepak et al. 2008), and powdery mildew caused by *Sphaerotheca aphanis* on strawberry (Kanto et al. 2007).

Silicon was shown to affect general of components of resistance in different plant-pathogen systems. Kema et al. (1996) suggested that silicon increases the latent period of *Mycosphaerella graminicola*, the causal agent of leaf blotch on wheat, while Seebold et al. (2001) found that the incubation period increased with increasing rates of silicon. Examples of

silicon-amendment affecting infection efficiency include the reduction of the number of sporulating lesions per leaf area of the rice blast pathogen *M. grisea* on rice (Volk et al. 1958), a result confirmed by Seebold et al. (2001), failure of powdery mildew fungus *Erysiphe graminis* to penetrate the epidermal cells of barley (Carver et al. 1987), decrease in lesion numbers caused by *Mycosphaerella pinodes* on pea leaves (*Pisum sativum* L.; Dann and Muir 2002) and for gray leaf spot on St. Augustinegrass (Brecht et al. 2007a), reduction in the number of *Podosphaera fuliginea* colonies on cucumber leaves by 43-94% depending on the age of the leaf (Menzies et al. 1991), decrease in the number of powdery mildew (*Uncinula necator*) colonies on grape leaves (Bowen et al. 1992), and of *Diplocarpon rosae* (causal agent of black spot) on roses (*Rosa hybrida* 'Meipelta'; Gillman et al. 2003).

The area colonized and lesion size determines the effective area for fungal sporulation and is affected by silicon in a number of plant-pathogen systems. Menzies et al. (1991) and Seebold (2001) reported decreases in the size of powdery mildew colonies on cucumber by 55-99% and blast lesion length by 40-80% on rice, respectively. The success of polycyclic plant pathogens depends on their ability to sporulate effectively to spread the pathogen maximally throughout the season. *Podosphaera fuliginea* colonies on cucumber germinated less when plants were grown with added silicon (Menzies et al. 1991), and *M. grisea* produced less spores per square millimeter of lesion on silicon-amended rice (Seebold et al. 2001).

Berger et al. (1997) proposed the rate of lesion expansion as a component of resistance. This decreased by 49% when 10 t/ha calcium silicate was applied to rice, compared to the non-amended control (Seebold et al. 2001). The vertical lesion extension of sheath blight caused by *Rhizoctonia solani* was measured over time, and the resulting area under the lesion extension

progress curve decreased when the rice plants were amended with silicon (Rodrigues et al. 2001, 2003b).

## Proposed Resistance Mechanisms

Two different, but not mutually exclusive mechanisms through which silicon can enhance plant resistance to pathogen infection have been proposed. The first hypothesis proposes that silica forms a mechanical barrier which prevents fungal penetration, while the second hypothesis proposes that silicon increases plant resistance through physiological changes. Experimental evidence exists to support both.

### Mechanical barrier

Upon translocation through the xylem, silicon was deposited in the leaf epidermal cell walls (Heath 1979; Heath et al. 1992; Kim et al. 2002), and it was proposed that the silica-cuticle double layer formed an impenetrable barrier against fungal penetration. Datnoff et al. (2007) suggested however, that Kim et al. (2002) did not consider the possibility that the pressure applied by the penetration peg of the blast fungus might be enough to puncture through the silicon layer.

Bowen et al. (1992) sprayed soluble silicon on grape leaves, and found that this treatment inhibited powdery mildew development on the leaves. They suggested that the silica layer that formed on the leaf surface was responsible for forming a physical barrier against fungal penetration. They also showed that silicon was deposited within the leaves at the actual fungal penetration sites, similar to the results reported by Kunoh and Ishizaki (1975) for barley, cucumber, morning glory (*Ipomoea nil* Roth cv. Murasaki), and wheat. More recent support for the mechanical barrier hypothesis comes from research that showed that the rice blast fungal appressorium penetration was reduced at higher levels of applied silica gel (Hayasaka et al. 2008).

In an experiment where soluble silicon was made available to cucumber plants and subsequently taken away, beneficial effects of silicon discontinued, even though high levels of silicon had already accumulated in the leaves (Samuels et al. 1991). If the impenetrable barrier of silica in the epidermis was solely responsible for cucumber resistance against powdery mildew, soluble silicon would no longer have been necessary once the barrier had been formed. Therefore, an alternative theory to the mechanical barrier hypothesis described above is that silicon plays an active role in the ability of plants to withstand pathogen infection.

**Physiological modifier**

Rodrigues et al. (2003a) found that cells from rice plants amended with silicon accumulated amorphous material around the fungal hyphae penetrating the cell, and that fungal hyphae thus surrounded, often were empty. When this phenomenon was further studied, leaf extracts from silicon-amended, pathogen-inoculated plants were found to produce higher levels of phytoalexins than leaf extracts from non-amended plants (Rodrigues et al. 2004). Several gene transcripts known to be involved in a plant defense response were differentially regulated in silicon-amended, pathogen-inoculated plants (Rodrigues et al. 2005). In response to the plant pathogen *Blumeria graminis*, which causes powdery mildew on oat, an increase in the specific activity of phenylalanine ammonia lyase (PAL) was detected, and determined to be less in silicon-amended plants. This implied that silicon decreased PAL activity. Hayasaka et al. (2008) who found some evidence that supported the mechanical barrier hypothesis, also concluded that silicon imparted physiological resistance to the rice blast fungus after penetration of the fungus, because only a fraction of the penetrated appressoria which logically were not denied entry by the physical barrier, became sporulating lesions.

## Hypotheses

Mainly as a result of the difficulties in preparing silicon-free solutions, the essentiality of the element silicon to plant growth, and development is still in question. However, silicon has been established in a small number of plant-pathogen systems to effectively resist pathogen infection, but the exact role of silicon remains in dispute. There is evidence of silicon playing a role in preventing and/or inhibiting fungal penetration, and at the physiological level, but these roles still are not well defined.

In the second chapter, four ornamental plant species are tested for their ability to accumulate silicon. The hypothesis is that silicon amendment of the containerized growth medium will result in higher silicon concentration as a percentage of dry weight when the growth medium is amended with a source of silicate than plants that are grown without silicon amendment. This will result in recommendations regarding the usefulness of silicon amendment in containerized systems especially for ornamentals.

The third chapter describes the discovery of a leaf spot disease on the ornamental grass, tigergrass, and the isolation of a fungus from the disease lesions. Two hypotheses are tested: 1) the isolated fungus is the causal agent of the leaf spot disease on tigergrass; 2) silicon amendment increases the resistance of tigergrass against the leaf spot causal agent. This will identify a new pathogen on tigergrass, and study the potential of silicon to decrease the plant disease in question. Decreasing/limiting plant disease with silicon, may lead to less fungicide applications.

In the fourth chapter the effect of silicon is studied at a physiological level. The hypothesis is that silicon affects gene expression in rice without any pathogen challenge. If this were true, this would imply that silicon performs a signaling role in the plant, and might be considered essential for plant health. The second hypothesis tested in this chapter is that silicon affects the

response of the rice plant to infection with the rice blast pathogen *Magnaporthe grisea*. Such a physiological response can provide insight into the molecular mechanisms that are involved in silicon-induced plant resistance. Data derived from this study can (upon confirmation) implicate silicon-induced signaling pathways that warrant further study. Ultimately this would lead not only to a better understanding of the rice defense mechanisms, but it might also lead to new ways to induce plant resistance, by activating the defense pathways in different ways.

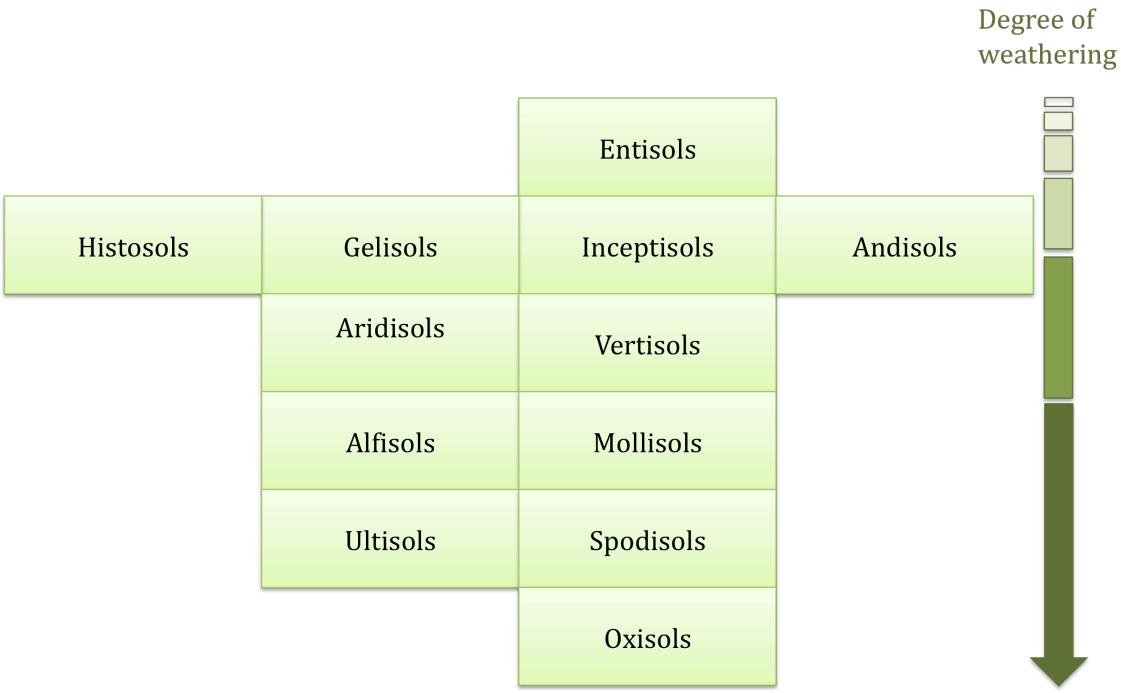


Figure 1-1 Soil orders and their general degree of weathering. Adapted from Brady and Weil (2002).

# CHAPTER 2
# SILICON AMENDMENT OF GROWING MEDIUM FOR THE CULTURE OF ORNAMENTAL DICOTYLEDONOUS PLANTS IN CONTAINERIZED SYSTEMS

## Introduction

The first indication that silicon might play a role in plant disease dates back to 1917, when Onodera found that there was less silicon in rice plants affected by rice blast disease than in healthy plants (Ishiguro 2001). Since monocotyledonous plants, especially grasses, accumulate silicon at much higher levels than dicotyledonous plants do, research on silicon accumulation has focused less on dicotyledonous plants. Plants are considered accumulators when they have more than 1% Si and a Si/Ca ratio greater than 1; intermediate accumulators contain 0.5-1% Si, or higher than 1%, but have a Si/Ca ratio smaller than 1; plants with less than 0.5% Si are non-accumulators (Ma et al. 2001b).

The number of reports of dicotyledonous plants taking up silicon–albeit at low levels–is growing. In 1939, Raleigh reported that silicon appeared to be essential for normal growth of beet (*Beta vulgaris* L.; Raleigh 1939). It became clear that the role of silicon had been underestimated when the greenhouse culture of horticultural crops in the Netherlands started using soilless medium in the 1980s (Voogt and Sonneveld 2001). When the silicon concentration of crops grown in soil was compared with that of crops grown on soilless medium, cucumber (*Cucumis sativus*), courgette (*Solanum melongena*), Heath aster (*Aster ericoïdes*), strawberry (*Fragaria* x *ananassa*), and rose (*Rosa* sp.) had lower levels of silicon when grown in soilless medium. A number of crops did not have different silicon levels, including carnation (*Dianthus caryophyllus*), gerbera (*Gerbera* sp.), lettuce (*Lactuca sativa*), and tomato (*Lycopersicon esculentum*). Frantz et al. (2005) found that New Guinea Impatiens accumulated silicon.

Takahashi and Miyake (1977) investigated the silicon concentration of different plant species grown in the same soil. They found that the average silicon concentration of

accumulators was 1.96%, while that of the non-accumulators was 0.25%. Highest accumulators were mosses and ferns among the lower plants, and monocotyledons among the higher plants. Hodson et al. (2005) studied the phylogenetic variation of silicon deposition by accumulating data from 125 studies, and found a correlation between higher-level phylogeny and silicon accumulation. Silicon uptake in dicotyledons has been reported for a number of species including cucumber (Miyake and Takahashi 1983), French bean (Heath 1979), miniature roses (Datnoff et al. 2006), New Guinea impatiens (Frantz et al. 2005), sunflower (Kamenidou 2008), and zinnia (Locke et al. 2006).

Silicon-amended plants have been reported to impart many beneficial characteristics unto plants, improving resistance against both abiotic and biotic stresses (Epstein 1994, 1999). For dicotyledons in particular, silicon appears to decrease the effects of high levels of manganese, even if the crop does not accumulate silicon (Voogt and Sonneveld 2001; Williams and Vlamis 1957). Kamenidou et al. (2008) found that silicon amendments incorporated in the soil, resulted in taller sunflower plants, while drenches with potassium silicate resulted in shorter plants, smaller flower diameter, and deformed flowers. A growing body of literature reports that silicon application increases resistance of plants to plant pathogens or disorders in dicotyledons. The grape-powdery mildew (Bowen et al. 1992), poinsettia-bract necrosis (McAvoy and Bernard 1996), rose-black spot (Gillman et al. 2003), and strawberry-powdery mildew (Voogt and Sonneveld 2001) complexes are good examples. An increased level of resistance against pathogen attack could reduce the amount and/or frequency of fungicide applications, and therefore be of great economic and environmental benefit (Alvarez et al. 2004; Seebold et al. 2004).

The goal of the experiments described in this chapter was to assess whether silicon amendment of some ornamental crops grown in containerized systems would result in higher silicon concentration per gram of dry tissue compared to non-amended plants. If such accumulation takes place, this would increase the number of plants known to accumulate silicon. Plants that accumulate silicon would then be tested for an increased ability to resist fungal pathogens in subsequent experiments. If silicon increases the ability of plants to withstand fungal infection, this might lead to a decrease in fungicide applications in nurseries and landscapes.

## Materials and Methods

Selected ornamentals were *Begonia* sp., *Impatiens* sp., and marigold (*Tagetes* sp.), and the monocotyledonous ornamental tigergrass (*Thysanolaena maxima*). For the ornamental dicotyledonous plants, the growing medium consisted of 400 ml Metro-Mix (Sun Gro Horticulture Canada, Vancouver, British Columbia) or Fafard 4P Mix soilless medium (Fafard, Agawam, MA). For the growing medium for tigergrass, 19 liter (a bucketful) of sand was mixed with 19 liter Metro-Mix or Fafard soilless medium and 100 ml Osmocote® (Scotts, Marysville, Ohio). Silicon was added in the form of Wollastonite W-20 (calcium silicate, 23.4% Si, R.T. Vanderbilt Company, Inc., Norwalk, CT), Excellerator (calcium silicate, 12.0% Si, Excell Minerals, Sarver, PA), or AgSil®25 (potassium silicate, 9.7% Si, PQ Corporation, Valley Forge, PA) at different rates up to 1.87 kg elemental silicon/$m^3$. AgSil25 was applied as a drench twice a week at the same rate of applied elemental silicon as Excellerator and Wollastonite. Control pots did not receive any amendment. All other treatments were incorporated in the soilless medium before planting.

Electrical conductivity (EC) measurements were conducted using the Field Scout® Direct Soil EC probe (Spectrum Technologies, Plainfield, IL). Before pH measurements, plants were fertilized with 1 gm/L Peters® 20-20-20 solution, 1-2 hours later ~20 ml de-ionized water was

applied to the pot and the pH of the flow-through was measured using the Ultrabasic UB-10 pH/mV meter (Denver Instrument Company, Arvada, CO).

Plants were placed into 100 mm Azalea pots (Kord Products, Toronto, Ontario, Canada) immediately after mixing the treatment with the growing medium. The first AgSil25 application was added to the pots immediately after planting. Pots were placed in a climate-controlled growth room with shelf systems and artificial lighting at levels of 4500-6000 lux (Extech Instruments Light Meter, Waltham, MA), where the temperature was maintained at 21-30°C; lights were on for 12 hours (Figures 2-1 and 2-2). AgSil25 was applied twice a week at a rate equivalent to 1.40 kg elemental silicon/$m^3$. All pots were fertilized once a week with Peters® 20-20-20 solution (The Scotts® Company LLC, Marysville, Ohio) at a nitrogen level of 200 ppm (1 gm Peter's/L).

Samples of the soilless medium were dried at 80°C for 3-4 days, passed through a #10 (2 mm) sieve and processed at the University of Florida's Everglades Research and Education Center Soil Testing Laboratory in Belle Glade for analysis of pH, and Si, Mg, and Ca concentration, at the beginning of the experiment and after harvesting of plant material at the end of the experiment. The method of analysis described below was obtained from the Soil Testing Laboratory, and were updated on July 7, 2007.

**Soil silicon measurements**

A measuring scoop was used to place 10 ml of each screened (10-mesh, 2 mm) medium sample into a 25 × 200 mm glass tube, and 25 ml 0.5 N acetic acid was added as extraction reagent. The tubes were capped and shaken to completely wet the soil, allowed to stand for 20 hours, and shaken for 50 min on end-to-end shaker. The extracts were filtered and collected.

Silicon standards of 0, 0.1, 0.2, 0.5, 0.75, and 1.0 mg/L silicon were prepared from a 1000 ppm silicon reference standard solution (Fisher Scientific, Fair Lawn, NJ), and treated the same way as the sample extracts for colorimetric measurements (Elliott and Snyder 1991). Of the extracts, 250 µl was pipetted into a 20 mL plastic scintilliation vial (Fisher Scientific, Pittsburgh, PA), 10 ml of water added, and mixed. For colorimetric analysis 150 µl of 1:1 hydrochloric acid dilution and 500 µl of 10% ammonium molybdate (adjusted to pH 7.8 with sodium hydroxide) were added, and allowed to stand for 5 minutes. Five hundred microliters of 20% tartaric acid was added, and the vials were allowed to stand for 2 min before adding 500 µl reducing agent. Reducing agent consisted of a mixture of two solutions: (A) 1.6 gm 1-amino naphtol-4-sulfonic acid and 0.8 gm sodium sulfite in 10 ml water, and (B) 100 gm sodium bisulfite in 800 ml water. After mixing the two solutions, the total volume was brought to 1000 ml with de-ionized water. Vials were allowed to stand for 5 minutes and were read within 30 minutes after adding the reducing agent. Absorbance was measured using a Brinkmann® PC910 colorimeter at a wavelength of 670 nm. A regression was done using the silicon standards to calibrate the probe. The results were calculated as mg Si/L using the following equation: (10250/250) × 2.5 × slope × absorbance.

**Leaf silicon measurements**

Leaf silicon measurement relied on the same colorimetric procedure as described for soil analysis, and was modified from Elliott and Snyder (1991). Plant tissue was dried in paper bags in a dry heat oven (Isotemp Oven, Fisher Scientific) at 80°C to constant weight (2-3 days). The dry tissue was ground using a Cyclotec™ 1093 sample mill (FOSS, Denmark), and stored in 20 ml plastic scintillation vials (Fisher Scientific, Pittsburgh, PA). From the dried, ground material, 100 mg was transferred to a 100 ml plastic high-speed polypropylene copolymer tube (Nalgene)

for digestion, the top part of which had been rinsed prior in 0.1 M NaOH solution. This minimized undigested plant material adhering near the top of the tube. Two ml of a 50% $H_2O_2$ solution were added to each tube, followed by 3 ml of 100% NaOH. The tubes were then placed for 1 hour in a 100°C water bath to initiate the tissue digestion. Then 2 ml of a 50% $H_2O_2$ were added to each tube. The tissue-containing tubes were autoclaved for 20 min, cooled to room temperature, and mixed using a vortex. If the tissue was not completely digested, an additional 2 ml of $H_2O_2$ were added and the autoclave cycle repeated. Samples typically took 4-6 rounds of $H_2O_2$ addition and autoclaving for complete digestion.

The volume of the tissue digest was adjusted to 50 ml with de-ionized water, and thoroughly mixed. Twenty ml was transferred to a 20 ml plastic scintillation vial (Fisher Scientific) for storage. For silicon concentration determination, an aliquot of 100 µl of the digested and diluted tissue were transferred to a new plastic vial, diluted 101-fold by adding 10 ml de-ionized water. One-way statistical analysis of variance (ANOVA) was performed using Statistical Analysis Software (SAS; SAS Institute, Cary, NC).

## Results

### Begonia

There was no statistically significant difference in the leaf silicon concentration of the treatments for leaves and stems (Table 2-1). Regression analysis did not support a linear curve with a slope significantly different from zero ($R^2$=0.0899), the estimated intercept for that model was 0.130. There was also no statistical support for a quadratic curve ($R^2$=0.0997). The estimated intercept for the quadratic model was 0.134, and is shown in Figure 2-3. To confirm these results, the experiment was repeated using amendment levels of 0 and 1.87 kg elemental silicon/$m^3$ (Table 2-2) to confirm the lack of difference in accumulation between control plants and plants amended with a high level of silicon. No statistically significant differences were

found in the silicon concentration of the leaves obtained from plants grown at the two different silicon levels. In all three experiments, silicon amendment of the medium did not affect the average dry weight of the silicon-amended plants compared to the control plants.

To assess whether different sources of silicon might result in accumulation of silicon in begonia, experiments were performed in which begonia was grown on shelves in a climate controlled growth room. The treatments consisted of a non-amended control, and amendment of the growing medium with Wollastonite, Excellerator, and AgSil25 to a level of 1.40 kg elemental silicon/$m^3$. The experiment was done twice and results are listed in Tables 2-3 and 2-4.

Contrary to the results with Wollastonite incorporated in the medium, begonia tissue accumulated significantly more silicon when a drench of soluble potassium silicate (AgSil25) was applied as a source of silicon in the growing medium, compared to control plants and plants amended with Excellerator or Wollastonite in both experiments. The silicon concentration of the tissue on a dry weight basis was 2.6 and 2.4-fold higher in AgSil25-treated plants compared to that of control plants for the two experiments, respectively. In the first experiment (Table 2-3) plants amended with Excellerator and Wollastonite had a lower numerical silicon concentration than control plants, while there was no statistically significant difference between these treatments in the second experiment.

The medium amended with AgSil25 has a pH of 8.69 at the end of the experiment, which is significantly higher than that of medium amended with Excellerator or Wollastonite. The pH of all the silicon-amendment treatments was higher than that of the control. AgSil25-amended medium also had a higher EC than silicon-amended media. Most remarkably, the dry weight of AgSil25-amended plants was less than 20% of the dry weight of control plants in both

experiments (Table 2-4). AgSil25-amended plants were very small compared to the other plants (Figure 2-4), which is also obvious from the dry weight measurements (Tables 2-3 and 2-4).

**Impatiens**

Impatiens Super Elfin White (Speedling, Sun City, FL) was grown using Wollastonite at 0, 0.47, 0.94, and 1.87 kg elemental silicon/$m^3$ in the greenhouse. The silicon concentration in the dried tissue was determined after 4 weeks of growth (Table 2-5). No significant difference was found between any of the treatments. Silicon treatment also had no effect on the dry weight of impatiens leaves. Regression analysis was performed with both a linear and a quadratic model. In the linear model there was no statistical support for a slope different from zero ($R^2$=0.1251), with an estimated intercept at 0.1909. With the quadratic model, a curve was fitted ($R^2$=0.3013) shown in Figure 2-5, with the formula:

$$y = -0.1111 + 0.2676 x + 0.1424$$

Where y is the silicon concentration (cg/gm) and x is the silicon amendment in kg elemental silicon per hectare. The estimated curve has a maximum silicon concentration of 0.30 at an amendment of 1.2 kg elemental silicon/$m^3$.

To confirm these results, impatiens was grown in medium amended with Wollastonite at 0 and 1.87 kg elemental silicon/$m^3$ in 3 additional experiments. The differences in the silicon concentrations of the impatiens leaves were not statistically significant (Table 2-6). Dry weight of the leaves was significantly different in the third experiment, with Wollastonite-amended plants being 46% smaller than those of control plants. Comparing the three experiments, the dry weight and level of silicon accumulation of the plants in the first experiment was smaller than in the second and third experiments.

To test whether alternative sources of silicon might result in significant silicon accumulation in impatiens, experiments were performed using AgSil25, Excellerator, and

Wollastonite as silicon sources (Tables 2-7 and 2-8). In these experiments, impatiens Super Elfin Lipstick plants grown in medium amended with silicon were significantly smaller than the control plants, especially when amended with AgSil25 (Figure 2-6, panel D). The pH and EC AgSil25-amended medium were significantly higher than that of control plants in both experiments.

In the experiments where different sources of silicon were used (Tables 2-7 and 2-8), the cultivar of impatiens used was Super Elfin Lipstick, and the silicon percentage of dry weight was higher than in the experiments where different amounts of Wollastonite were added (Tables 2-5 and 2-6) to the impatiens cultivar Super Elfin White.

Although the silicon concentration of Wollastonite-amended plants was 43% higher than that of control plants in the first experiment (Table 2-7), this difference was not statistically significant. By contrast, in the second experiment, the 24% increase in silicon concentration of Excellerator and Wollastonite-amended leaves compared to control leaves was statistically significant. Dry weights of Excellerator and Wollastonite-amended plants were 45 and 36% smaller than control plants respectively in the first experiment (Table 2-7), while the dry weight of AgSil25 amended plants was 69% lower than that of control plants, and 43 and 52 % lower than that of Excellerator and Wollastonite-amended plants. Smaller Excellerator and AgSil25-amended plants can be seen in Figure 2-6, panels C and D, which also shows that plants from both these treatments were also chlorotic. In the second experiment (Table 2-8) all the AgSil25-amended plants died, and the silicon concentration could not be analyzed for this treatment. The dry weight of the Excellerator and Wollastonite-amended plants was no different from that of the control plants.

**Marigold**

Marigold plants were grown for 4 weeks in 100 mm pots (Kord Products, Toronto, Ontario, Canada) under greenhouse conditions in growing medium amended with the equivalent of 0, 0.94, and 1.87 kg elemental silicon/$m^3$. As shown in Table 2-9, there was no statistically significant difference in silicon concentration in the leaves among the different silicon levels. There was no difference between the dry weight of leaves from silicon-amended and control plants. Regression was performed for a linear and a quadratic model. A linear model resulted in a slope not significantly different from zero, and an intercept of 0.204 ($R^2$=0.1756). The quadratic model also predicted parameters not significantly different from zero, with an estimated intercept at 0.215 ($R^2$=0.2791, shown in Figure 2-7).

This experiment was repeated twice with only levels of 0 and 1.87 kg elemental silicon/$m^3$. The results are listed in Table 2-10. In the first experiment, there was no significant difference between silicon-amended and control plants, and no difference in silicon concentration. In the second experiment, dry weight of leaves from plants amended with Wollastonite was 25% lower than control leaves. There was no difference in the silicon concentration of leaves amended with silicon compared to the control. In contrast to the results in Table 2-9, the silicon concentration of leaves grown with amendment levels of 1.87 kg elemental silicon/$m^3$ was not significantly lower than control plants, and was similar in both experiments.

Alternative sources of silicon were tested in two experiments to test whether different silicon sources might result in silicon accumulation in marigold. AgSil25, Excellerator and Wollastonite were all applied at a rate equivalent to 1.40 kg elemental silicon/$m^3$. In both experiments (Table 2-11 and Table 2-12), AgSil25-amended pots had very high pH (means of 8.87 and 9.03).

In the first experiment (Table 2-11) only one plant amended with AgSil25 survived, and it remained very small (Figure 2-8). The dry weights of AgSil25, Excellerator, and Wollastonite-amended leaves were 51%, 44%, and 44% less than that of control plants. The silicon concentration of plants grown in medium amended with Excellerator and Wollastonite in the first experiment (Table 2-11) was actually lower than that of the control plants, except for the single plant grown on medium amended with AgSil25, which survived and had a higher silicon concentration than both the control and Excellerator and Wollastonite-amended plants. In the second experiment (Table 2-12) none of the AgSil25-amended plants survived, and thus no silicon concentration could be determined for these plants. No statistically significant differences were found in the dry weight of leaves from control plants compared to the silicon-amended plants, and similarly, no difference was found in the silicon concentration of the leaves.

**Tigergrass**

Accumulation of silicon was tested in tigergrass using the equivalent of 0, 0.47, 0.94, and 1.87 kg elemental silicon/$m^3$ added to the growing medium. The experiment was repeated twice, results are reported in Tables 2-13 and 2-14, and the rate response curves are in Figure 2-9 and 2-10 respectively. There was a statistically significant uptake of silicon for the different rates of silicon applied in both experiments. The dry weight of plants amended with 1.87 kg elemental silicon/$m^3$ was lower than control plants in both experiments. The silicon concentration of tigergrass amended with 1.87 kg elemental silicon/$m^3$ is 2.5-fold greater than the silicon concentration of control plants in the first experiment, and 2.9-fold greater in the second experiment.

Linear and quadratic regression analysis was performed for both experiments. For the first experiment, linear regression estimated a slope of 0.2553, and an intercept of 0.4030 ($R^2$=0.7244). The quadratic model was a better fit than the linear model ($R^2$=0.7990), with a

maximum of 0.84% at an amendment rate of 1.81 kg elemental silicon/$m^3$ (Figure 2-9). The formula with estimated parameters is:

$$y=-.1527\ x^2 + 0.5520\ x + 0.3395$$

In this formula y is silicon concentration (%, cg/gm) and x is the silicon amendment (kg elemental silicon/$m^3$). In the second experiment, the linear model estimated a slope of 0.5752, and an intercept of 0.6653 ($R^2$=0.9016), but the quadratic model was again a better fit ($R^2$=0.9438). This model estimated the maximum silicon concentration at 1.71 cg/gm at a silicon amendment of 2.30 kg elemental silicon/$m^3$. The corresponding formula is:

$$y=-0.2139\ x^2 + 0.9861\ x + 0.5720$$

Different sources of silicon were tested for their effect on tigergrass silicon concentration. AgSil25, Excellerator, and Wollastonite were used at rates equivalent to 1.40 kg elemental silicon/$m^3$. This experiment was performed only once and the results are listed in Table 2-15.

Plants amended with Excellerator and Wollastonite had 4.9 and 4.4 times the percent silicon relative to control plants. Plants amended with AgSil25 had 2.1 times the amount of silicon as control plants, but this difference was not significant (Table 2-15). There was no significant difference in the dry weight of the plants from the different treatments.

## Discussion

According to the definition of silicon non-accumulators by Ma et al. (2001b) begonia is not an accumulator of silicon. The level of silicon as a percentage of dry weight varied between experiments, but was typically between 0.2 and 0.3 cg/gm, and did not exceed 0.4 cg/gm when silicon was incorporated in the medium as Excellerator or Wollastonite. In the experiment performed with different rates of silicon amendment, regression analysis did not support a linear or quadratic curve with parameters different from zero, so there was no rate response. A higher silicon concentration (0.82 cg/gm and 0.89 cg/gm for the two experiments) was noted when

soluble potassium silicate (AgSil25) was applied as a drench. However, plants treated with AgSil25 performed very poorly, grew very little, and often died (Figure 2-4). Amendment with AgSil25 increased the pH to 8.86 and 8.69 in the two experiments, which may cause serious micronutrient deficiencies. In addition, the EC was very high. A likely cause is the failure of the plants to grow, as a result of which the plants did not take up nutrients from the medium. These nutrients then accumulated over the course of the experiment. These results indicate that the criteria used to categorize silicon accumulators apart from non-accumulators need to be placed in the context of a "marketable plant". Clearly, AgSil25 as applied in this study does not result in a marketable plant.

Because AgSil25 is a liquid source of soluble silicon is immediately available to the plant, as opposed to the granular forms of silicon amendment (Excellerator and Wollastonite), it might be possible to apply less AgSil25 to the pots, limiting the increase in pH. This could shed light on begonia's ability to uptake silicon. Under the conditions of the experiments described in this study, amendment of soilless medium with silicon does not result in a statistically significant increase in the silicon concentration of begonia. The use of AgSil25 as described in this study cannot be recommended to ornamental plant growers as it results in too high a pH to be of practical use.

Impatiens 'Super Elfin White' grown on soilless medium amended with Wollastonite at rates of 0 to 1.87 kg elemental silicon/$m^3$ did not accumulate silicon, even though silicon concentration was 1.8-2 times the amount in control plants. Regression using a linear and a quadratic model, resulted in a better fit for a quadratic curve, weakly supporting the hypothesis thatimpatiens had a rate response to silicon amendment. In subsequent experiments, comparing amendments of 0 and 1.87 kg elemental silicon/$m^3$, the numerical difference between the

treatment means was much smaller. Using different sources of silicon for the soil amendment at a rate equivalent to 1.40 kg elemental silicon/$m^3$, higher levels of silicon in plant tissue were measured. Plants grown in medium amended with AgSil25, Excellerator, and Wollastonite had 63%, 14%, and 43% more silicon than the control, respectively, in the first experiment but these differences were not statistically significant. In the second experiment, however, Excellerator and Wollastonite-amended impatiens 'Super Elfin Lipstick' had 24% more silicon on a dry weight basis than control plants, and this difference was statistically significant. Although most of the experiments carried out in this study showed no statistically significant differences, it cannot be ruled out that impatiens accumulates silicon. Possibly, experiments with a larger number of replicates might result in more consistent results.

Frantz et al. (2005) reported that New Guinea impatiens (cv. 'Pure Beauty Purple') grown in soilless medium amended with 2.0 mM potassium silicate were stiffer to the touch, and had sharper serrated edges than control plants that did not receive potassium silicate. When the leaves were analyzed with scanning electron microscopy and energy dispersive X-ray analysis, they were found to have silicon "scales" on the serrated edges possibly located near hydathodes. The percentage of silicon as a percentage of dry weight was not reported in that study. It is possible that impatiens accumulates silicon, but the amount was so small that it was not (consistently) measurable under the conditions used in this study. Another possibility, which was observed for all three ornamentals in this study, is that these plants need very small amounts of silicon and that enough silicon was supplied with the soilless medium and/or irrigation water that additional silicon amendment does not result in an increase in silicon concentration. It is extremely difficult to keep experiments silicon free (Epstein 1994, 1999), since uncharged silicic acid passes through ion-exchangers; even de-ionized water has some silicon (Epstein 1999).

As with begonia, AgSil25 was not likely to be a practical source of silicon for impatiens under the conditions used in this experiment. Plants typically remained very small (Figure 2-6, panel D), and often died; those that were alive were chlorotic. Impatiens appears to be more sensitive to increases in pH than begonia. Begonia did not show obvious changes in appearance or chlorosis when the medium was amended with Excellerator (Figure 2-4, panel C) while impatiens did (Figure 2-6, panel C). Both plant species showed decreased growth and chlorosis when the medium was amended with AgSil25.

When marigold was grown in soilless medium amended with Wollastonite at rates from 0 to 1.87 kg elemental silicon/$m^3$, plants amended with 0.94 and 1.87 kg elemental silicon/$m^3$ actually had lower amounts of silicon per dry weight in the leaves, but the difference was not statistically significant. There was no rate response in marigold, since no slope estimated with linear regression differed significantly from zero. In two separate experiments where Wollastonite was applied at rates equivalent to 0 and 1.87 kg elemental silicon/$m^3$, the amount of silicon as a percentage of dry weight was similar for both treatments and experiments (Table 2-10), with levels of 0.22 and 0.23 cg/gm silicon.

Using different sources of silicon for medium amendment, the mean pH of pots amended with AgSil25 was as high as 8.87 and 9.03 in two experiments (Table 2-12 and 2-12). At a pH greater than 9, silicic acid ($Si(OH)_4$) disassociates into silicate ion ($(OH)_3(SiO^{-1})$) and becomes unavailable to the plant. As with begonia and impatiens, using AgSil25 under the conditions described in this study is impractical, but it might be possible to lower the concentration of potassium silicate applied in the form of AgSil25 to decrease the pH and still measure a difference in silicon concentration.

For begonia and marigold, amendment of the soilless growing medium with Wollastonite and Excellerator does not result in increased silicon concentration of the leaf tissue. It is possible that different results can be obtained with AgSil25 if the application rate is changed. There is weak support for impatiens accumulating silicon in a rate-dependent manner, following a quadratic relationship. Future research could implement strategies to maintain a better pH balance for all silicon-amended treatments as was done by adding $HNO_3$ and decreasing $KNO_3$ by Voogt and Sonneveld (2001) and by adding $H_2SO_4$ by Frantz et al. (2005), although it is questionable whether that would be practical from a grower's point of view.

It is possible that there are differences in silicon uptake from one cultivar to another. Neither Super Elfin White impatiens nor Super Elfin Lipstick impatiens showed significant difference in silicon uptake, but the latter did have higher levels of silicon that the Super Elfin White. In addition, since disease susceptibility of the plants used in this study was not tested, it cannot be ruled out that plants that do not have measurable increases in silicon concentration benefit from silicon-amendment of the growing medium. From the examples seen in this study it is clear that such a process would require re-designing the fertilization to offset the increase in pH caused by silicates.

Another possibility is that the ornamental plants used in this study do not accumulate silicon to a level significantly different from that of control plants, while still benefiting from the amendment in terms of increased disease resistance. According to the criteria used by Ma et al. (2001b), sunflower would not be considered an accumulator, but Kamenidou et al. (2008) did determine that the level of silicon in silicon-amended plants was statistically higher than that of control plants, and that silicon-amended sunflowers were taller than control plants. In addition, when the medium was amended with soluble silicon (potassium silicate), the plants were shorter,

had a smaller flower diameter, and deformed flowers, indicating that potassium silicate is not a practical way of amending the medium for sunflowers. In zinnia, silicon amendment resulted in a delayed powdery mildew symptom development (Locke et al. 2006). This means that it is possible for plants to benefit from silicon even if it is not considered an accumulator based on the silicon concentration of the plants tissue.

The ornamental grass tigergrass, accumulated silicon and generated a silicon rate-response curve similar those seen for turfgrasses (Datnoff et al. 2007; Datnoff and Nagata 1999; Datnoff and Rutherford 2003; Nanayakkara et al. 2008ab). In both experiments, the rate response curve fit a quadratic model. Since tigergrass belongs to the family Poaceae, this is no surprise. With regard to the different sources of silicon used, Excellerator and Wollastonite resulted in higher levels of silicon in the leaves compared to leaves of control and AgSil25-amended plants. No significant differences in mean dry weight were seen between the silicon-amended plants and the control plants, not even for AgSil25, indicating that tigergrass can better tolerate AgSil25 and a high medium pH than the ornamental dicotyledons used in this study.

In conclusion, begonia and marigold did not accumulate silicon, while there was weak statistical support for silicon accumulation in impatiens. Tigergrass accumulated silicon in a rate-dependent manner, and was used in subsequent experiments to test the hypothesis that silicon accumulation caused an increase in resistance against a fungal pathogen.

Table 2-1 Effect of silicon rate on percent dry weight accumulation of silicon in begonia

| Silicon amendment (kg elemental silicon/$m^3$) | Si (%, cg/gm)[x] | |
|---|---|---|
| | Leaves | Stems |
| 0 | 0.13 a[y] | 0.15 a |
| 0.94 | 0.14 a | 0.21 a |
| 1.87 | 0.18 a | 0.17 a |

[x]Silicon concentration of *Begonia* sp. leaves and stems as a percentage of dry weight.
[y]Means in each column followed by different letters are significantly different based on Fisher's protected LSD ($\alpha$=0.05).

Table 2-2 Dry weight and silicon concentration of begonia leaves grown at 0 and 1.87 kg elemental silicon/$m^3$

| Amendment (kg elemental silicon/$m^3$) | Experiment 1 | | Experiment 2 | |
|---|---|---|---|---|
| | Leaf dry weight (gm) | Si (cg/gm)[x] | Leaf dry weight (gm) | Si (cg/gm)[x] |
| 0 | 0.90 a[y] | 0.39 a | 0.77 a | 0.29 a |
| 1.87 | 0.52 a | 0.48 a | 0.52 a | 0.22 a |

[x]Silicon concentration of *Begonia* sp. leaves as a percentage of dry weight.
[y]Means in each column followed by different letters are significantly different based on Fisher's protected LSD ($\alpha$=0.05).

Table 2-3 Effect of different silicon sources applied at a rate of 1.40 kg elemental silicon/$m^3$ on growing medium pH, EC, and begonia dry weight and silicon concentration (first experiment)

| Treatment | pH | EC (mS/cm) | Leaf dry weight (gm) | Si (cg/gm)[x] |
|---|---|---|---|---|
| Control | 6.35 c[y] | 1.95 b | 0.41 a | 0.32 b |
| AgSil25 | 8.86 a | 4.81 a | 0.12 b | 0.82 a |
| Excellerator | 7.89 b | 2.75 b | 0.34 a | 0.19 c |
| Wollastonite | 7.60 b | 2.02 b | 0.41 a | 0.22 c |

[x]Silicon concentration of *Begonia* sp. leaves as a percentage of dry weight.
[y]Means in each column followed by different letters are significantly different based on Fisher's protected LSD ($\alpha$=0.05).

Table 2-4 Effect of different silicon sources applied at a rate of 1.40 kg elemental silicon/$m^3$ on growing medium pH, EC, and begonia dry weight and silicon concentration (second experiment)

| Treatment | pH | EC (mS/cm) | Leaf dry weight (gm) | Si (cg/gm)[x] |
|---|---|---|---|---|
| Control | 5.85 c | 2.01 b | 0.87 a | 0.37 b |
| AgSil25 | 8.69 a | 3.86 a | 0.17 b | 0.89 a |
| Excellerator | 7.53 b | 1.98 b | 0.63 a | 0.40 b |
| Wollastonite | 7.46 b | 1.77 b | 0.78 a | 0.33 b |

[x]Silicon concentration of *Begonia* sp. leaves as a percentage of dry weight.
[y]Means in each column followed by different letters are significantly different based on Fisher's protected LSD ($\alpha$=0.05).

Table 2-5 Silicon concentration of impatiens leaves grown in medium amended with different levels of Wollastonite

| Treatment (kg elemental silicon/$m^3$) | pH | EC (mS/cm) | Leaf dry weight (gm) | Si (cg/gm)[x] |
|---|---|---|---|---|
| 0 | 5.6 b[y] | 1.83 ab | 0.61 a | 0.14 a |
| 0.47 | 6.9 a | 1.67 b | 0.60 a | 0.26 a |
| 0.94 | 7.1 a | 2.09 a | 0.56 a | 0.28 a |
| 1.87 | 7.2 a | 1.66 b | 0.55 a | 0.26 a |

[x]Silicon concentration of *Impatiens* sp. leaves as a percentage of dry weight.
[y]Means in each column followed by different letters are significantly different based on Fisher's protected LSD ($\alpha$=0.05).

Table 2-6 Silicon concentration of impatiens leaves grown at 0 and 1.87 kg elemental silicon/$m^3$

| Amendment (kg elemental silicon/$m^3$) | Experiment 1 | | Experiment 2 | | Experiment 3 | |
|---|---|---|---|---|---|---|
| | Dry weight (gm) | Si (%)[x] | Dry weight (gm) | Si (cg/gm) | Dry Weight (gm) | Si (cg/gm) |
| 0 | 0.54 a[y] | 0.14 a | 1.0 a | 0.27 a | 1.3 a | 0.27 a |
| 1.87 | 0.57 a | 0.15 a | 0.9 a | 0.24 a | 0.7 b | 0.26 a |

[x]Silicon concentration of *Impatiens* sp. leaves as a percentage of dry weight.
[y]Means in each column followed by different letters are significantly different based on Fisher's protected LSD ($\alpha$=0.05)

Table 2-7 Silicon concentration in impatiens grown in medium amended with different sources of silicon at a rate of 1.40 kg elemental silicon/$m^3$ (first experiment)

| Treatment | pH | EC (mS/cm) | Leaf dry weight (gm) | Si (cg/gm)[x] |
|---|---|---|---|---|
| Control | 6.2 c[y] | 1.78 c | 0.42 ab | 0.42 a |
| AgSil25 | 8.8 a | 4.15 a | 0.13 c | 0.57 a |
| Excellerator | 8.1 b | 2.48 b | 0.23 bc | 0.48 a |
| Wollastonite | 7.8 b | 2.21 bc | 0.27 ab | 0.60 a |

[x]Silicon concentration of *Impatiens* sp. leaves as a percentage of dry weight.
[y]Means in each column followed by different letters are significantly different based on Fisher's protected LSD ($\alpha$=0.05)

Table 2-8 Silicon concentration in impatiens grown in medium amended with different sources of silicon at a rate of 1.40 kg elemental silicon/$m^3$ (second experiment)

| Treatment | pH | EC (mS/cm) | Leaf dry weight (gm) | Si (cg/gm)[x] |
|---|---|---|---|---|
| Control | 6.0 c[y] | 2.17 b | 0.45 a | 0.46 b |
| AgSil25 | 8.0 a | 3.20 a | not done[z] | not done[z] |
| Excellerator | 7.5 b | 2.35 b | 0.38 a | 0.57 a |
| Wollastonite | 7.3 b | 2.34 b | 0.39 a | 0.57 a |

[x]Silicon concentration of *Impatiens* sp. leaves as a percentage of dry weight.
[y]Means in each column followed by different letters are significantly different based on Fisher's protected LSD ($\alpha$=0.05).
[z]All plants treated with AgSil25 died in this experiment.

Table 2-9 Silicon concentration in the leaves of marigold grown in pots amended with different levels of silicon applied as Wollastonite

| Treatment (kg elemental silicon/$m^3$) | pH | EC (mS/cm) | Leaf dry weight (gm) | Si (cg/gm)[x] |
|---|---|---|---|---|
| 0 | 6.8 a[y] | 1.74 a | 0.45 a | 0.21 a |
| 0.94 | 7.0 a | 1.68 a | 0.45 a | 0.15 a |
| 1.87 | 6.9 a | 2.40 a | 0.48 a | 0.16 a |

[x]Silicon concentration of *Tagetes* sp. leaves as a percentage of dry weight.
[y]Means in each column followed by different letters are significantly different based on Fisher's protected LSD ($\alpha$=0.05).

Table 2-10 Dry weight and silicon concentration of marigold leaves grown at 0 and 1.87 kg elemental silicon/$m^3$

| Amendment (kg elemental silicon/$m^3$) | Experiment 1 | | Experiment 2 | |
|---|---|---|---|---|
| | Leaf dry weight (gm) | Si (cg/gm)[x] | Leaf dry weight (gm) | Si (cg/gm)[x] |
| 0 | 1.94 a[y] | 0.22 a | 2.06 a | 0.23 a |
| 1.87 | 1.64 a | 0.23 a | 1.54 b | 0.22 a |

[x]Silicon concentration of *Tagetes* sp. leaves as a percentage of dry weight.
[y]Means in each column followed by different letters are significantly different based on Fisher's protected LSD ($\alpha$=0.05).

Table 2-11 Silicon concentration of marigold amended with different sources of silicon applied at 1.40 kg elemental silicon/$m^3$ (first experiment)

| Treatment | pH | EC (mS/cm) | Leaf dry weight (gm) | Si (cg/gm)[x] |
|---|---|---|---|---|
| Control | 6.4 d[y] | 1.85 a | 0.41 a | 0.20 b |
| AgSil25 | 8.9 a | 5.51 b | 0.20 b | 0.29 a |
| Excellerator | 8.1 b | 1.71 a | 0.23 b | 0.17 bc |
| Wollastonite | 7.8 c | 2.19 a | 0.23 b | 0.12 c |

[x]Silicon concentration of *Tagetes* sp. leaves as a percentage of dry weight.
[y]Means in each column followed by different letters are significantly different based on Fisher's protected LSD ($\alpha$=0.05).

Table 2-12 Silicon concentration of leaf tissue of marigold amended with different sources of silicon applied at 1.40 kg silicon/$m^3$ (second experiment)

| Treatment | pH | EC (mS/cm) | Leaf dry weight (gm) | Si (cg/gm)[x] |
|---|---|---|---|---|
| Control | 6.0 c | 1.97 b | 0.37 a | 0.28 a |
| AgSil25 | 9.0 a | 2.63 a | - | - |
| Excellerator | 7.3 b | 2.15 ab | 0.26 a | 0.30 a |
| Wollastonite | 7.2 b | 1.71 b | 0.33 a | 0.28 a |

[x]Silicon concentration of *Tagetes* sp. leaves as a percentage of dry weight.
[y]Means in each column followed by different letters are significantly different based on Fisher's protected LSD ($\alpha$=0.05).

Table 2-13 Silicon concentration of tigergrass leaves for different levels of medium amendment with Wollastonite (first experiment)

| Treatment (kg elemental silicon/$m^3$) | pH | EC (mS/cm) | Dry weight (gm) | Si (cg/gm)[x] |
|---|---|---|---|---|
| 0 | 6.2 b[y] | 2.02 ab | 2.92 a | 0.34 c |
| 0.47 | 6.8 ab | 1.22 ab | 2.78 ab | 0.55 b |
| 0.94 | 7.0 a | 2.64 a | 2.16 ab | 0.74 ab |
| 1.87 | 7.2 a | 2.64 a | 1.78 b | 0.84 a |

[x]Silicon concentration of tigergrass leaves as a percentage of dry weight.
[y]Means in each column followed by different letters are significantly different based on Fisher's protected LSD ($\alpha$=0.05).

Table 2-14 Silicon concentration of tigergrass leaves for different levels of medium amendment with Wollastonite (second experiment)

| Treatment (kg elemental silicon/$m^3$) | pH | EC (mS/cm) | Dry weight (gm) | Si (cg/gm)[x] |
|---|---|---|---|---|
| 0 | 6.0 c | 2.10 a | 2.3 ab | 0.57 d |
| 0.47 | 6.3 b | 2.10 a | 2.6 ab | 1.04 c |
| 0.94 | 6.4 b | 1.90 a | 2.9 a | 1.32 b |
| 1.87 | 7.3 a | 1.93 a | 1.4 b | 1.67 a |

[x]Silicon concentration of tigergrass leaves as a percentage of dry weight.
[y]Means in each column followed by different letters are significantly different based on Fisher's protected LSD ($\alpha$=0.05).

Table 2-15 Silicon accumulation of tigergrass grown with different sources of silicon at a rate equivalent to 1.40 kg elemental silicon/$m^3$

| Treatment | pH | EC (mS/cm) | Dry weight (gm) | Si (cg/gm)[x] |
|---|---|---|---|---|
| Control | 6.0 b[y] | 1.49 b | 0.50 a | 0.22 b |
| AgSil25 | 7.00 a | 2.89 a | 0.48 a | 0.47 b |
| Excellerator | 7.2 a | 1.70 b | 0.69 a | 0.96 a |
| Wollastonite | 6.6 a | 1.97 b | 0.69 a | 1.08 a |

[x]Silicon concentration of tigergrass leaves as a percentage of dry weight.
[y]Means in each column followed by different letters are significantly different based on Fisher's protected LSD ($\alpha$=0.05).

Figure 2-1 Experimental setup for marigolds in the greenhouse.


Figure 2-2 Experimental setup of begonia, marigold, and impatiens containers in the climate-controlled growth room with fluorescent lighting.

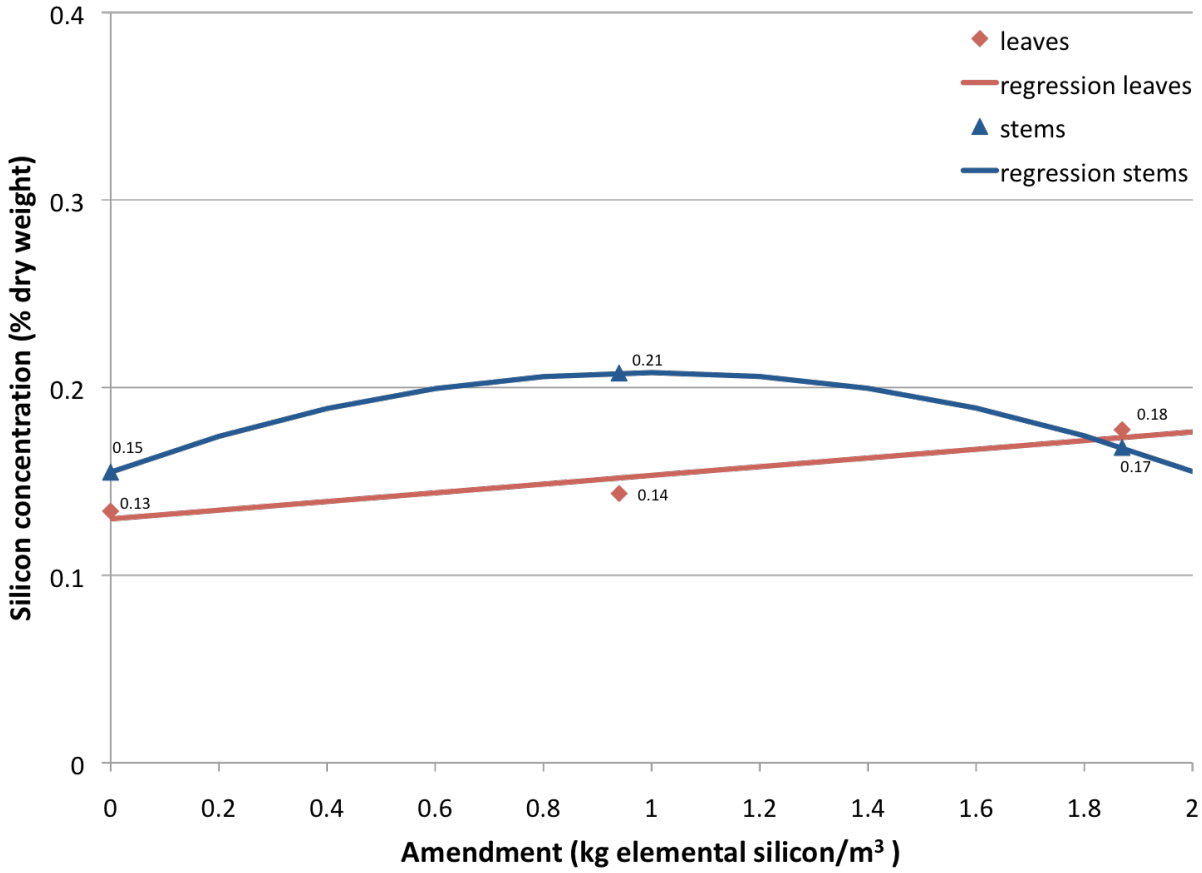


Figure 2-3 Silicon concentration of begonia leaves and stems after four weeks of growth on different levels of silicon amendment. The solid lines represent the regression curves $y= 0.0232 x + 0.13$ ($P=0.2775$, $R^2=0.0997$, $n=5$) for leaves, and $y= -0.0529 x^2 + 0.106 x + 0.1549$ for stems ($P=0.7595$, $R^2=0.1262$, $n=5$). The blue triangles represent the mean silicon concentration of stems, and the red squares represent the mean silicon concentration of leaves.

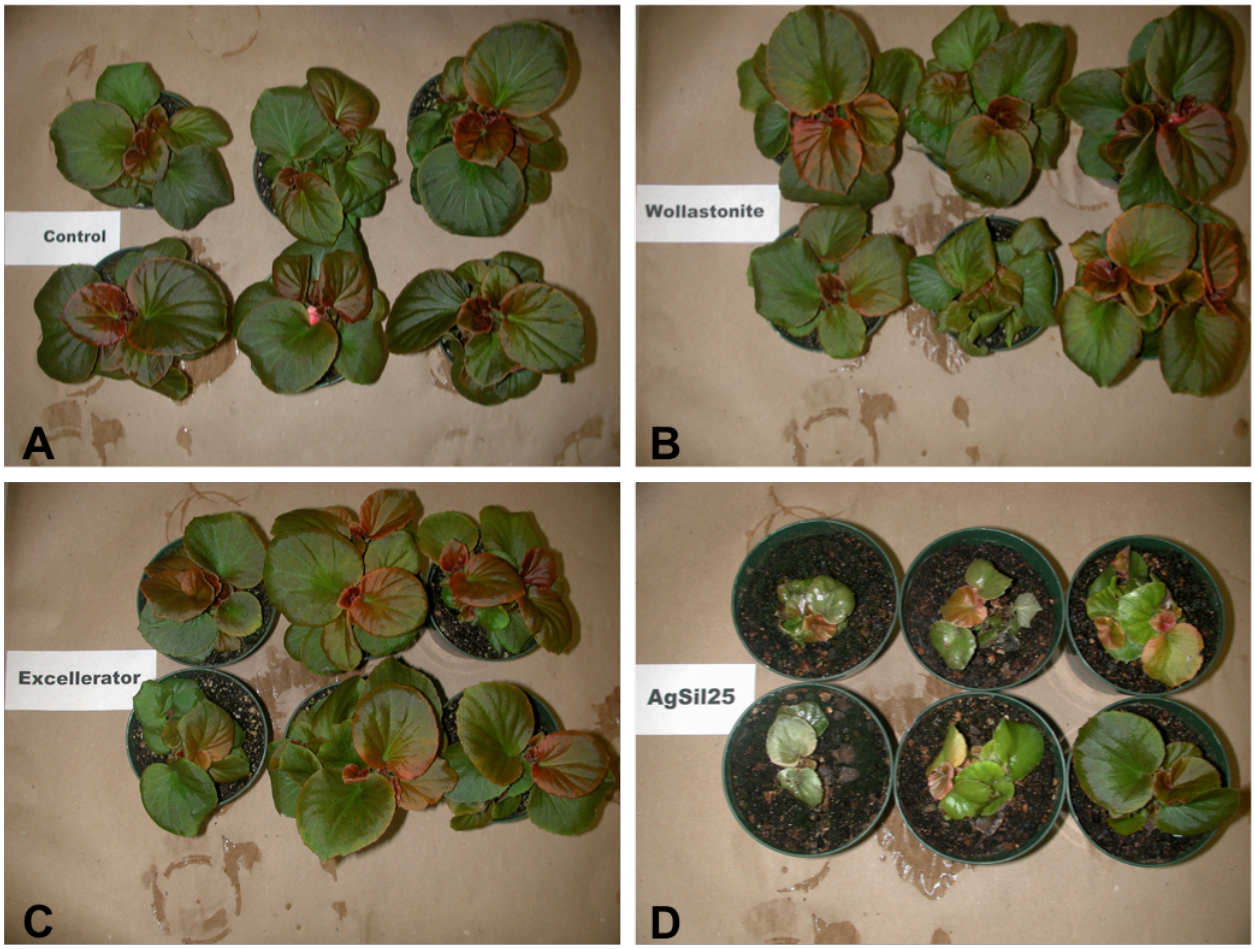


Figure 2-4 Begonia plants treated with different sources of silicon after four weeks of growth. A. Non-amended control, B. Wollastonite, C. Excellerator, D. AgSil25.

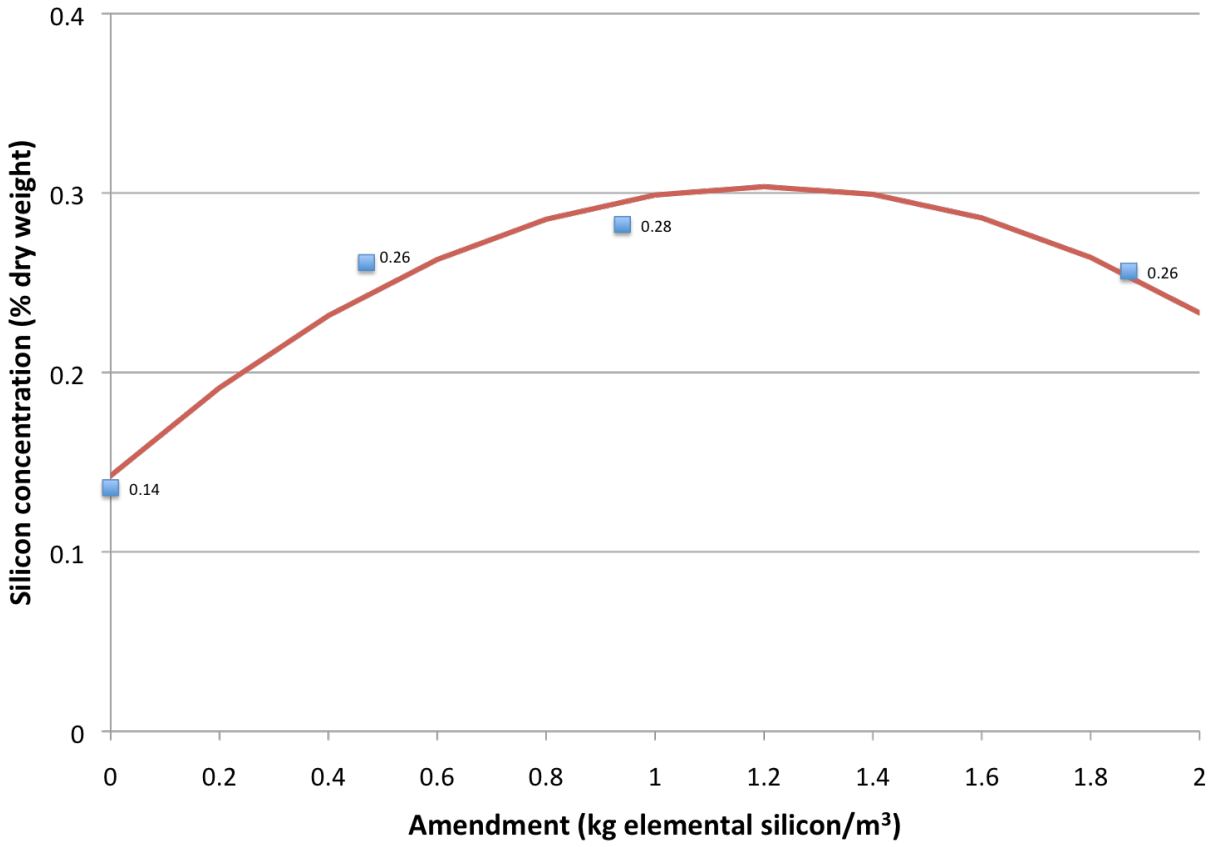


Figure 2-5 Silicon concentration of impatiens leaves and stems after four weeks of growth on different levels of silicon amendment. The solid line represents the regression curve $y= -0.1111\ x^2 + 0.2676\ x + 0.1424$ ($P=0.0475$, $R^2=0.3013$, $n=5$). The blue squares represent the mean silicon concentration of impatiens leaves.

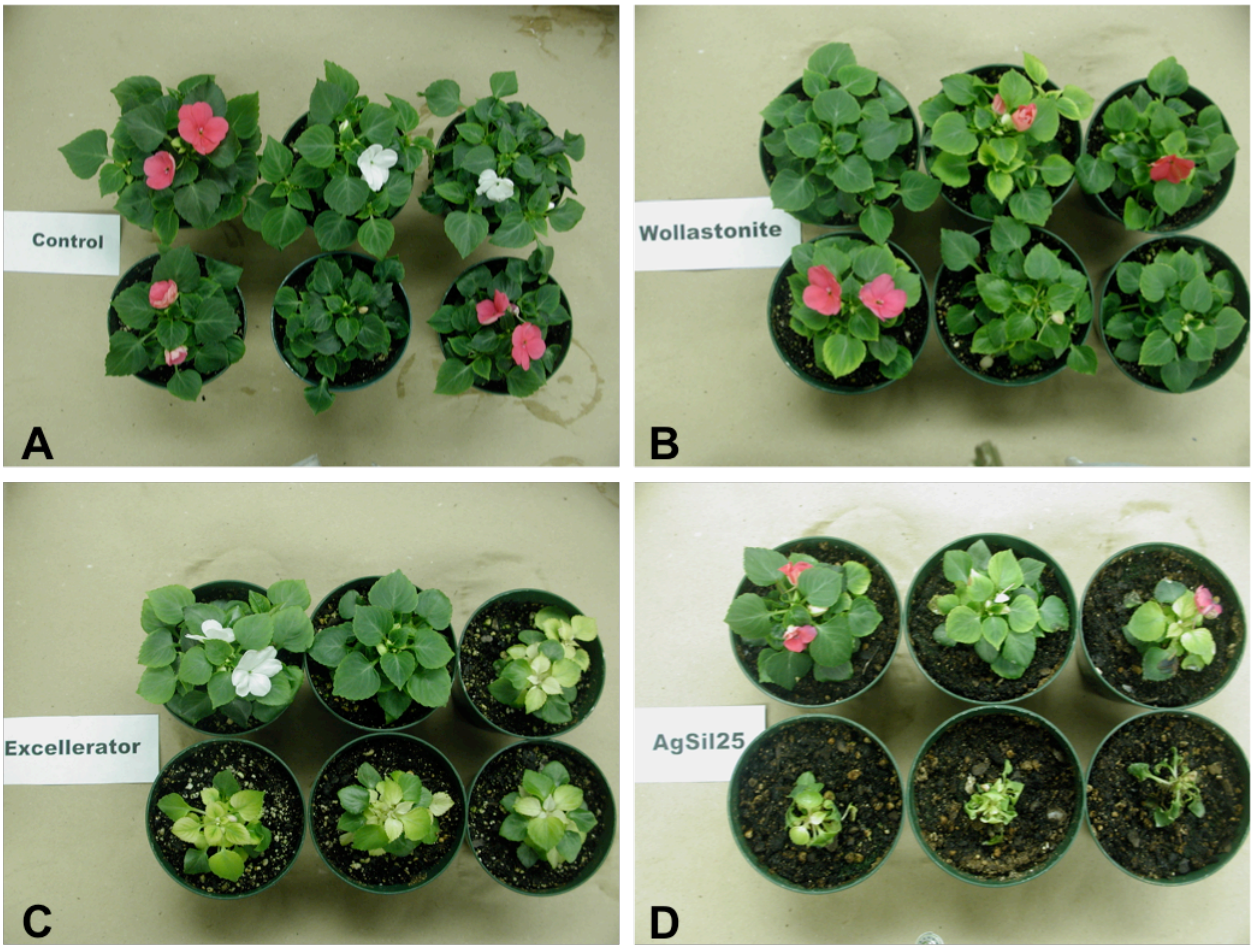


Figure 2-6 Impatiens plants amended with different sources of silicon after four weeks of growth. A. Non-amended control, B. Wollastonite, C. Excellerator, D. AgSil25.

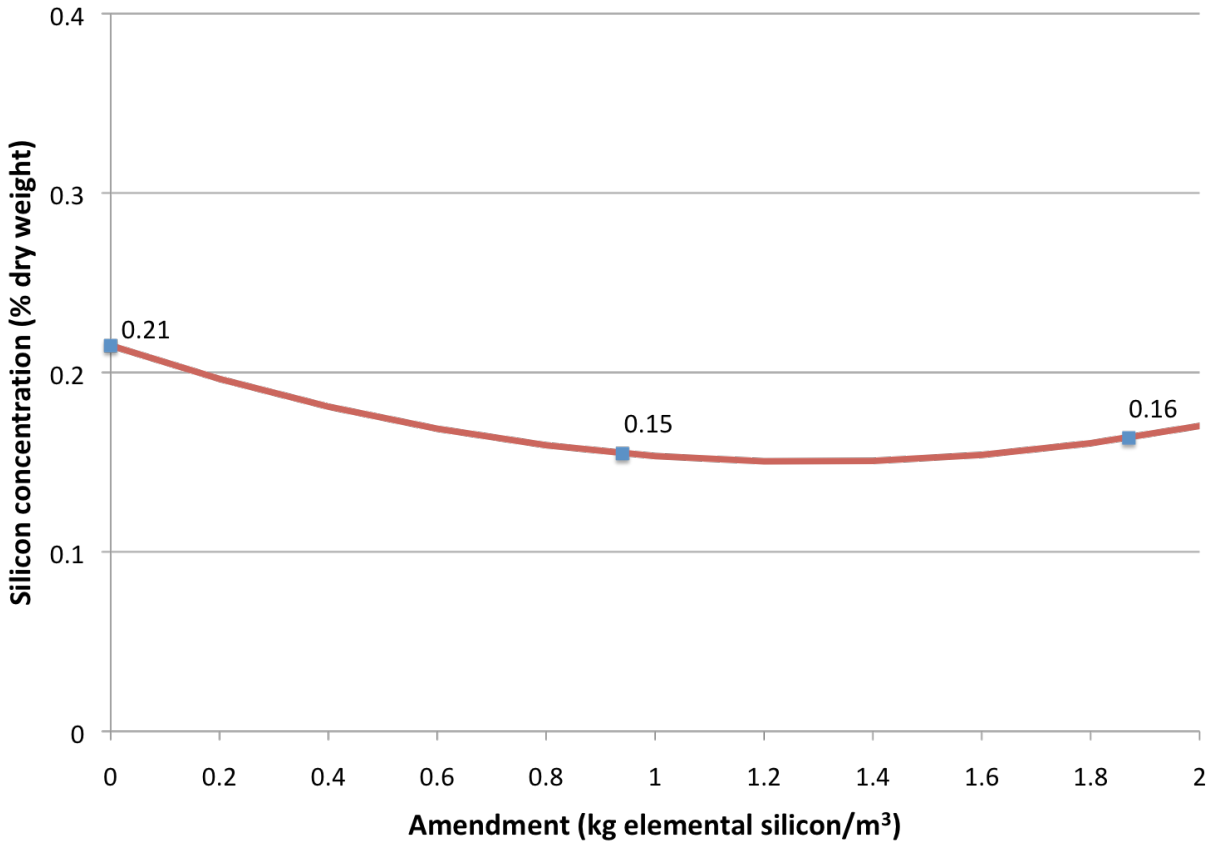


Figure 2-7 Silicon concentration of marigold leaves after four weeks of growth on different levels of silicon amendment. The solid line represents the regression curve $y= 0.0392 x^2 – 0.1008 x + 0.215$ ($P=0.1403$, $R^2=0.2791$, $n=5$). The blue squares represent the mean silicon concentration of marigold leaves.


Figure 2-8 Marigold plants amended with different sources of silicon after four weeks of growth. A. Non-amended control, B. Wollastonite, C. Excellerator, D. AgSil25.

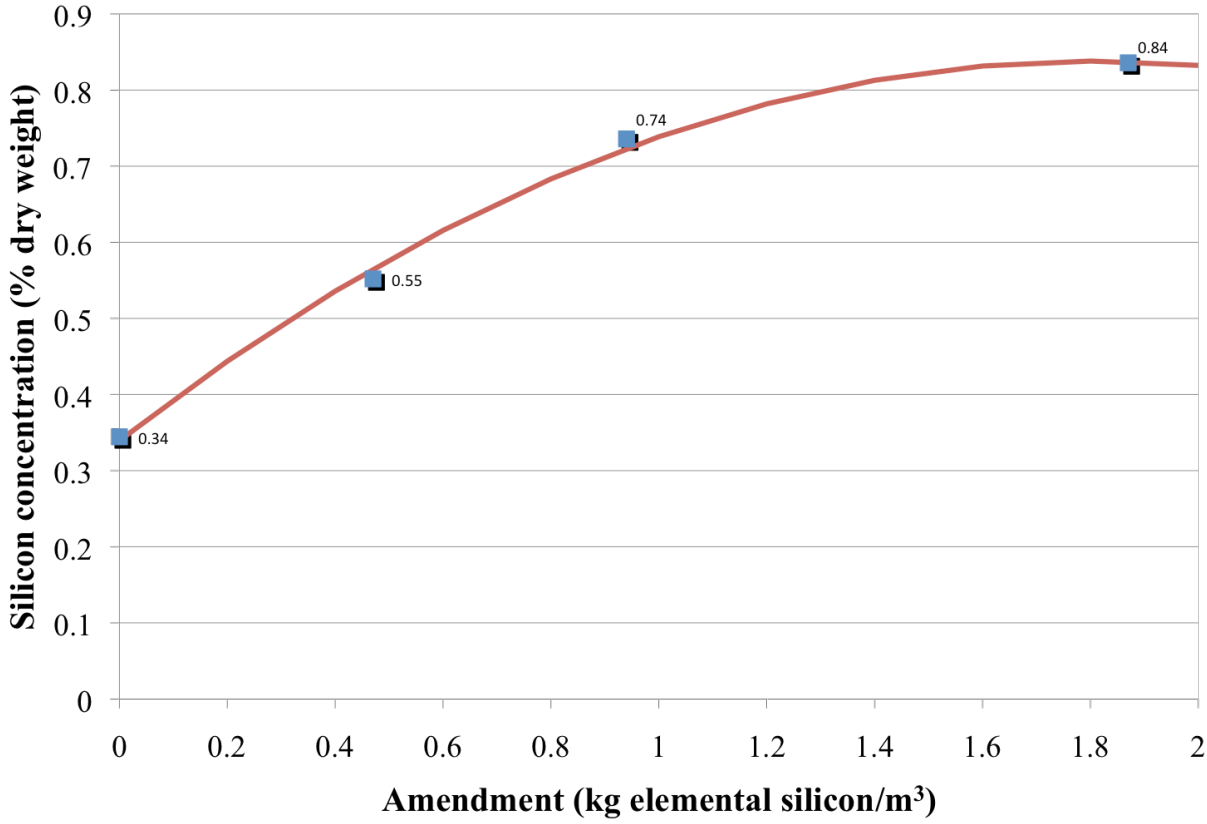


Figure 2-9 Rate-dependent silicon accumulation of tigergrass (first experiment). The solid line represents the quadratic regression curve $y = -0.1527 x^2 + 0.5519 x + 0.3395$, ($P<0.0001$, $R^2=0.7990$, $n=5$). The squares represent the mean silicon concentration of tigergrass.

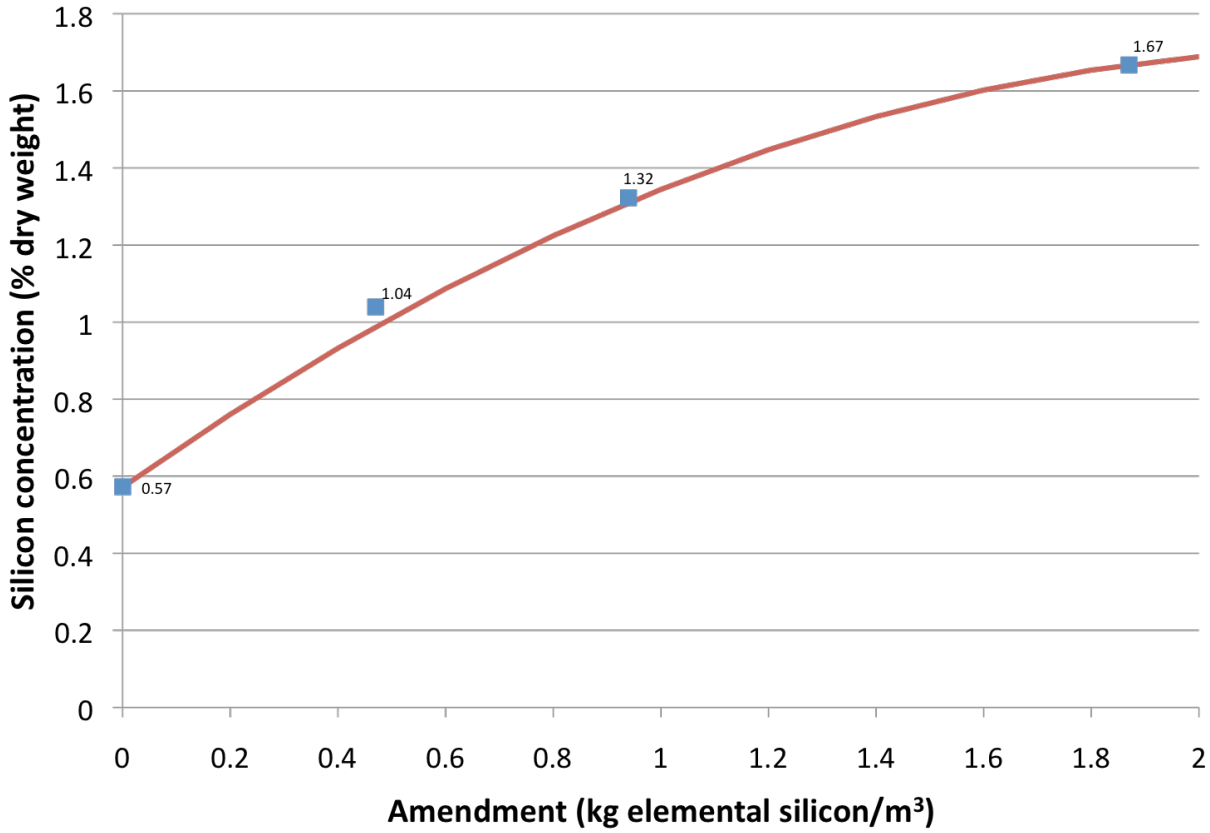


Figure 2-10 Rate-dependent silicon accumulation of tigergrass (second experiment). The solid line represents the quadratic regression curve $y = -0.2139\ x^2 + 0.9861\ x + 0.5719$, ($P<0.0001$, $R^2=0.9438$, $n=5$). The blue squares represent the mean silicon concentration of tigergrass.

CHAPTER 3
EFFECT OF SILICON ON TIGERGRASS DISEASE RESISTANCE AGAINST TIGERGRASS LEAF SPOT

## Introduction

### *Exserohilum rostratum*

*Exserohilum rostratum* (Drechsler) Leonard & Suggs (Leonard and Suggs 1974) is the anamorph of *Setosphaeria rostrata* Leonard (Leonard 1976) Sivanesan lists the following synonyms:

*Helminthosporium halodes* Drechsler
*Helminthosporium rostratum* Drechsler
*Helminthosporium halodes* Drechsler var. *tritici* Mitra
*Helminthosporium halodes* Drechsler var. *elaeidicola* Kovachich
*Bipolaris halodes* (Drechsler) Shoem.
*Bipolaris rostrata* (Drechsler) Shoem.
*Drechslera halodes* (Drechsler) Subram. & Jain
*Helminthosporium apatternae* K.S. Deshpande & K.B. Deshpande
*Drechslera rostrata* (Drechsler) Richardson & Fraser
*Exserohilum halodes* (Drechsler) Lenard & Suggs
*Luttrellia rostrata* (Drechsler) Gonorstaĭ

Drechsler (1923) initially described two distinct species. *E. rostratum* and *E. halodes*; the *E. rostratum* conidia were more rostrate and larger, while the *E. halodes* conidia were elliptical and smaller. These differences were not consistent over time, and often conidia of the two species were indistinguishable. Honda and Aragaki (1978a) found that temperature affected conidial morphology, with lower temperatures resulting in longer conidia. Leonard (1976) suggested that *E. halodes* was synonymous with *E. rostratum*, and described the conidia as follows:

> "Conidia porogenous, acrogenous becoming pseudopleurogenous, elliptical or narrowly obclavate-rostrate, brown or olivaceous, thick-walled except in a small subhyaline region at the apex and a similar region surrounding the hilum which protrudes as a darkened cylinder or truncate cone from the end of the basal cell. Conidia usually straight, 1- to 15-septate (rarely more), the basal septum darker and thicker than intermediate septa, 15-190 (rarely more) × 7-29 μm, germ tubes produced from subhyaline region of end cells and

growing parallel to conidial axis. Conidiophores cylindrical, simple, olivaceous brown, 40-180 μm, 1- to 6-spetate, with conidia produced on geniculations at intervals of 5-30 μm."

The teleomorph is heterothallic, and matings were more successful when the cultures were preconditioned by growth at low temperatures (4°C) before mating opposite mating types on a sterilized substrate (barley grains or pieces of Johnson grass; Leonard 1976).

The original genus *Helminthosporium* was divided in two subgenera: *Eu-Helminthosporium* with fusoid conidia that only germinated from the terminal cells, and *Cylindro-Helminthosporium* with cylindrical multiseptate conidia that could germinate from any cell (Drechsler 1923). The subgenus *Cylindro-Helminthosporium*, members of which were often associated with *Pyrenophora* teleomorphs, became the genus *Drechslera* Ito (Sivanesan 1987). Shoemaker proposed the genus name *Bipolaris* for *Eu-Helminthosporium*, which was often associated with *Cochliobolus* teleomorphs, especially if the conidia did not have a protuberant hilum (Sivanesan 1987). Only one species of *Bipolaris* with a protuberant hilum, *Bipolaris turcica* (Pass.), had a teleomorph stage associated with it, and it belonged to the genus *Trichometasphaeria* Munk (Luttrell 1958). More anamorphs belonging to different anamorph genera were identified with protuberant hila, and a *Trichometasphaeria* teleomorph. Disagreement about the taxonomy of species belonging to the former *Helminthosporium* complex continued for some time (Sivanesan 1987).

In 1974, Leonard and Suggs erected a new anamorph genus, *Exserohilum*, for *Bipolaris* species that had a protruding hilum, and the associated teleomorph genus was called *Setosphaeria*. They included all species within the complex with a protruding hilum in the new genus *Exserohilum*. The genera *Bipolaris*, *Exserohilum*, and *Drechslera*, were associated with the teleomorph genera *Cochliobolus*, *Setosphaeria*, and *Pyrenophora*, respectively. It proved particularly important to note the temperature at which cultures were grown when observing

protuberance of the hilum. Honda and Aragaki (1978b) found that the hilum of *E. rostratum* was at least partially inhibited at temperatures above 34ºC, which could lead to misidentification.

*Exserohilum rostratum* is common on grasses, occurs in soil, and is widely distributed worldwide (Kucharek 1973; Sivanesan 1987; Whitehead and Calvert 1959; Young et al. 1947). Whitehead and Calvert (1959) reported the fungus as the causal agent of ear rot of corn and leaf spot of thirteen different grasses, while Kucharek (1973) reportedly isolated it from rotted corn stalks in Florida in 1971 and confirmed it as the cause of the disease.

**Silicon in tigergrass**

Increased disease resistance in monocotyledons as a result of silicon application has been shown in numerous host-pathogen systems. Rice-rice blast (*Oryza sativa* L. -*Magnaporthe grisea* (T.T. Hebert) M.E. Barr) is the best-studied system (Volk et al. 1958; Datnoff et al. 1991; Seebold et al. 2000). Others include wheat-powdery mildew (*Triticum aestivum* L.-*Blumeria graminis* f. sp. *tritici*; Bélanger et al. 2003), and barley-powdery mildew (*Hordeum vulgare* L.-*Erysiphe graminis* D.C. Hordei; Carver et al. 1987).

Tigergrass (*Thysanolaena maxima* (Roxb.) Kuntze) accumulated silicon ranging from a concentration of 0.2 cg/gm dry weight when the growing medium was not amended with silicon up to a concentration of 1.6 cg silicon/gm dry weight when the growing medium was amended with 1.87 kg elemental silicon/$m^3$. The rate response fit a quadratic model, and was similar to those of turfgrasses for which silicon-accumulation was demonstrated: bermudagrass (Datnoff and Rutherford 2003), perennial ryegrass (Nanayakkara et al. 2008a,b), and St. Augustinegrass (Brecht et al. 2004, 2007a; Datnoff and Nagata 1999). In each of those cases, the increased silicon concentration of silicon-amended turfgrass correlated with disease suppression of leaf spot and melting out on bermudagrass, gray leaf spot on both perennial ryegrass and St. Augustinegrass. These results also provided evidence that silicon-related disease resistance is not

solely limited to diseases caused by biotrophs, such as the cereal-powdery mildew examples mentioned above.

Tigergrass is a commercial containerized and landscape ornamental grass from the family Poaceae similar in appearance to bamboo (Saikia 1992). In the summer of 2006, a leaf spot was first noticed in a South Florida nursery, and the disease has since then been observed in several nurseries and landscapes throughout Miami-Dade County and the Florida Keys in Monroe County (A. Palmateer, *personal communication*). In addition, the same leaf spot symptoms were observed on young transplants from a production greenhouse.

In experiments reported in Chapter 2, silicon concentration in tigergrass was determined to increase with silicon amendment. The goal of the research reported in this chapter was to 1) characterize the pathogen that was responsible for the leaf spot symptoms on tigergrass, 2) determine whether silicon had a direct effect on pathogen growth, and 3) test whether silicon amendment increased tigergrass resistance to a fungal pathogen.

## Materials and Methods

### Pathogen Characterization

Tigergrass plants were donated for research by Agristarts III, located in Apopka, Florida. Tigergrass leaf pieces each containing part of a lesion were excised with a small amount of surrounding asymptomatic tissue, surface sterilized for 45 seconds in 50 ml 10% commercial NaOCl solution (Clorox®, Clorox Company, Oakland, CA) with a drop of detergent (Tween 20), and rinsed three times in sterile de-ionized water. The leaf pieces were picked up with sterile tweezers, excess surface water removed by briefly placing on filter paper (Whatman #1), and placed in the center of V8-juice agar plates. V8-juice agar was prepared by mixing 1 can (330 mL) V8-juice (Campbell Soup Company, Camden, NJ), 670 mL de-ionized water, 3 mg calcium carbonate, and 15-20 gram agar, and autoclaving. Single-spore isolates were obtained by

transferring individual germinating conidia to fresh plates of V8-juice agar media. Cultures were maintained in an incubator at 21.1-26.3°C, with fluorescent lighting at 970 lux, 12 hours light, 12 hours dark. Tentative identification was done using Sivanesan's dichotomous key (Sivanesan 1987) based on conidial morphology.

Koch's postulates were performed by growing single-spore isolates on V8-juice agar medium, harvesting conidia from 7-10 day-old cultures, re-suspending the conidia in sterile water with Tween 20 to varying inoculum densities of $10^3$ to $10^5$ conidia/ml, and spraying onto symptomless tigergrass plants with a Crown #8211 Sprā-tool (Gardnerville, NV). Inoculated plants were placed in plastic bags for 24 hours after inoculation to maintain high humidity and kept at in an air-conditioned greenhouse (temperatures ranged from 22-32 °C.)

For confirmation of species identity, polymerase chain reaction was performed with the MyCycler™ thermal cycler (Bio-Rad laboratories, Hercules, CA) using Internal Transcribed Spacer region primers, ITS1 and ITS4 (Lott et al. 1993), with genomic fungal DNA isolated with the Extract ′n Amp kit (Sigma-Aldrich). PCR products were cleaned with the QIAquick® PCR purification kit (Qiagen), and sequenced by the ICBR sequencing core at the University of Florida. BLAST (Altschul et al. 1990) searches were performed, and sequences were aligned with Clustal W (Larkin et al. 2007).

To visualize conidia in the process of infecting tigergrass leaves, leaf sections with clearly visible lesions were cleared by heating (but not boiling) for 5-10 minutes in lactophenol blue, rinsed briefly in lactophenol essentially as described by Dhingra and Sinclair (1995). Excess lactophenol was then removed by placing the leaf sections on a paper towel for a few seconds. Slides were prepared with lactophenol, and lesions were photographed using an Olympus BX51 bifocal microscope (Olympus, Japan) with an Olympus DP12 digital camera (Olympus, Japan).

Conidial size was determined by measuring the width and length of 50 conidia derived from several isolates using an the bifocal microscope at a 400-fold magnification.

**Fungal Growth on Soluble Silicon**

The effect of soluble silicon on growth of the fungal pathogen *in vitro* was assessed by placing plugs of mycelium obtained with a # 1 hole punch (4 mm) from a 3-4 day old culture of a single-spore isolate on V8-juice agar plates containing 0, 1, 2, 4, and 8 ml AgSil™25 per 100 ml V8-juice agar, which corresponded to 0, 34, 69, 139, and 277 mM elemental silicon, respectively.

To prepare the media, AgSil25 was 1:1 diluted with de-ionized water and filter sterilized using a 0.22 μm vacuum driven disposable filtration system (Millipore Corporation, Billerica, MA). V8-juice agar was prepared by mixing 1 can (340 ml) V8 juice and 3 gm $CaCO_3$ per liter, and autoclaving. Diluted, filter-sterilized AgSil25 and V8-juice agar were mixed before pouring into petri-dishes to achieve the desired amounts of AgSil25 per 100 ml as previously mentioned. Since AgSil25 has a pH of 11.3, amending V8-juice agar with this product greatly affected the final pH of the medium. To assess the effect of pH on the growth of *E. rostratum*, V8-juice agar plates also were amended with potassium hydroxide (KOH), which resulted in media with similar pH levels as AgSil25. Colony growth on each plate was measured by taking two measurements at 90º angles. Percent inhibition of growth (PIG) was calculated with the following formula:

$$PIG = \frac{(C - T) \times 100}{C}$$

C = average diameter (cm) of colonies on control plates

T = average diameter (cm) of colonies on the treatment plates

At the end of the experiment, 9 days after transfer, the mycelium was scraped off the medium, air dried for 3-4 days in sterile plastic petri-dishes under the laminar airflow bench, pooled for each treatment, and analyzed for silicon concentration (to test whether the fungus had taken up silicon) using the same technique used for determining silicon concentration in leaf tissue as described in the Materials and Methods section of Chapter 2. Digestion of fungal tissue only required a single autoclave-induced digestion (compared to 4-6 cycles for leaf tissue).

**Effect of Silicon on Disease Development**

Rate-dependent silicon accumulation of tigergrass was reported in Chapter 2. To assess the effect of silicon on *E. rostratum* infection, tigergrass plants were grown for 4 weeks in 100 mm Azalea pots (Kord Products, Toronto, Ontario, Canada). The treatments were, no amendment control, AgSil25, Excellerator, and Wollastonite at rates equivalent to 1.40 kg elemental silicon/$m^3$. Amendments were included at the time of planting the tigergrass plugs, except for the treatment with AgSil25, which was applied at time of planting and twice a week for the duration of the experiment (four weeks). Because the tigergrass arrived with leaf spot symptoms from the supplier, the tigergrass plugs were trimmed to the soilline, and allowed to re-grow. Growing medium was prepared by mixing a 19 liter bucket of Metro-Mix (Sun Gro Horticulture Canada, Vancouver, British Columbia) or Fafard 4P Mix soilless medium (Fafard, Agawam, Massachussetts) with a 19 liter bucket of sand and 100 ml of Osmocote® (Scotts, Marysville, Ohio).

On the day of inoculation, one plant of each treatment was harvested to determine silicon concentration of the leaves. Three pots of each treatment containing plants of comparable size were selected for inoculation. Plants were kept on a NSF® Shelf System (Protrend Co., Taipei, Taiwan), outfitted with fluorescent light bulbs and clear, heavy-duty plastic sheeting 102 μm thick (Contractor's Choice, Olympic General Corporation, Reno, NV), and enclosed completely

using Velcro® in order to maintain a high relative humidity (Figure 3-1). Plants were inoculated with conidia of *E. rostratum* harvested from 9 day-old cultures. The conidial concentration was adjusted to $5 \times 10^3$ conidia/ml, and a drop of surfactant (Tween20) was added to the conidial suspension. Tigergrass plants were inoculated with the conidial suspension using a Crown #8211 Sprā-tool (Gardnerville, NV), by uniformly spraying the plants until runoff. The plants were placed in the enclosed humidity chamber (Figure 3-1). After 24 hours, the front plastic cover of the humidity chamber was removed. Plants were monitored for symptom development and symptoms were scored using the Horsfall-Barratt scale (Horsfall and Barratt 1945) every 24 hours up to 14 days.

Two weeks after inoculation, the plants were harvested for determination of leaf silicon concentration. The electrical conductivity (EC) of the pots was measured using the Field Scout® Direct Soil EC probe (Spectrum Technologies, Inc., Plainfield, IL) one hour after the pots were watered with 1 gm/L Peters® 20-20-20. At this time, 20 mL de-ionized water was poured over the pots and the flow-through was collected for pH measurement. The leaves were separated from the stems, dried for 2-3 days at 80°C in a dry heat oven (Isotemp Oven, Fisher Scientific), and ground finely using the Cyclotec™ 1093 sample mill (FOSS, Denmark). One hundred milligram of the ground tissue was weighed and placed in 100 ml plastic high-speed polypropylene copolymer tubes (Nalgene) with loose-fitting plastic caps for digestion. As a positive check, 100 mg of rice tissue was digested in the same manner as the samples. Sodium hydroxide solution (100%) was added to each tube (3 ml) in the fume hood, 2 ml hydrogen peroxide were added to each tube. The tubes were then placed into a 95-100°C water bath for one hour to initiate the digestion process, and to prevent spilling of undigested plant material during autoclaving. The tubes were placed into racks in the autoclave for a 20-minute liquid

cycle, and allowed to cool to room temperature. For the next round of autoclave-induced digestion, 2 ml hydrogen peroxide was added to each tube before autoclaving for 20 minutes as before. The digestion was repeated until the digested liquid became clear, and no leaf pieces were visible. The digestates were then diluted and silicon concentration determined using the colorimetric method described in the Materials and Methods section of Chapter 2.

## Results

Tigergrass plants often arrived with leaf spot symptoms (Figure 3-2) from the supplier. Symptoms begin as small tan spots or flecks, which enlarged into tan-colored lesions that elongated elliptically between the veins, sometimes with a yellow halo. Older lesions turned necrotic. Individual lesions in close proximity could coalesce into large necrotic elliptical spots to blotches, sometimes interspersed with chlorosis. Infected leaf tips turned light brown to brown, curled and turned yellow away from the leaf tip. All plants had to be cut back completely, to begin each experiment with healthy plants. This confirmed that the disease is a serious problem in the nursery, although during a discussion with the greenhouse manager of AgriStartsIII, it became clear that it was not perceived as such (D. Hartman, *personal communication*). A targeted or regular fungicide spray schedule of either Dithane® (Mancozeb, Dow Agrosciences, Indianapolis, IN), Medallion® (Fludioxonil, Syngenta, Greensboro, NC), and Cleary 3336™ , and Chipco 26 GT (Iprodione, Bayer, Montvale, NJ) was maintained to adequately control disease symptoms in the nursery greenhouse.

### Pathogen Identification

A dematiaceous fungus was consistently isolated from naturally occurring lesions on tigergrass (Figure 3-2), and identified as *Exserohilum rostratum* based on conidial morphology (Figures 3-3 and 3-4) according to Sivanesan's key (Sivanesan 1987).

Inoculation of tigergrass with conidia of *E. rostratum* at different concentrations resulted in symptoms 1-3 days post-inoculation (dpi), with symptoms appearing as early as 12 hours after inoculation when sprayed with $10^5$ conidia/ml. Increasing inoculum density corresponded with increasing levels of disease severity (Figure 3-5) and decreasing incubation times. High levels of inoculum ($10^5$ conidia/ml) showed symptoms as early as 12 hours after inoculation, while at lower inoculation density levels symptoms appeared 2-5 days after inoculation. *Exserohilum rostratum* was re-isolated from the lesions resulting from the spray inoculation experiments.

**Fungal Characterization**

*Exserohilum rostratum* grew on all tested media (water, potato dextrose, V8-juice, and lactose caseine hydrolysate agars), but sporulated most profusely and reliably on V8-juice agar, which was used for subsequent experiments. Sporulation typically only occurred after the mycelium had reached the edge of the petridish, 6-7 days after transfer to the medium. The mycelium was initially white in culture, turning dark brown as the culture aged. The conidiophores were brown and geniculate. Conidiogenesis was blastic and sympodial. The conidia were brown, straight to slightly curved, pseudoseptate, with the terminal septa dark and thick, with a protruding hilum, 11-18 μm × 56-128 μm (Figure 3-3), most often germinating from both terminal cells of the conidium (Figure 3-4).

The inoculum density of *E. rostratum* affected the final disease severity on tigergrass (Figure 3-5). Based on the results depicted in Figure 3-5, all subsequent experiments were performed with $5 \times 10^3$ conidia/ml, harvested from cultures 7-11 days old.

The ITS1/4 sequences derived from several isolates were identical, and Blastn (Altschul et al. 1990) results indicated 100% alignment with the *E. rostratum* sequence deposited in Genbank (gi:76555872). This confirmed the identity of the fungal plant pathogen as *E. rostratum*. The

Clustal W (Larkin et al. 2007) sequence alignment with the closest blast hit (*E. rostratum*) is displayed in Figure 3-6. One of the Blastn resulted with a very high similarity (99% over 92% of the sequence) with gi:55586034, *Alternaria* sp.. This sequence is most likely mis-identified by the depositors, since there were no other similar *Alternaria* Blast hits. In addition, the conidial morphology of *E. rostratum* was sufficiently distinct from those of *Alternaria*.

To visualize the conidia in association with lesions, leaf sections from leaves obtained from artificially inoculated plants with lesions were cleared with lactophenol blue. Figure 3-7 shows lesions on tigergrass associated with *E. rostratum* conidia. *Exserhilum rostratum* formed a very pronounced bulbous appressorium on one of the germ tubes. The appressoria (Figure 3-8) had two distinct cell walls and could clearly be seen to penetrate the tigergrass leaf surface.

**Effect of Soluble Silicon on Fungal Growth *In Vitro***

To assess the effect of soluble silicon on growth of *E. rostratum*, plugs obtained with a #1 hole punch (4 mm) from 3-day old *E. rostratum* cultures were transferred to the center of a petri-dish with V8-juice agar amended with different amounts of AgSil25, and to plates amended with different amounts of potassium hydroxide (KOH) to increase the pH. During initial experiments attempts were made to lower the pH of the medium. This failed, as clumps formed immediately in the V-8 juice agar, likely due to polymerization of silicon.

Colony diameters were measured every 24 hours for each of three replicates per treatment. Results of the first experiment are summarized in Table 3-1, and graphically depicted in Figure 3-9. Both high pH and high silicon concentration suppressed the growth of *E. rostratum* on V8-juice agar, as indicated by final colony diameter and percent inhibition (Table 3-1). However, the inhibition observed on medium amended with silicon cannot be explained simply by increasing pH. KOH-amended medium with pH level of 10.3 still supported fungal growth that was not significantly different from that of the control (4.6% inhibition). However, a pH of 10.8 in plates

with 4 ml AgSil25/100 ml resulted in 85.9% inhibition. In addition, on KOH plates, a pH of 11.2 mycelial growth was only 55% inhibited, while on plates with 277 mM silicon with a pH of 11.3, growth was 100% inhibited.

*E. rostratum* did not grow at all on medium with 277 mM silicon. An initial experiment also included 550 mM silicon, on which the fungus also did not grow. After 2 weeks, the non-growing plugs were transferred to plain V8-juice agar to verify whether the effect of AgSil25 was fungistatic or fungitoxic. The plugs grew normally, and looked morphologically no different than control cultures after the transfer, indicating that the fungus had not died on the silicon-amended agar.

In a separate experiment, five replicates of each treatment were used with the following treatments: control (no amendments of the medium), four levels of potassium silicate: 34, 68, 138, and 277 mM silicon (corresponding to 1, 2, 4, and 8 ml AgSil25/100 ml medium; Si 1, Si 2, Si 4, and Si 8), and four levels of potassium hydroxide (KOH 1, KOH 2, KOH 3, and KOH4) with pH adjusted to approximately the same level as the four potassium silicate treatments. The results are summarized in Table 3-2. As in the first experiment, high pH negatively affected colony growth at the highest level only (by 42.5 %, KOH 4), while a pH of 10.3 resulted in a 5.3% inhibition of growth, which was not statistically significant compared to the control. In contrast, soluble silicon at a level of 139 mM silicon (pH 10.4) resulted in a 71.3% inhibition of growth. The 277 mM silicon treatment (pH 10.6) resulted in complete (100%) inhibition of growth of *E. rostratum*, while KOH 4 (pH 10.7) resulted in 42.5% inhibition of growth.

In a third repeat of the experiment, six replicates were used per treatment, and the same treatments were used (KOH 1 through 4, Si 1, 2, 4, and 8), with the KOH treatments adjusted to approximately the same pH as the four silicon treatments. The results are summarized in Table 3-

3. As in the first and second experiment, *E. rostratum* did not grow at all on the highest level of silicon (277 mM silicon, pH 10.9), while there was growth on KOH 4 (pH 11.1), albeit 33.2 % inhibited. On medium with 139 mM silicon, *E. rostratum* only started growing on the 4$^{th}$ day after transfer, growth was inhibited 90.5% after 5 days (Figure 3-12).

To test whether *E. rotratum* took up silicon from the amended medium, the mycelium was carefully scraped off the plates, and allowed to dry for silicon analysis. Since each plate yielded very small amount of dry fungal tissue, the mycelium from all samples per treatment was pooled for analysis. No autoclave-induced digestion of the fungal tissue was necessary. The results are in Table 3-4. *Exserohilum rostratum* grown on plates amended with AgSil25 does not appear to accumulate silicon.

**Effect of Silicon on Resistance of Tigergrass to *Exserohilum rostratum*.**

Tigergrass plugs were grown in 100 mm pots for 4 weeks using 5 treatments: no-amendment control, and AgSil25, Excellerator, and Wollastonite at a rate equivalent to 1.40 kg elemental silicon/m$^3$ as described in the materials and methods section, and three replicates per treatment were inoculated with $5 \times 10^3$ conidia/ml. Inoculated plants were placed in the high humidity chamber (87-100%, Figures 3-1) immediately after inoculation for 24 hours. The front plastic sheet was removed, and plants were monitored for disease development over 14 days. Disease symptoms were scored using the Horsfall-Barratt scale (Horsfall and Barratt 1945). At the time of inoculation, one plant from each treatment was harvested to determine silicon concentration in the leaves (Table 3-5). At the end of the experiment (day 14), tigergrass plants were harvested for the determination of the silicon leaf concentration.

The silicon concentration of the leaf tissue differed depending on the treatment. Control plants had low levels of silicon (0.23%), Excellerator and Wollastonite-treated plants had high levels of silicon (1.08 and 0.96, respectively), and AgSil25-treated plants had an intermediate

level of silicon. Disease symptoms appeared on the 5th day after inoculation, except for the plants in pots amended with AgSil25, on which symptoms appeared a day later. The disease progress curve, (Figure 3-13), final disease severity (Figure 3-15), and final Area Under Disease Progress Curve (AUDPC, Figure 3-14), showed that pots treated with AgSil25 had the lowest AUDPC 14 days after inoculation. Although plants amended with Excellerator and Wollastonite had lower means of final disease severity and AUDPC than the control, this difference was not significant.

In a second experiment, plants were inoculated at 6 weeks after planting and monitored for 19 days. On the day of inoculation one plant of each treatment was harvested for silicon analysis, and all plants were harvested at the end of the experiment to determine silicon concentration of the leaves. The results of the tigergrass silicon analyses for the second experiment are listed in Table 3-6. Silicon-amended plants had significantly more silicon than control plants, both at the time of inoculation and at the time of harvest, 19 days later. As in the first experiment, although no statistical analysis was done on the plants harvested on day 0, because only one plant was harvested per treatment, the data does suggest that the silicon-amended pants continued to accumulate silicon during the 19 days after inoculation.

Symptoms were visible on the 6th day after inoculation for control and Wollastonite-amended plants. On Excellerator-amended plants the first symptoms appeared on the 7th day, and on AgSil25-amended plants on the 8th day after inoculation. The disease progress curves are shown in Figure 3-16. The disease severity on plants amended with AgSil25 remained generally below that of other treatments until the 18th day after inoculation, while disease severity on control was highest throughout the experiment. Leaf spot severity on Excellerator and Wollastonite-amended plants was intermediate between that of the control and AgSil25-amended plants. From the 9th to the 15th day, control plants had a significantly higher disease severity than

on silicon amended plants. Control, AgSil25, Excellerator, and Wollastonite-amended plants had final disease severities of 16.1, 14.8, 14.2, and 14.1%, respectively, which were not significantly different between any of the treatments (Figure 3-18).

The final AUDPC was significantly decreased by 46% in AgSil25-amended plants, by 34% in Excellerator-amended plants, and 32% in Wollastonite-amended plants in comparison to the controls (Figure 3-17). The final AUDPC's of the silicon-amended plants did not differ statistically from each other, and AgSil25-amended plants, as in the first experiment, had numerically the lowest final AUDPC.

## Discussion and Conclusions

*Exserohilum rostratum* (Drechsler) Leonard & Suggs was positively identified as the causal agent of tigergrass leaf spot disease using morphological and molecular characteristics, and performing Koch's postulates. This fungal pathogen has not previously been reported on tigergrass before, although it is known to be common on grasses, other plants and substrates, and in soil (Sivanesan 1987). Even relatively low levels of inoculum ($10^3$ conidia/ml) resulted in substantial disease severity in artificial inoculation experiments. Inoculation of tigergrass at high inoculum densities ($10^5$ conidia/ml) caused such severe leaf spot symptoms that the coalescing lesions resulted in widespread necrosis and death of the leaf. This implied that the disease has the potential to be severe under natural conditions. Indeed, tigergrass supplied by the grower were often heavily infected. *Exserohilum rostratum* is the anamorph of *Setosphaeria rostrata* Leonard. The species is heterothallic, producing a teleomorph *in-vitro* after culturing different mating types at low temperature for 1-3 months (Sivanesan 1987). From a commercial marketing point of view, any disease symptoms on the plants at the time of sale would be completely unacceptable.

Most conidia of *E. rostratum* germinated bipolarly. The fungus produced prominent appressoria that were associated with tigergrass lesions. Individual lesions expanded longitudinally, and were for the most part restricted to between the leaf veins. However, multiple lesions could coalesce into larger diseased areas that might span the veins. Symptoms appeared at times very rapidly, implying that fungal toxins may be involved in the infection process. Indeed, the genus *Exserohilum* belongs to what was formerly called the genus *Helminthosporium*, to which a number of species belong that produce host-specific toxins (Sivanesan 1987).

Inhibition of mycelial growth on medium amended with soluble silicon was reported by Kaiser et al. (2005) for several fungi, including *Drechslera* spp., that are closely related to *Exserohilum* spp. (Sivanesan 1987). In that study however, the plugs that failed to grow on silicon-amended medium were not transferred to media without silicon to test whether the effect was fungistatic or fungicidal. Bi et al. (2006) did a similar experiment, but used sodium silicate to amend the medium and found that mycelial growth of *Alternaria alternata*, *Fusarium semitectum*, and *Trichothecium roseum* were diminished. The authors did transfer the plugs to non-amended medium after the experiment, found that they grew normally, and concluded the effect was fungistatic. Several factors make it hard to draw definitive conclusions from that experiment. First, the source of silicon was added to the medium before autoclaving, which would have caused polymerization of the silicate into insoluble silica gel, thereby changing the amounts of soluble silicon. Second, sodium silicate would have caused an increase in medium pH, and the effect of pH was not tested. Finally, no control was added to investigate whether the effect could have been due to the increased concentration of sodium.

In contrast, in this study, filter-sterilized potassium silicate solution was added to V-8 juice agar once it had cooled to 55° C, which prevented polymerization. Soluble silicon in the growing medium inhibited mycelium growth of *E. rostratum* significantly, and the results were consistent in three separate experiments. The effect was determined to be fungistatic, since the plugs grew normally when they were transferred to regular V-8 juice agar at the end of the experiment, and could not be explained exclusively by the increased pH of the soluble silicon-amended medium, or the increased concentration of potassium in the medium.

There is some evidence that spraying potassium silicate decreases fungal infection. On grape leaves, soluble silicon sprays inhibited powdery mildew (*Uncinula necator*) development (Bowen et al. 1992), while on cucumber foliar applications of silicon, suppressed powdery mildew (*Podosphaera xanthii*; Liang et al. 2005). Bi et al. (2006) dipped Hami melons in a sodium silicate solution and found that this inhibited mycelial growth of *A. alternata*. However, it is unclear whether this was due to silicate or sodium, since the control treatment consisted of dipping in water. This is particularly important because Aharoni et al. (1997), in a similar experiment, found that melons dipped in sodium bicarbonate showed reduced decay caused by *A. alternata*, *Fusarium* spp., and *Rhizopus stolonifer* without testing whether this was due to sodium or bicarbonate. In addition, Bi et al. (2006) did not measure the silicon concentration of the melon rind to determine whether silicon was present.

It is possible that in the *E. rostratum*-tigergrass system soluble silicon plays a role in the *in-planta* infection process, when the penetration peg penetrates the epidermal wall and goes through the relatively high silicon concentration silica depositions. An inhibition of penetration peg growth might result in the plant having more time to rally defense mechanisms against the fungal attack, or the defense mechanisms may be more effective on an impeded penetration peg.

An alternative interpretation could be that the fungus penetrates the silica layer underneath or above the cuticle, thereby releasing silicate monomers in locally high concentration, and that this decreases the rate of penetration peg advancement. In contrast, Kema et al. (1996) concluded that polymerized silicon did not prevent penetration of *Mycosphaerella graminicola* in wheat, but that does not exclude the possibility that penetration peg development is slowed or weakened. In these experiments, *E. rostratum* did not accumulate silicon itself, but the measurements may not be accurate. If silicon is present at low concentrations in the medium, it may not be possible with the techniques used in this study to measure the difference in silicon concentration of fungal mycelium grown on plates with and without silicon. With higher silicon concentrations there was no fungal growth, so silicon concentration could not be determined.

In the first of two experiments, only AgSil25-amendment applied at 1.40 kg elemental silicon/$m^3$ was associated with a statistically significant decreased disease severity and AUDPC of tigergrass to *E. rostratum* infection. However, the final disease severity and AUDPC was not significantly different with AgSil25 amendment from that of the other silicon amendments. Plants amended with AgSil2 accumulated 56% less silicon than plants amended with Excellerator and 50% less than plants amended with Wollastonite in this experiment. In the second experiment, the final disease severity was not significantly different between any of the silicon treatments. The fact that the AUDPC of AgSil25-amended plants was 86% less than the control in the first experiment, but only 46% less in the second experiment was likely due to the disease severity on AgSil25-amended plants ending up with the same final disease severity as control plants towards the end of the experiment.

The difference in final AUDPC in the first experiment is likely due to the delayed appearance of the symptoms on AgSil25-amended plants (2 days later), even though the final

disease severity was not different. Similarly, in the second experiment the final AUDPC was significantly different between the control and all silicon-amended plants (Figure 3-18). At different points of time during the experiment, the silicon-amended treatments did have a significantly lower disease severity than control plants, particularly during the second experiment. From day 9 through 15 after inoculation, the disease severity on control plants was significantly higher than all silicon-amended plants. This no longer held true for Wollastonite-amended plants on day 16, while Excellerator-amended plants and AgSil25-amended plants caught up on day 17 and 18 respectively. This difference in disease severity between control and silicon amended plants throughout the observation period explains the difference in AUDPC between control plants and silicon-amended plants.

Brecht et al. (2004) noted a similarly decreased final AUDPC in the gray leaf spot-St. Augustinegrass system, even when the final disease severity was not different from non-amended plants, due to differences in disease severity at different points of time during the experiment. An increased incubation period was correlated with increasing rates of silicon application in the rice-rice blast system (Seebold et al. 2001), but not clearly affected by silicon amendment in the rice-sheath blight (*Rhizoctonia solani*) system (Rodrigues et al. 2003b).

In light of results reported herein, there is ample opportunity to continue research in the tigergrass-*Exserohilum* system. It may be useful to investigate different rates of silicon in addition to the use of different silicon sources to provide more detail as to their relationship between silicon concentration and disease severity and AUDPC. Other components of tigergrass resistance might be affected, but which were not assessed in this study are lesion size and the rate of lesion expansion. Further research in this system might yield more generalized principles of the basis of silicon-correlated disease resistance.

In conclusion, *E. rostratum* was confirmed to be the causal agent of tigergrass leaf spot. Silicon amendment increased the resistance of tigergrass to *E. rostratum* as determined by the lower AUDPC for silicon-amended plants compared to that of the control plants.

Table 3-1 Effect of potassium hydroxide and potassium silicate on colony diameter and percent inhibition of growth of *Exserohilum rostratum* after 5 days, first experiment

| Medium amendment | pH | Diameter (cm)[x] | Inhibition (%)[y] |
|---|---|---|---|
| Control (no amendment) | 6.1 | 8.0 c[z] | 0 c |
| KOH 1[w] | 7.0 | 7.7 c | 4.6 c |
| KOH 2 | 9.0 | 7.6 c | 5.8 c |
| KOH 3 | 10.3 | 7.7 c | 4.6 c |
| KOH 4 | 11.2 | 3.6 b | 55.2 b |
| Si 1 (34 mM Si) | 9.3 | 7.9 c | 1.2 c |
| Si 2 (68 mM Si) | 10.1 | 6.9 c | 13.7 c |
| Si 4 (139 mM Si) | 10.8 | 1.1 a | 85.9 a |
| Si 8 (277 mM Si) | 11.3 | 0 a | 100 a |

[w]KOH1 through 4 media were pH adjusted with potassium hydroxide the same pH as the Si1 through 8 media.
[x]Average diameter of colonies on V8-juice agar amended with potassium hydroxide or potassium silicate 5 days after transfer.
[y]Percent inhibition was calculated by expression the difference in colony diameter of control and treatment plates as a percentage of the average diameter on the control plate
[z]Means in each column followed by different letters are significantly different based on Fisher's protected LSD ($\alpha$=0.05).

Table 3-2 Effect of potassium hydroxide on colony diameter and percent inhibition of growth of *Exserohilum rostratum* after 5 days, second experiment

| Treatment | pH | Diameter (cm)[x] | Inhibition (%)[y] |
|---|---|---|---|
| Control (no amendment) | 6.4 | 8.0 a[z] | 0 a |
| KOH 1[w] | 8.5 | 7.5 a | 5.5 e |
| KOH 2 | 9.1 | 7.6 a | 5 e |
| KOH 3 | 10.3 | 7.6 a | 5.3 e |
| KOH 4 | 10.7 | 4.6 c | 42.5 c |
| Si 1 | 8.7 | 7.7 a | 3.5 e |
| Si 2 | 9.3 | 6.3 b | 20.6 d |
| Si 4 | 10.4 | 2.3 d | 71.3 b |
| Si 8 | 10.6 | 0 e | 100 a |

[w]KOH1 through 4 media were pH adjusted with potassium hydroxide the same pH as the Si1through 8 media.
[x]Average diameter of colonies on V8-juice agar amended with potassium hydroxide or potassium silicate 5 days after transfer.
[y]Percent inhibition was calculated by expression the difference in colony diameter of control and treatment plates as a percentage of the average diameter on the control plate
[z]Means in each column followed by different letters are significantly different based on Fisher's protected LSD ($\alpha$=0.05).

Table 3-3 Effect of potassium hydroxide on colony diameter of *Exserohilum rostratum* after 5 days, third experiment

| Treatment | pH | Diameter (cm)[x] | Inhibition (%)[y] |
|---|---|---|---|
| Control (no amendment) | 6.2 | 8.2 a[z] | 0 f |
| KOH 1[w] | 9.2 | 8.1 a | 1.4 f |
| KOH 2 | 9.8 | 7.4 b | 10.3 e |
| KOH 3 | 10.7 | 6.5 c | 20.9 d |
| KOH 4 | 11.1 | 5.5 d | 33.2 c |
| Si 1 (34 mM Si) | 9.1 | 7.9 a | 4.5 f |
| Si 2 (68 mM Si) | 9.9 | 6.3 c | 23.2 d |
| Si 4 (139 mM Si) | 10.5 | 0.8 e | 90.5 b |
| Si 8 (277 mM Si) | 10.9 | 0 f | 100 a |

[w]KOH1 through 4 media were pH adjusted with potassium hydroxide the same pH as the Si1through 8 media.
[x]Average diameter of colonies on V8-juice agar amended with potassium hydroxide or potassium silicate 5 days after transfer.
[y]Percent inhibition was calculated by expression the difference in colony diameter of control and treatment plates as a percentage of the average diameter on the control plate
[z]Means in each column followed by different letters are significantly different based on Fisher's protected LSD ($\alpha$=0.05).

Table 3-4 Silicon concentration (cg/gm) of *Exserohilum rostratum* grown on V-8 juice agar with soluble silicon amendment

| Treatment | Experiment 1[y] | Experiment 2 | Experiment 3 |
|---|---|---|---|
| Control (no amendment) | 0.006 | 0 | 0.002 |
| KOH 1 | 0.007 | 0.009 | 0 |
| KOH 2 | 0.005 | 0.011 | 0.005 |
| KOH 3 | 0.005 | 0.006 | 0.002 |
| KOH 4 | 0.002 | 0.002 | 0 |
| Si 1 (34 mM Si) | 0.005 | 0.002 | 0.005 |
| Si 2 (68 mM Si) | 0.002 | 0.004 | 0.002 |
| Si 4 (139 mM Si) | Not done[z] | 0.002 | Not done |
| Si 8 (277 mM Si) | Not done | Not done | Not done |

[y]No statistical analysis was done, since there were no replicates.
[z]No growth

Table 3-5 Silicon concentration of tigergrass leaves at the time of inoculation (day 0) and the time of harvest (day 14), first experiment

| Treatment | Si (cg/gm) on day 0 | Si (cg/gm) on day 14 |
|---|---|---|
| Blank control | 0.21 | 0.23 a[x] |
| AgSil25 | 0.36 | 0.48 b |
| Excellerator | 0.50 | 1.08 c |
| Wollastonite | 0.49 | 0.96 c |

[x]Means followed by different letters are significantly different based on Fisher's protected LSD ($\alpha$=0.05).

Table 3-6 Silicon concentration of tigergrass leaves at the time of inoculation (day 0) and the time of harvest (day 19), second experiment

| Treatment | Si (cg/gm) on day 0 | Si (cg/gm) on day 19 |
|---|---|---|
| Blank control | 0.26 | 0.30 b[x] |
| AgSil25 | 0.97 | 1.08 a |
| Excellerator | 1.00 | 1.17 a |
| Wollastonite | 0.89 | 1.16 a |

[x]Means followed by different letters are significantly different based on Fisher's protected LSD ($\alpha$=0.05).

Figure 3-1 Shelf enclosed in plastic used for keeping plants at high humidity for the first 24 hours after inoculation.

Figure 3-2 Tigergrass showing leaf spot symptoms on day of receipt from the supplier. Note the tan colored lesions that are typical for the disease.

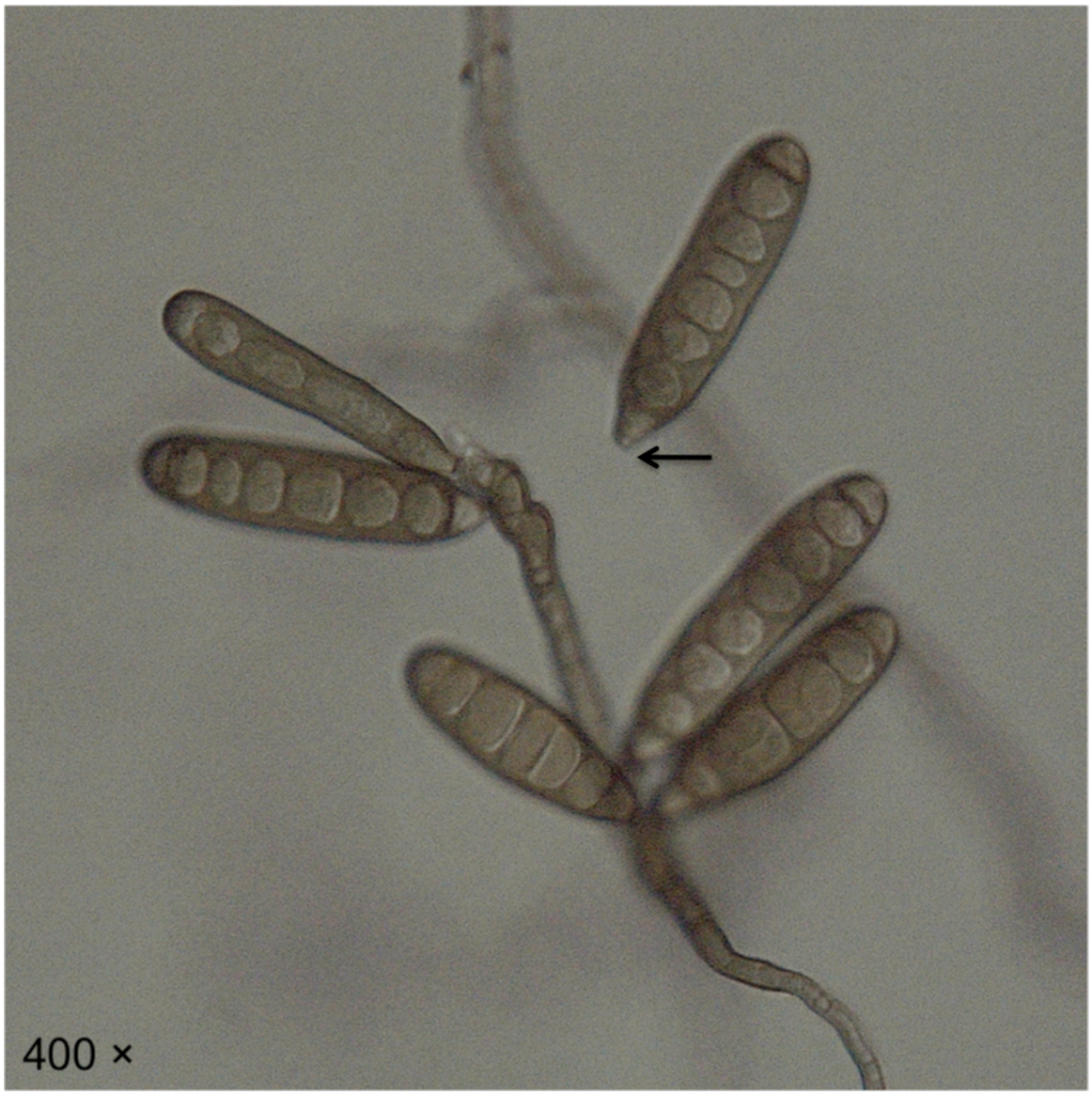

Figure 3-3 Conidia of *Exserohilum rostratum*. Note the protruding hilum (arrow), which distinguishes the genus *Exserohilum* from the genus *Bipolaris*.


Figure 3-4 Bipolar conidial germination of *Exserohilum rostratum*.


Figure 3-5 The effect of inoculum density on *Exserohilum rostratum* leaf spot development on tigergrass. Numbers on the right indicate the number of conidia/ml. Increasing concentration results in higher disease severity.

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0706019         ---CATTACACAACAAAAATATGAGGGTGTGGTTTGCTGGCAACAGCGTCCGCCCCAAGT 57
gi_76555872     GATCATTACACAACAAAAATATGAGGGTGTGGTTTGCTGGCAACAGCGTCCGCCCCAAGT 60
                   ************************************************************
0706019         ATTTTTCACCCATGTCTTTTGCGCACTTTTTGTTTCCTGGGCGAGTTCGCTCGCCACCAG 117
gi_76555872     ATTTTTCACCCATGTCTTTTGCGCACTTTTTGTTTCCTGGGCGAGTTCGCTCGCCACCAG 120
                ************************************************************
0706019         GACCCAACCATAAACCTTTTTTTTATGCAGTTGCAATCAGCGTCAGTATAATAATTCAATT 177
gi_76555872     GACCCAACCATAAACCTTTTTTTTATGCAGTTGCAATCAGCGTCAGTATAATAATTCAATT 180
                ************************************************************
0706019         TATTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAAT 237
gi_76555872     TATTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAAT 240
                ************************************************************
0706019         GCGATACGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGC 297
gi_76555872     GCGATACGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGC 300
                ************************************************************
0706019         GCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGCGTCATTTGTACCCTCAAGCTTTGC 357
gi_76555872     GCCCTTTGGTATTCCAAAGGGCATGCCTGTTCGAGCGTCATTTGTACCCTCAAGCTTTGC 360
                ************************************************************
0706019         TTGGTGTTGGGCGTCTTTTTGTCTCTCCCCTTGTTGGGGGAGACTCGCCTTAAAACGATT 417
gi_76555872     TTGGTGTTGGGCGTCTTTTTGTCTCTCCCCTTGTTGGGGGAGACTCGCCTTAAAACGATT 420
                ************************************************************
0706019         GGCAGCCGACCTACTGGTTTTCGGAGCGCAGCACAAATTTGCGCCTTCCAATCCACGGGG 477
gi_76555872     GGCAGCCGACCTACTGGTTTTCGGAGCGCAGCACAAATTTGCGCCTTCCAATCCACGGGG 480
                ************************************************************
0706019         CGGCATCCAGCAAGCCTTTGTTTTCTATAACAAATCCACATTTTGACCTCGGATCAGGTA 537
gi_76555872     CGGCATCCAGCAAGCCTTTGTTTTCTATAACAAATCCACATTTTGACCTCGGATCAGGTA 540
                ************************************************************
0706019         GGGATACCCGCTGAACTTAAGCATATCAATA 568
gi_76555872     GGGATACCCGCTGAACTTAA----------- 560
                ********************
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Figure 3-6 ITS sequence alignment. ITS1/4 sequences derived from *Exserohilum rostratum* isolate 0706019 compared with the ITS sequence from GenBank gi:76555872 *Exserohilum rostratum*.

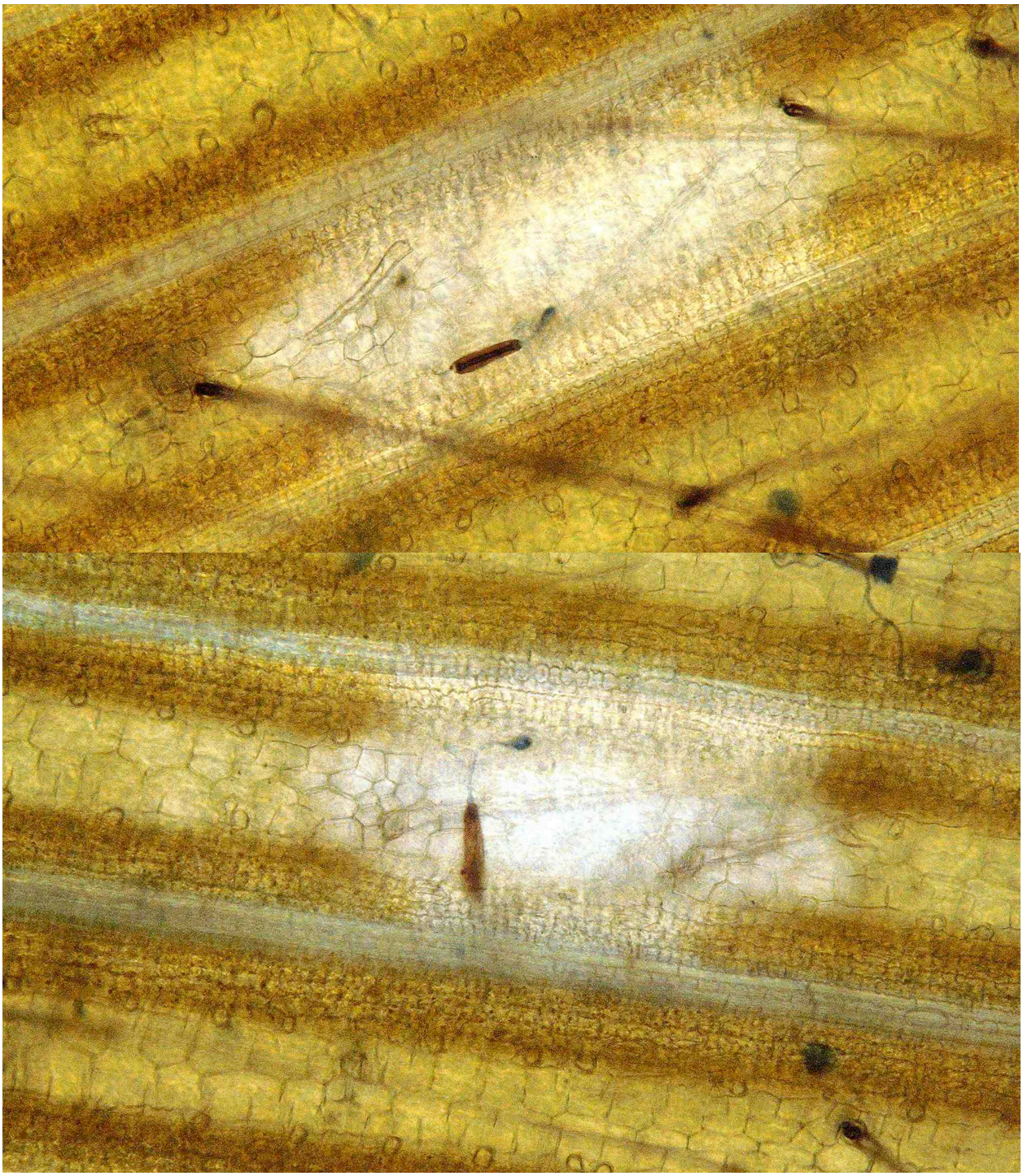

Figure 3-7 Association of conidia of *Exserohilum rostratum* with lesions on tigergrass. The light-colored areas of the leaves are the lesions. *Exserohilum rostratum* conidia in this picture have formed an appressorium in the infection process

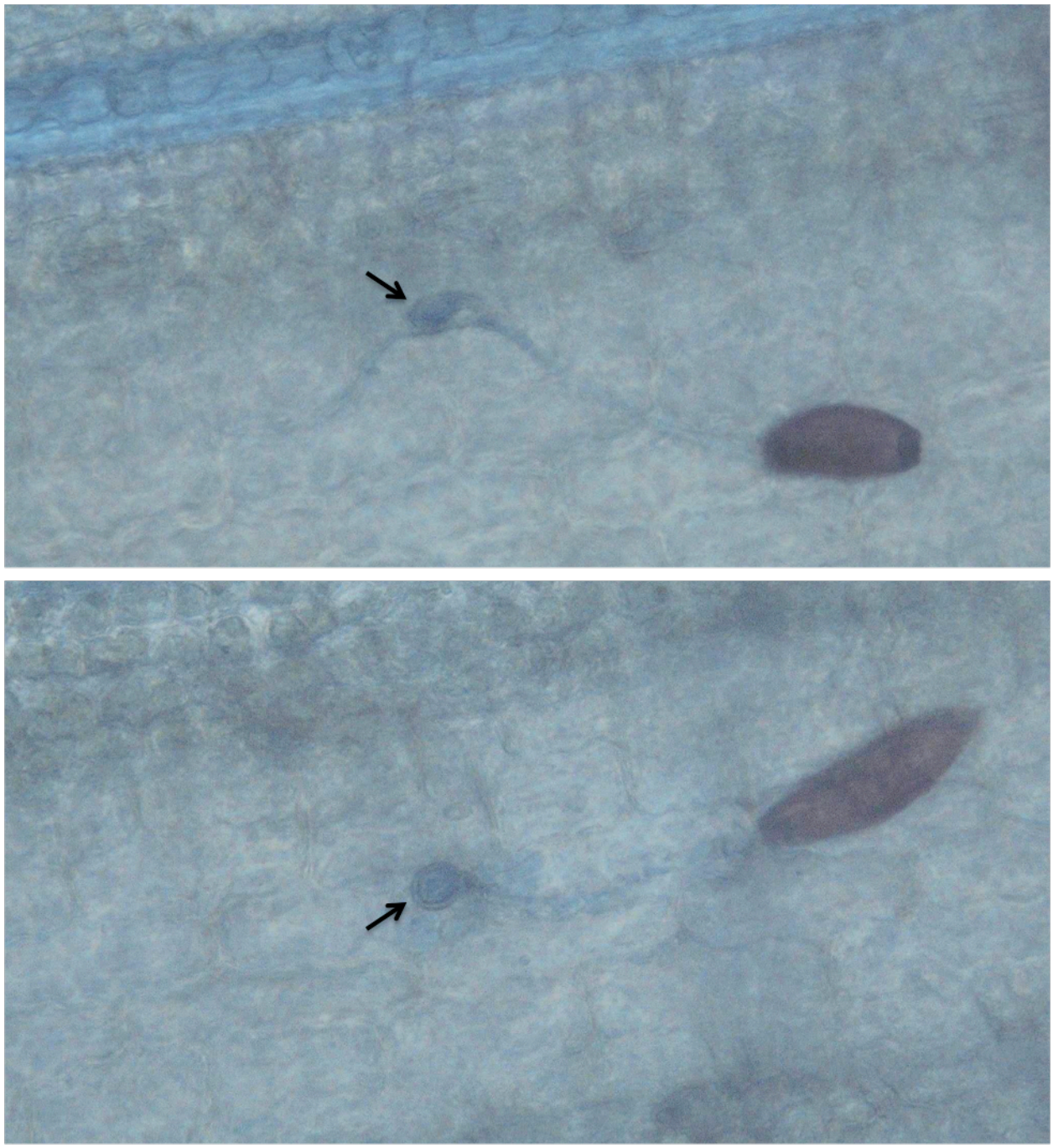

Figure 3-8 *Exserohilum rostratum* forms distinct appressoria (arrows) in the process of infecting tigergrass. The leaves were cleared and stained with lactophenol blue..

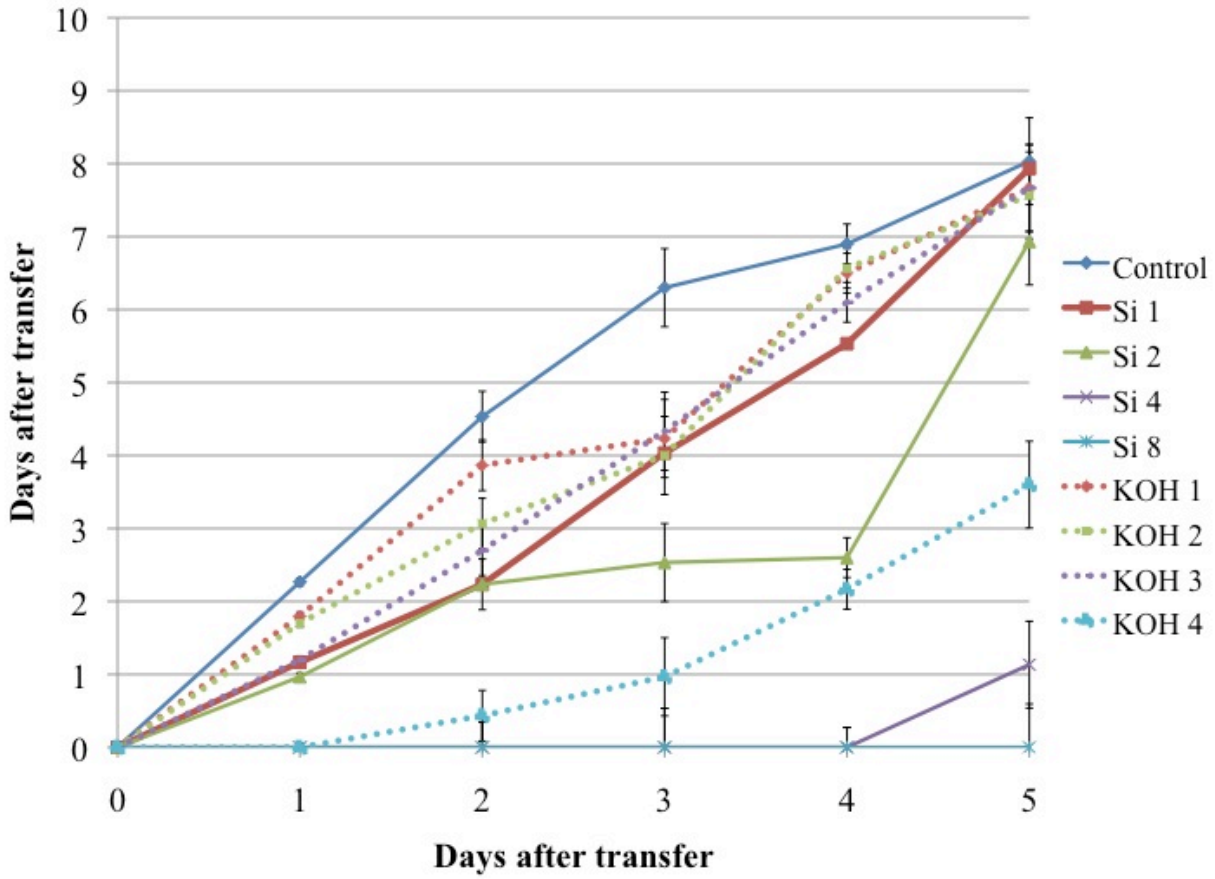


Figure 3-9 Growth of *Exserohilum rostratum* on V8-juice agar amended with soluble silicon (AgSil™25) or potassium hydroxide (KOH) first experiment. Silicon concentrations Si 1: 34 mM, Si 2: 69 mM, Si 4: 139 mM, Si 8: 277mM. Media KOH 1-KOH4 were adjusted to the same pH as the four silicon-amended media at the start of the experiment.

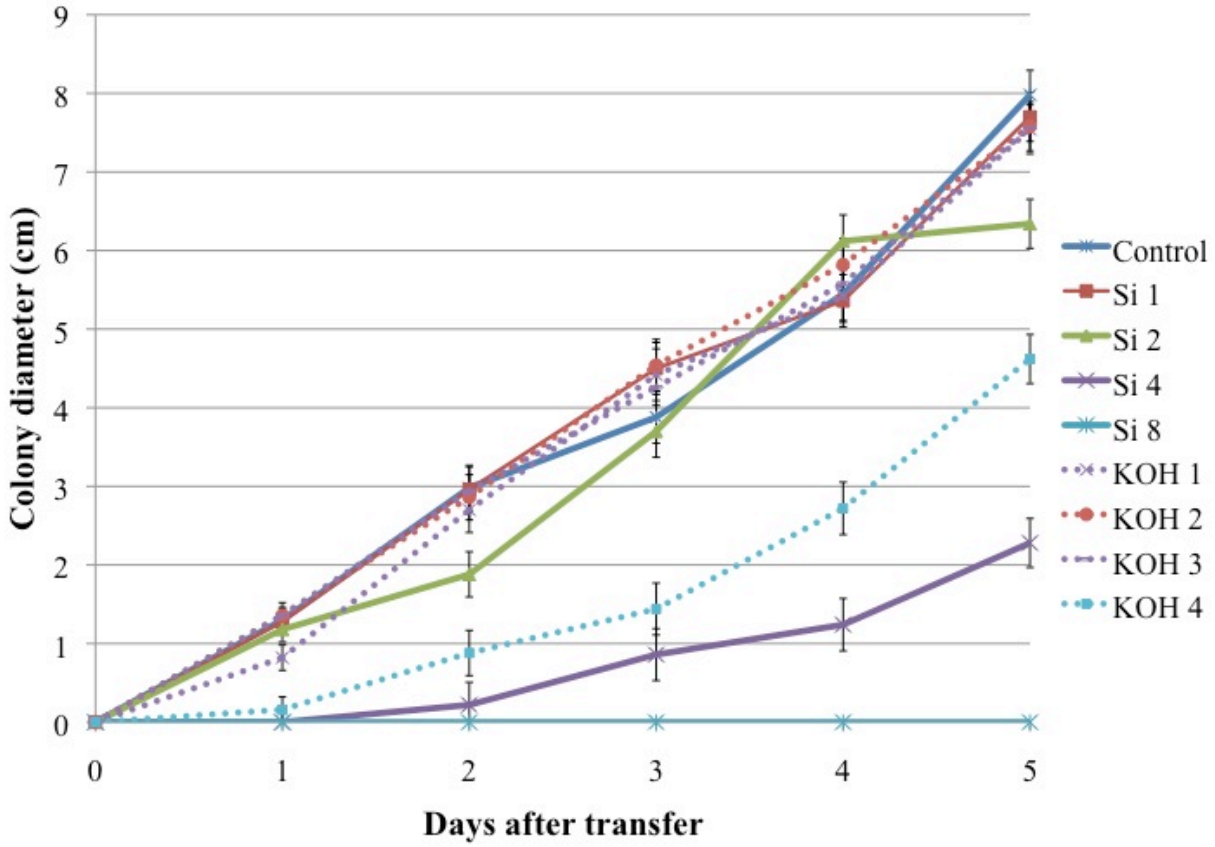


Figure 3-10 Growth of *Exserohilum rostratum* on V8-juice agar amended with soluble silicon (AgSil™25) or potassium hydroxide (KOH), second experiment. Silicon concentrations Si 1: 34 mM, Si 2: 69 mM, Si 4: 139 mM, Si 8: 277mM. Media KOH 1-KOH4 were adjusted to the same pH as the four silicon-amended media at the start of the experiment.

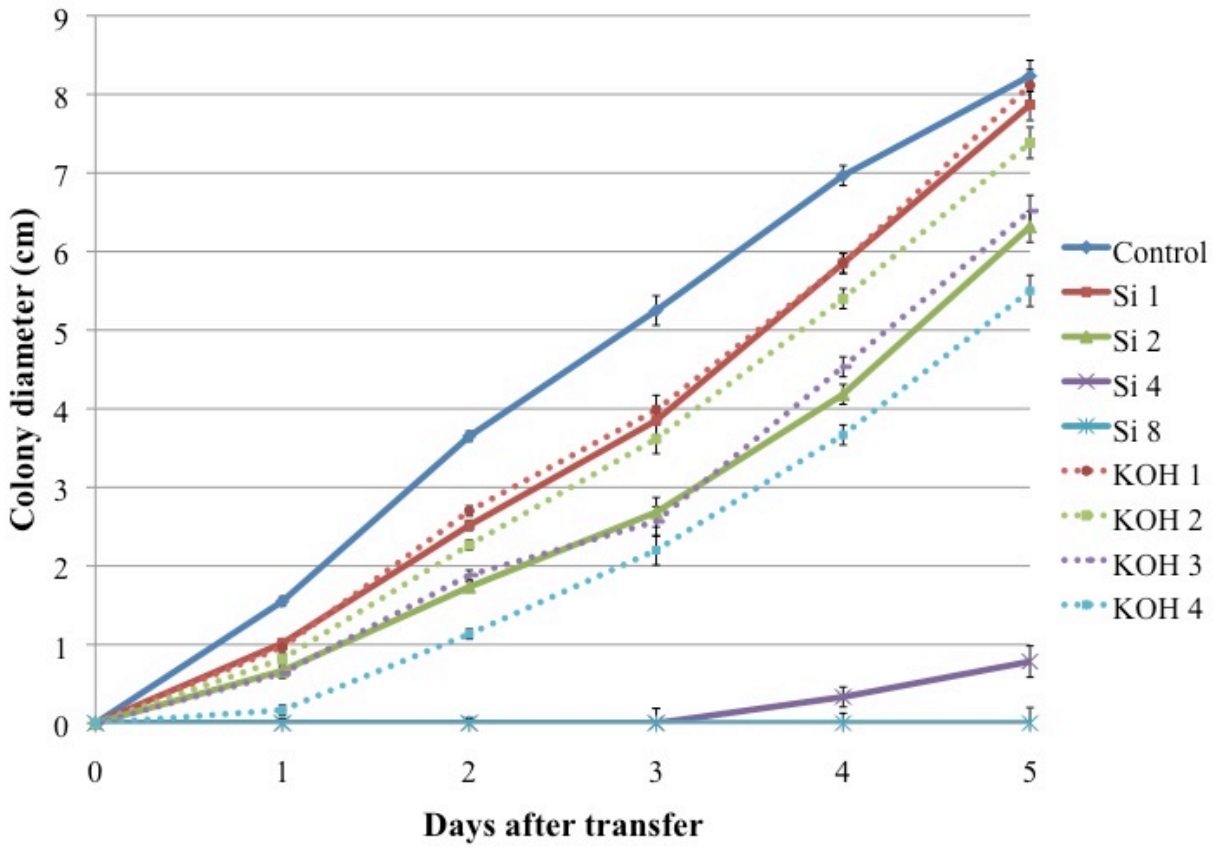


Figure 3-11 Growth of *Exserohilum rostratum* on V8-juice agar amended with soluble silicon (AgSil™25) or potassium hydroxide (KOH), third experiment. Silicon concentrations Si 1: 34 mM, Si 2: 69 mM, Si 4: 139 mM, Si 8: 277mM. Media KOH 1-KOH4 were adjusted to the same pH as the four silicon-amended media at the start of the experiment.

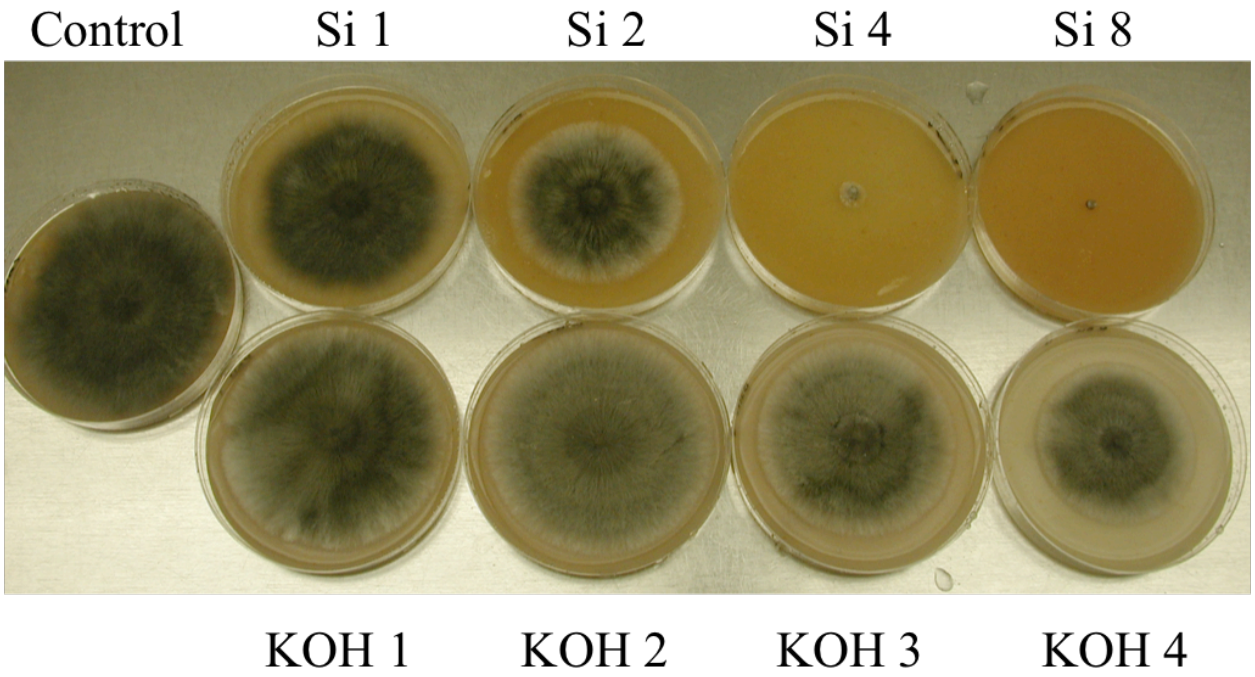


Figure 3-12 Growth inhibition of *Exserohilum rostratum in vitro* on medium containing soluble silicon. Silicon concentrations Si 1: 34 mM, Si 2: 69 mM, Si 4: 139 mM, Si 8: 277mM. Media KOH 1-KOH4 were adjusted to the same pH as the four silicon-amended media at the start of the experiment. Note that the fungus did not grow on media with higher silicon concentrations, while there was still growth on KOH media adjusted to the same pH.

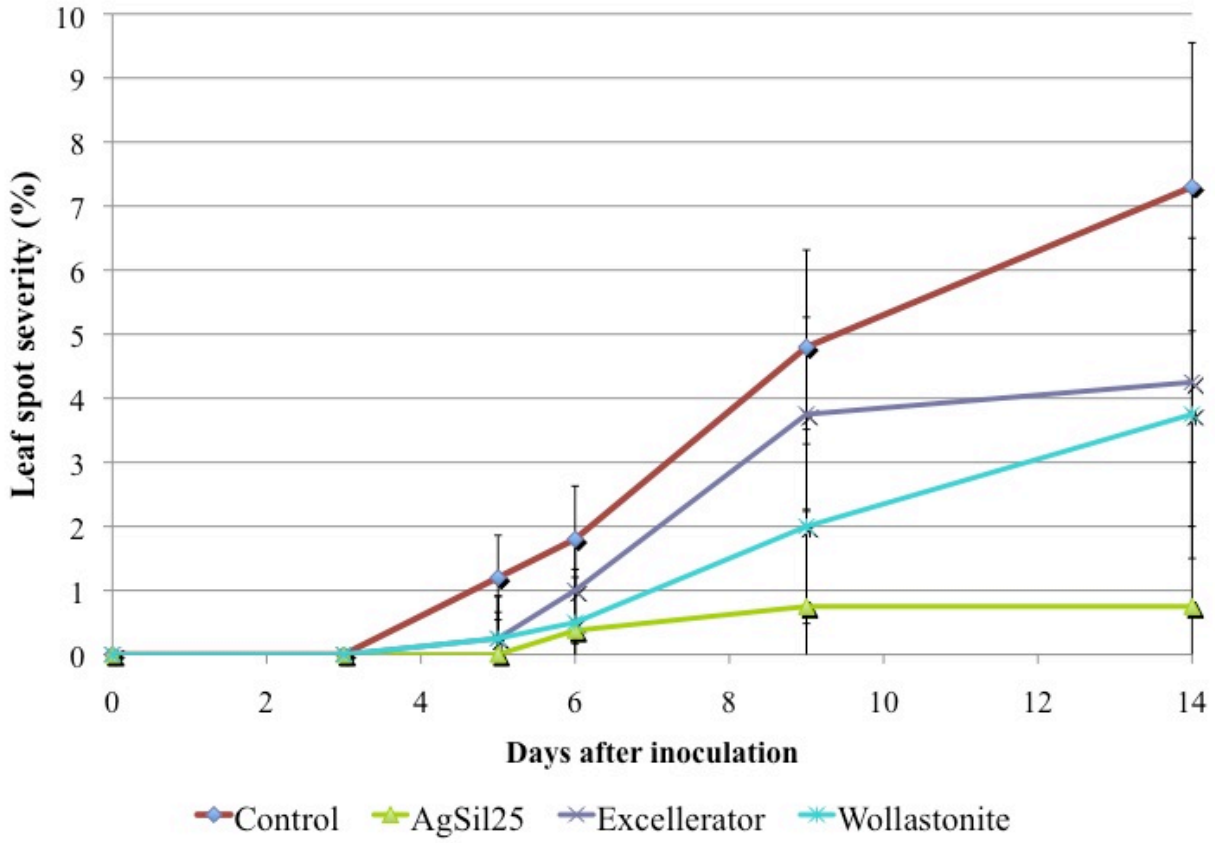


Figure 3-13 Development of tigergrass leaf spot severity caused by *Exserohilum rostratum* ($5 \times 10^3$) over a 14-day period. Data were arcsine transformed before anova was performed, first experiment.

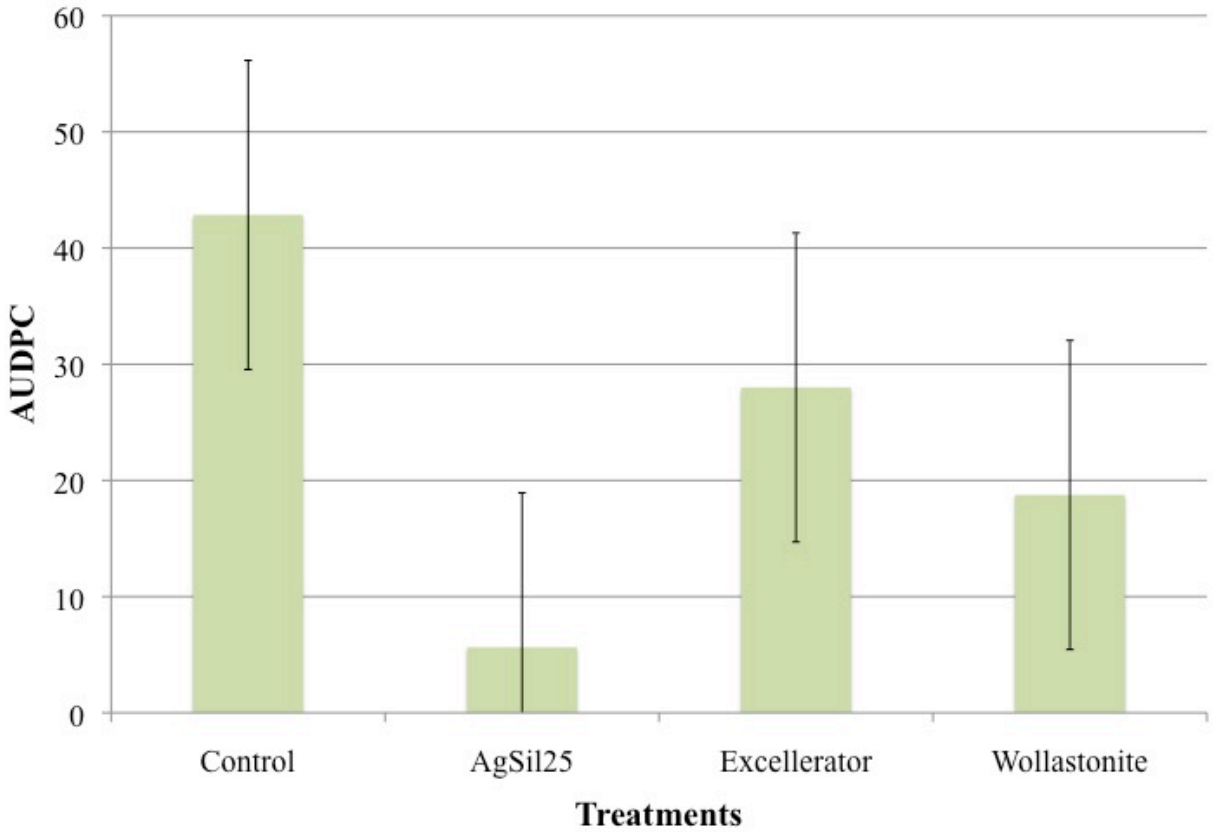


Figure 3-14 Final Area Under Disease Progress Curve (AUDPC) for tigergrass leaf spot caused by *Exserohilum rostratum*, first experiment.

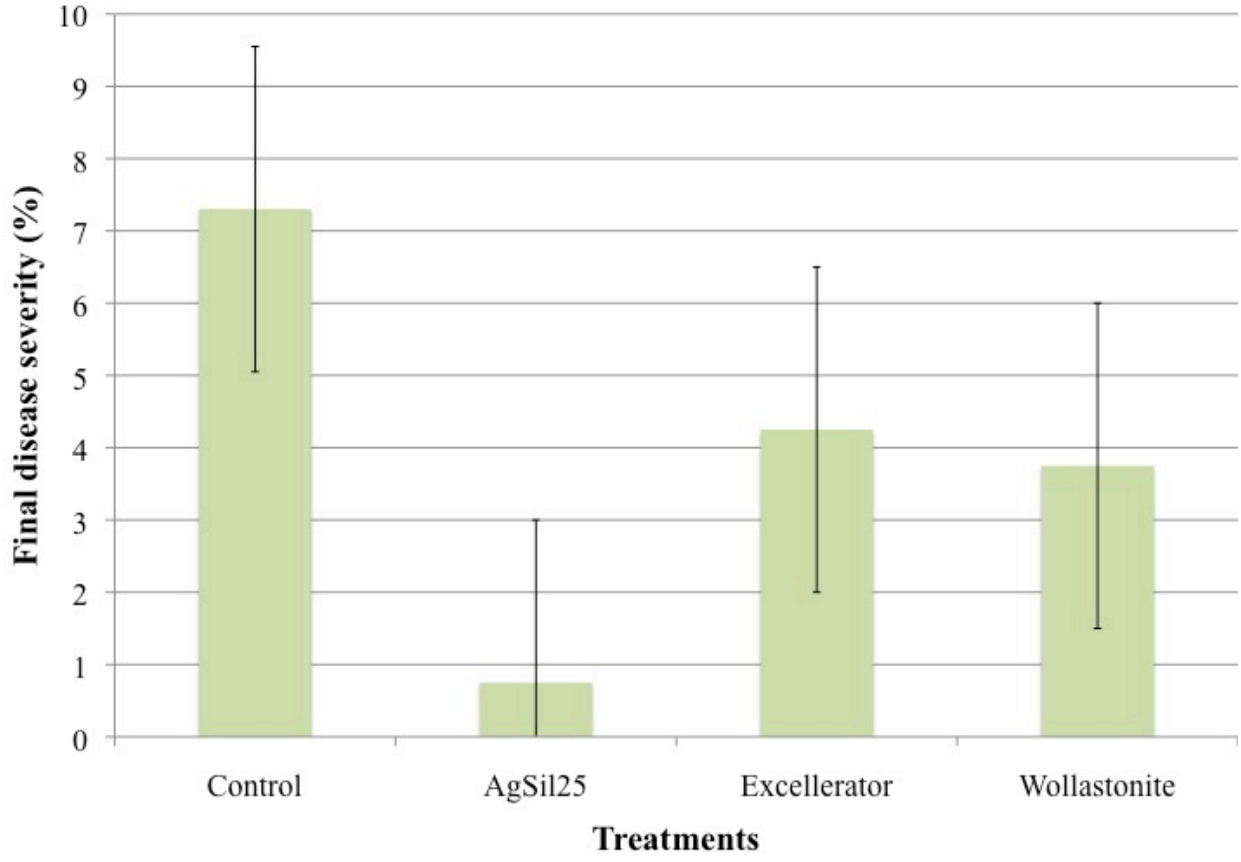


Figure 3-15 Final tigergrass leaf spot severity caused by *Exserohilum rostratum*, first experiment.

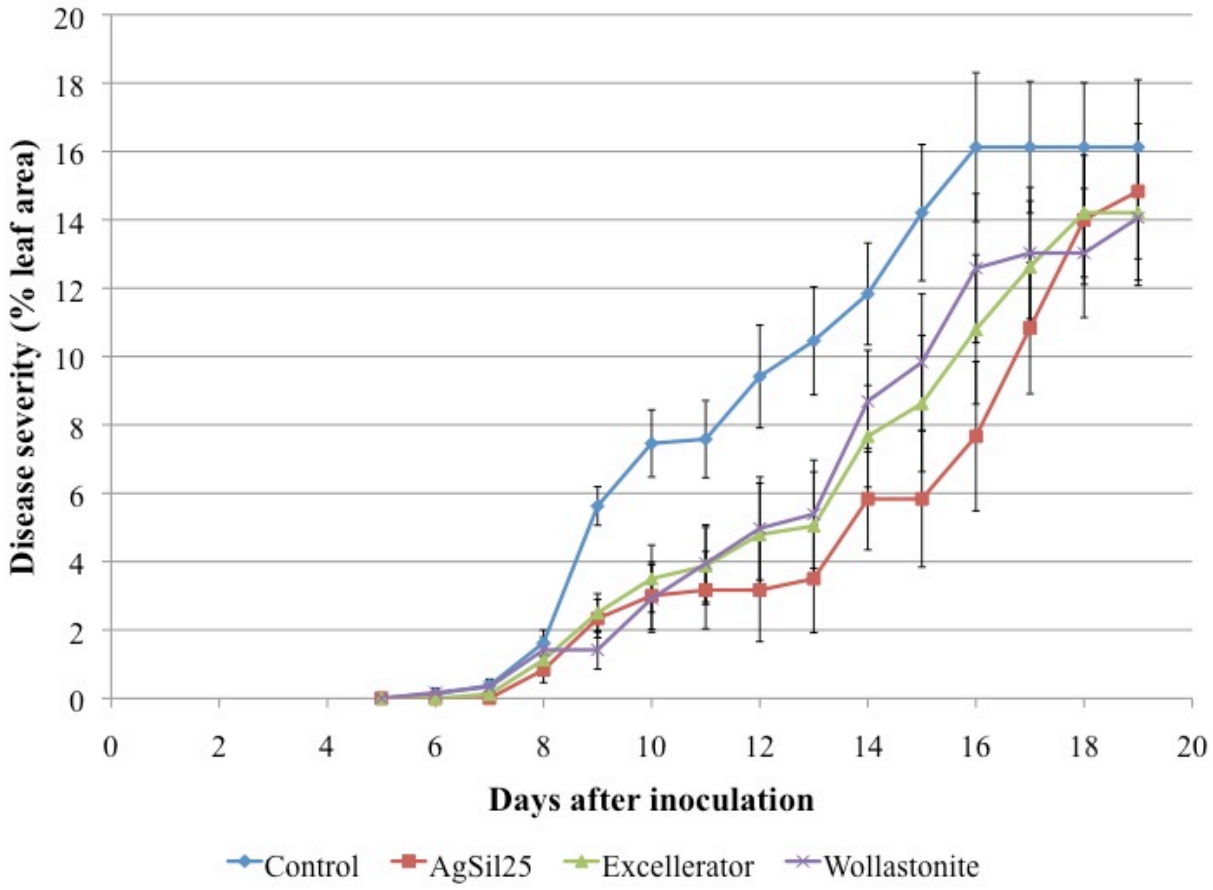


Figure 3-16 Development of tigergrass leaf spot severity caused by *Exserohilum rostratum* ($5 \times 10^3$) over a 19-day period. Data were arcsine transformed before anova was performed, second experiment.

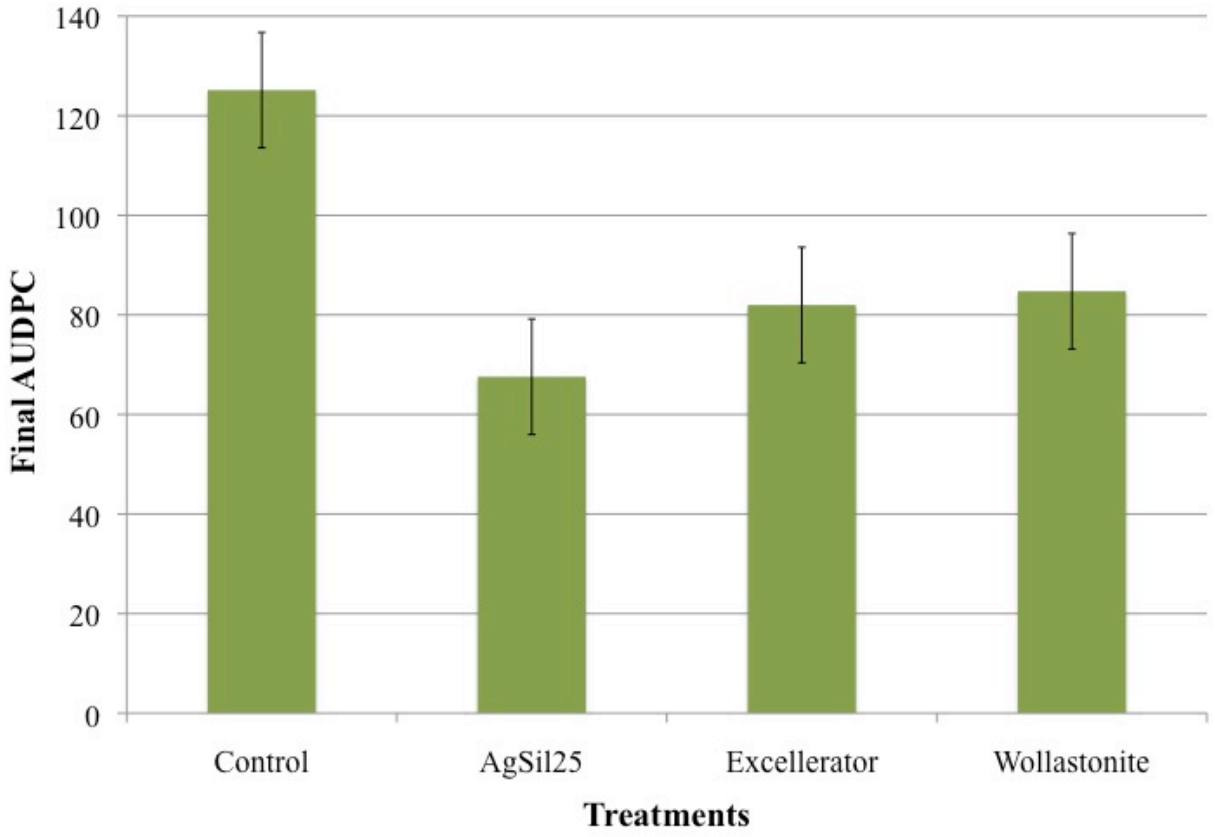


Figure 3-17 Final Area Under Disease Progress Curve (AUDPC) for tigergrass leaf spot, 19 days after inoculation, second experiment.

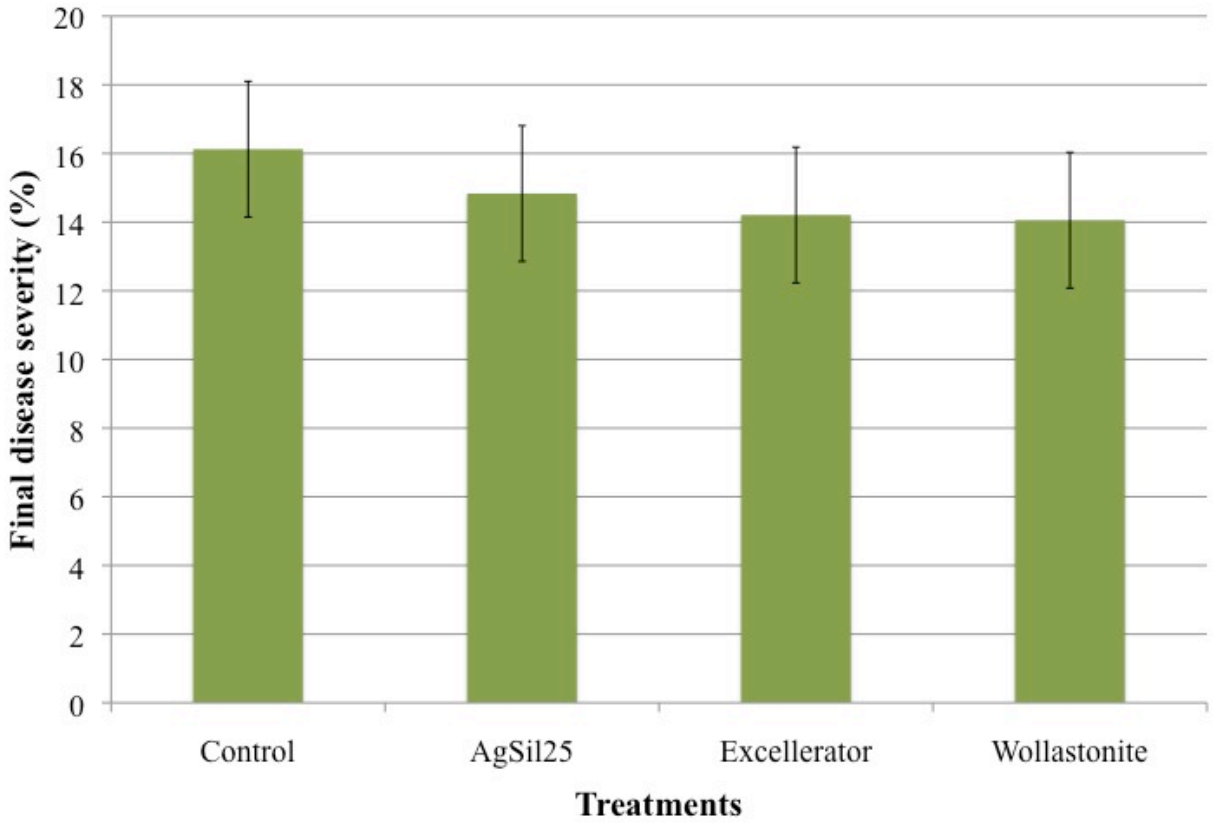


Figure 3-18 Final disease severity of tigergrass at 19 days after inoculation with *Exserohilum rostratum*, second experiment.

CHAPTER 4
EFFECT OF SILICON AMENDMENT ON GENE EXPRESSION PATTERNS IN RICE

## Introduction

Rice cultivars lacking major resistance genes are nevertheless highly resistant to the rice blast pathogen *Magnaporthe grisea* (T.T. Hebert) M.E. Barr when supplemented with silicon, comparable to the level obtained by application of a fungicide (Datnoff and Rodrigues 2005). Seebold et al. (2004) showed that in upland rice, leaf and neck blast could be controlled with silicon at least as effectively as with fungicide application, if disease severity was low. At high disease severity, fungicide applied at 10% the normal rate in addition to silicon amendment was sufficient to control the disease. Sheath blight of rice caused by *Rhizoctonia solani* J.G. Kuhn was reduced between 17% and 82% by silicon application equivalent to 10 t/ha, depending on the inherent resistant of the rice cultivar (Rodrigues et al. 2001).

Two main hypotheses have been proposed to explain the resistance of silicon-amended plants to infection by fungal pathogens. One hypothesis proposes that the insoluble silicon layer deposited on the epidermal cells prevents fungal penetration (Volk et al. 1958), and is referred to as the "mechanical barrier hypothesis." An alternative hypothesis proposes that silicon affects the response of the rice plant at a biochemical level. For example in cucumber, Chérif et al. (1992) found that resistance against *Pythium ultimum* was enhanced even though silicon did not accumulate at penetration points under saturated humidity. Evidence for both hypotheses has been reported.

In support of the physical barrier hypothesis, X-ray analysis (Kim et al. 2002) and NMR spectroscopy (Park et al. 2006) showed the presence of a layer of silicon in the epidermal cell wall of rice leaves, with a gradient of increasing amounts of silicon towards the outside of the cell (Kim et al. 2002). Kim et al. (2002) did not detect any silicon in the epidermal cytoplasm,

but Neumann and De Figueiredo (2002) reported on the presence of silicon in the cytoplasm of several heavy-metal tolerant plants and *Arabidopsis thaliana*. Carver et al. (1987) found that higher silicon levels correlated with an increase in failed fungal penetrations. Several lines of evidence support the role of silicon as a molecular signal. 1) The activation of the terpenoid pathway was indicated by the accumulation of diterpenoid isoprenoid phytoalexins in rice leaves of plants amended with silicon and inoculated with *M. grisea* (Rodrigues et al. 2004). 2) The surrounding of fungal hyphae *in planta* by an amorphous phenolic-like compound was associated with, and served as another indicator of events that played a role at a molecular level in the silicon-mediated blast resistance of rice (Rodrigues et al. 2003a). 3) The inoculation of silicon-amended rice plants with *M. grisea* resulted in the differential expression of a number of known plant defense genes (Rodrigues et al. 2005).

Fauteux et al. (2006) were the first to study the interaction at a genome wide level between silicon amendment and a fungal plant pathogen. In a microarray study that involved the entire *A. thaliana* genome, they found a limited number of differentially expressed genes in silicon-amended plants compared to control plants, while powdery mildew (*Erysiphe cichoracearum* DC)-inoculated plants compared to control plants had almost 4000 differentially expressed genes. They concluded that silicon had no direct effect on *A. thaliana* metabolism, and that the effect of silicon was limited to attenuating the reaction of the host to pathogen infection, by decreasing the number of differentially expressed genes in response to pathogen infection in silicon-amended plants.

The objective of the study reported in this chapter was to describe the effect of silicon on the molecular response of rice to pathogen infection on a genome-wide scale. To this end, microarrays containing ~44,000 probes representing 41,863 transcripts were used in a loop

design to identify genes differentially expressed in the rice cultivar Monko-to with and withouth silicon amendment in response to inoculation with *M. grisea* 86-137. Differentially expressed genes were identified in silicon-amended plants and pathogen-inoculated plants versus control plants, and in the interaction between silicon-amendment and pathogen-inoculation.

## Materials and Methods

The technical part of this experiment was performed in Japan, in the laboratory of Dr. Jian Feng Ma. This chapter focuses on the analysis of the data performed at the University of Florida. Rice (japonica cultivar Monko-to) was grown hydroponically in half-strength Kimura B solution with (2 mM) or without silicon [treatments Si and C (control), respectively]. At the four-five leaf stage, half of both the control and silicon-amended plants were inoculated with the rice blast pathogen, *M. grisea* 86-137. This resulted in four treatments (Table 4-1). Monko-to contains no known major resistance genes against *M. grisea*, and is highly susceptible without silicon amendment.

Whole plants of each treatment were harvested 24 hours after inoculation, and immediately frozen in liquid nitrogen for mRNA extraction. cDNA was prepared from all the mRNA populations by reverse transcription, divided into two groups each, one of which was labeled with Cy-3 (labeled blue in Figure 4-1), the other with Cy-5 (labeled red). The experiment was set up in a loop-design that hybridized two cDNA population samples labeled with different colors to the same slide (with a duplicate slide containing the reverse color labels). This resulted in 8 slides as indicated in Figure 4-1. The cDNA from control plants was thus hybridized to the same slide as silicon-amended plants (C vs Si), and to the same slide as pathogen-inoculated plants (C vs P). Silicon-amended and pathogen-inoculated plants were also hybridized to the same slide as silicon-amended-and-pathogen-inoculated plants (Si vs SiP and P vs SiP, respectively).

Spot intensity data was normalized using quantile normalization (Bolstad et al. 2003), followed by mixed-model analysis of variance (Littell et al. 1996) with treatment as a fixed effect. Significant differentially expressed genes were identified using a t-test (Jin et al. 2001) and corrected for multiple tests using a modified false-discovery rate using the Bioconductor QVALUE software packages (Storey 2002; Storey and Tibshirani 2003; Storey et al. 2004) running under the R 2.7.2 environment for statistical computing and graphics (Ihaka and Gentleman 1996; http://www.r-project.org/).

Differentially expressed genes for each treatment comparison was based on both the significance to the q-value calculated by QVALUE, and the foldchange in expression. Genes with a q-value<0.001 were selected, and further narrowed down by taking only into account genes with greater than 3-fold up- or down regulation. Data were plotted using R. (Ihaka and Gentleman 1996; http://www.r-project.org/).

## Results

The Volcano plots in Figures 4-2 through 4-5 illustrate the distribution of data points, the significance of the measured differences in expression levels, and the selection of differentially expressed genes for each of the four comparisons. Each dot represents one of 41863 spots on the microarray. The black dots represent genes with a low significance ($q \geq 0.001$), the dotted horizontal line is $q=0.001$. The blue dots represent spots that have $q<0.001$, but a foldchange<3. The red dots are the genes selected as differentially regulated ($q<0.001$, foldchange>3). The number of differentially expressed genes for each of the arrays is indicated in Figure 4-1 as the number of up- and down-regulated genes for each pair of treatments compared. Differentially expressed genes were divided into categories based on known or suspected function or activity. The distribution of differentially expressed genes for each comparison of treatments into defense genes, unknown genes, and other genes, is in Table 4-3 and Figure 4-6. The category of "other"

genes is further divided and differentially expressed genes in each of the categories are listed in appendices A through J with their expression profiles (whether the genes up and/or downregulated for each of the treatment comparisons).

**Silicon amendment**

In plants that were grown in the presence of silicon, 221 genes were differentially expressed compared to control plants. Of these, 105 genes were upregulated, and 116 genes were downregulated (Figures 4-1 and 4-2). Nine of the upregulated and 19 of the downregulated genes are known or implicated to be involved in defense and stress pathways.

Among the genes that are differentially regulated in silicon-amended plants compared to control plants are a metal transporter-encoding gene (Os07g0258400), peroxidase precursor-encoding genes (Os01g0378100, Os01g0963200, Os03g0235000), 11 putative plant resistance proteins, and a Type-1 pathogenesis related protein-encoding gene (Os10g0191300). Among the transcription factor-encoding genes that are differentially regulated in the silicon-amended plants, six genes are unique, and three are WRKY transcription factor-encoding genes. There are many genes differentially regulated that are involved in cellular housekeeping processes, their expression profiles are listed in appendix E. Forty-three percent of all differentially expressed genes are of unknown function.

**Pathogen inoculation**

Not surprisingly, a large number of differentially expressed genes in this study were in the comparison of control plants and pathogen-inoculated plants (738), most were upregulated (618). Among the differentially expressed gene in response to pathogen-inoculation, 59 were upregulated and 13 were downregulated defense or stress-related genes (Table 4-3, Figure 4-6, appendix A), 65 were upregulated transcription factor-encoding genes, and 13 were downregulated transcription factor-encoding genes (appendix B), and 20 genes involved in

hormone signaling pathways (appendix D). Interestingly, all genes that were categorized as calcium signaling related, were upregulated in the pathogen-inoculated plants compared to the control plants (appendix H). Also 65 genes were identified as kinase/phosphatase encoding genes (59 upregulated, 6 downregulated). Thirty seven percent of differentially regulated genes in response to the pathogen were unknown.

**Silicon-pathogen interaction**

The main purpose of this study was to investigate the interaction between silicon amendment and pathogen inoculation on the transcriptional profile of rice. The comparisons Si vs SiP, and P vs SiP illustrate this interaction (Figures 4-1, compare Figure 4-4 with Figure 4-5, Figure 4-6). When the response of the plant to pathogen infection was compared between control (C vs P) and silicon-amended plants (Si vs SiP), the silicon-amended plants responded with 298 differentially expressed genes, while the control plant responded with more than 738. P vs SiP compared two groups of pathogen-inoculated plants, and showed that there was a difference between silicon-amended plants and non-amended plants. In silicon-amended rice, 63 genes were upregulated in response to infection bv *M. grisea*, while 123 genes were downregulated compared to non-amended rice, of which 54 genes were unique. Compared to 59 upregulated pathogenicity/stress and 13 downregulated related genes in response to pathogen inoculation of control plants, only 30 were upregulated in response to pathogen inoculation of silicon-amended plants, and two were downregulated. Similarly, the number of transcription factor-encoding genes in response to pathogen inoculation of silicon-amended plants (32 upregulated and two downregulated genes) was lower than the 65 upregulated and 13 downregulated once in response to inoculation of non-amended ones.

Two genes putatively involved in the auxin signaling pathway (auxin conjugate hydrolase encoding Os01g0706900 and auxin responsive SAUR family protein encoding Os08g0452500)

were upregulated in response to pathogen inoculation non-amended plants, but not in response to pathogen inoculation of silicon-amended plants. Of the 18 genes encoding putative calcium signaling pathways that were upregulated in response to pathogen inoculation of control plants, ten were upregulated upon inoculation of silicon-amended plants.

## Discussion and Conclusions

From the volcanoplots derived from the data collected in this study, it is clear that a large number of genes were significantly differentially expressed. There were also genes that had a large but not significant foldchange. It was therefore crucial to base initially the cutoffs on significance values, and narrow down the pool of differentially expressed genes by thresholding the foldchange cutoff. Dividing the treatment plants at least in groups of two before isolating mRNA, instead of isolating mRNA from the pooled plants, would have resulted in two biological replicates of each sample.

Evidence for a role of silicon as molecular signal was apparent from the differential expression of 221 genes in the control versus silicon-amended (C vs Si) comparison, and the fact that 28 of those genes are known or suspected to be involved in defense or stress response by the plant. The differential expression of a metal transporter gene (Os07g0258400) is of interest because silicon is reported to alleviate heavy metal stress (Ma et al. 2001b; Williams and Vlamis 1957). Heavy metal transport/detoxification protein-encoding genes were differentially regulated; however, one was unique to the Si-SiP comparison (expressed in response to pathogen infection of silicon-amended plants) and three in response to pathogen infection of non-amended plants.

Gene expression in silicon-amended rice was markedly different from expression in non-amended plants, which indicates that silicon-amendment had an effect on metabolic activity in

rice. This is in contrast to the results obtained in a similar study done with the *Arabidposis thaliana*-powdery mildew interaction (Fauteux et al. 2006), which found only two genes differentially expressed in silicon-amended plants compared to control plants. One of these encoded an esterase lipase thioesterase family protein, the other a multicopper oxidase type I family protein (At1g21860); a weak homolog (25% identical, 44% similar) of the latter was also downregulated (Os11g06415) in silicon-amended rice. Fauteux et al. (2006) concluded that "Si alone has apparently no effect on the metabolism of plants growing in a controlled environment (e.g., unstressed), thus confirming its nonessentiality in plant growth; supplying silicon alleviates the stress such as one imposed by a pathogen." From the results of the experiment described in this chapter, it appears that silicon differs from *A. thaliana* in this respect and does affect rice metabolism. This may be a difference in the role of silicon between *Arabidopsis* and rice, and/or a difference in relationship between the host plants and their respective pathogens in the systems studied. Powdery mildew of *Arabidopsis* is caused by *Erysiphe cichoracearum*, an obligate parasite, while *M. grisea* is a hemi-biotroph initially acting as a biotroph, and switching to a necrotrophic mode of pathogenicity. A biotroph requires the host cells to be alive, whereas a necrotroph seeks to kill the host and live off the dead material.

The differences found between the transcriptional response of the rice-rice blast and the *Arabidopsis*-powdery mildew systems could also be a reflection of the fact that silicon appears to have many benefits to rice, including resistance against lodging, higher yields, greater requirement for silicon during flowering and seed-set (Datnoff et al. 2001, Epstein 1991, Ma et al. 1989, Savant et al. 1997) while increased resistance of *A. thaliana* to powdery mildew is the only reported benefit of silicon in *A. thaliana* (Ghanmi et al. 2004).

The results described herein are harder to reconcile with the report by Watanabe et al. (2004) who did an experiment using ~9000 rice genes. They found 20 candidate transcripts differentially regulated, and confirmed five by reverse transcription polymerase chain reaction. They included transcripts for a zinc finger protein, a metallthionein-like protein, chlorophyll a/b binding protein, XA21 family member, and a carbonic anhydrase. The methodology used in Watanabe's study was very different than the one used in this study, and therefore it is hard to draw parallels or far-reaching conclusions on comparing the two studies. The arrays Watanabe et al. (2004) used contained less than 9000 transcripts, each sample was hybridized to one sample, and transcriptional levels were determined by comparison with tubulin and actin expression levels, and conclusions were drawn based on foldchange only. All the genes that were upregulated in the study by Watanabe et al. (2004) were represented on the microarrays used in this study. The transcript corresponding to the zinc finger-encoding gene Watanabe et al. (2004) found upregulated, was not upregulated in the study described herein, and neither was the chlorophyll a/b binding transcript, the metallothioneinlike protein- or the carbonic anhydrase transcript.

In this study, like the study by Watanabe et al. (2004) resistance gene family members were differentially expressed in silicon-amended plans compared to control plants. For example, Os11g0625900, is downregulated in both C vs Si, and C vs P plants in this study. The level of downregulation in silicon-amended compared to control plants in this study (1.14-fold) is comparable to the level found by Watanabe et al. (2004) in silicon-amended plants, but in response to pathogen infection, it decreases 14.9 fold in this study.

Compared to the results obtained by Rodrigues et al. (2005), this study confirms the involvement of chitinases in the defense response of rice after *M. grisea* inoculation, although

the timing is different between the two studies. Rodrigues et al. (2005) did not see significant chitinase transcript accumularion in northern blot analysis until 36 hours after inoculation in non-amended plants, while in this study there is an increase already at 24 hours after inoculation. As in the study by Rodrigues et al. (2005) this increase was not apparent in silicon-amended plants. Again in agreement, this study showed that phenylalanine lyase and peroxidases are upregulated after pathogen infection of non-amended plants. Regarding PR-1 gene expression, no conclusions can be drawn by comparing the two studies, since Rodrigues et al. (2005) did not see any difference at 24 hours after inoculation.

Defense/stress-related genes differentially regulated in the P vs SiP comparison are of particular interest, because they identify the differences in gene expression after pathogen inoculation which is associated with the presence of silicon. Among these were genes potentially part of the ethylene signaling pathway, a gene encoding a thaumatin/pathogenesis-related protein (Os12g0568900) predicted to be secreted (Nakai and Horton 1999), a class III peroxidase (Os07g0677500), and a number of transcription factors, and protein kinases that are not differentially expressed in the C vs P comparison.

More than simply attenuating the plant response to the pathogen as Fauteux et al. (2006) concluded in the *Arabidopsis*-powdery mildew experiments, a substantially different pattern of expression was noted in this experiment. Not only did the Si vs SiP comparison have 440 differentially expressed genes *less* than the C vs P comparison, the two comparisons had only 236 genes in common. In the rice-rice blast system, silicon affected the interaction between host and pathogen at the molecular level.

Since a number of defense/stress response-related genes were differentially regulated, a possibility is that silicon-amendment is responsible for preconditioning plants to react to stress.

This seemed to be reflected in the fact that infection with *M. grisea* resulted in less than half the number of differentially expressed genes in silicon-amended plants than in non-amended plants (298 vs 738). There are several possibilities to interpret the response: 1) silicon attenuated the reaction to the pathogen infection by directly preventing differential expression of rice genes, 2) genes differentially expressed in silicon-amended plants acted directly on the pathogen effectors, attenuating the effect they have on the rice plant, which then indirectly resulted in less genes being differentially regulated, 3) targets of the pathogen effector genes may be turned off by silicon amendment, blocking the pathogen's access to signal transduction pathways required for pathogenicity, 4) genes differentially expressed in silicon-amended plants may not require further differential regulation upon pathogen infection. These possibilities are not mutually exclusive.

In conclusion, in rice 221 genes are differentially regulated when amended with silicon compared to control plants suggesting that silicon may be an integral part of rice physiology, and that silicon might be essential for rice. Silicon amendment changes the rice response to rice blast infection at the gene transcription level, implying a role for silicon in one or more plant defense signaling pathways.

Table 4-1 Microarray experiment treatments

| Treatment[z] | Silicon amendment | Pathogen inoculation |
|---|---|---|
| C | − | − |
| Si | + | − |
| P | − | + |
| SiP | + | + |

[z]Control plants (C) were not amended with silicon, nor inoculated with *M. grisea*. Treatments consisted of plants amended with silicon (Si), inoculated with *M. grisea* (P), or both (SiP).

Table 4-2 Number of up- and down regulated genes unique for each comparison of treatments

| Treatment Comparison | Upregulated | Downregulated | Total |
|---|---|---|---|
| C-Si | 16 | 35 | 52 |
| P-SiP | 29 | 25 | 54 |
| C-P | 273 | 39 | 312 |
| Si-SiP | 42 | 12 | 54 |

Table 4-3 Categories of differentially expressed genes for each treatment comparison

| | Up/Downregulation | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Defense | Up | 9 | 6 | 59 | 30 |
| Defense | Down | 16 | 8 | 13 | 2 |
| Other | Up | 50 | 25 | 348 | 156 |
| Other | Down | 45 | 74 | 48 | 14 |
| Unknown | Up | 46 | 32 | 211 | 86 |
| Unknown | Down | 55 | 41 | 59 | 10 |
| Total | | 221 | 186 | 738 | 298 |

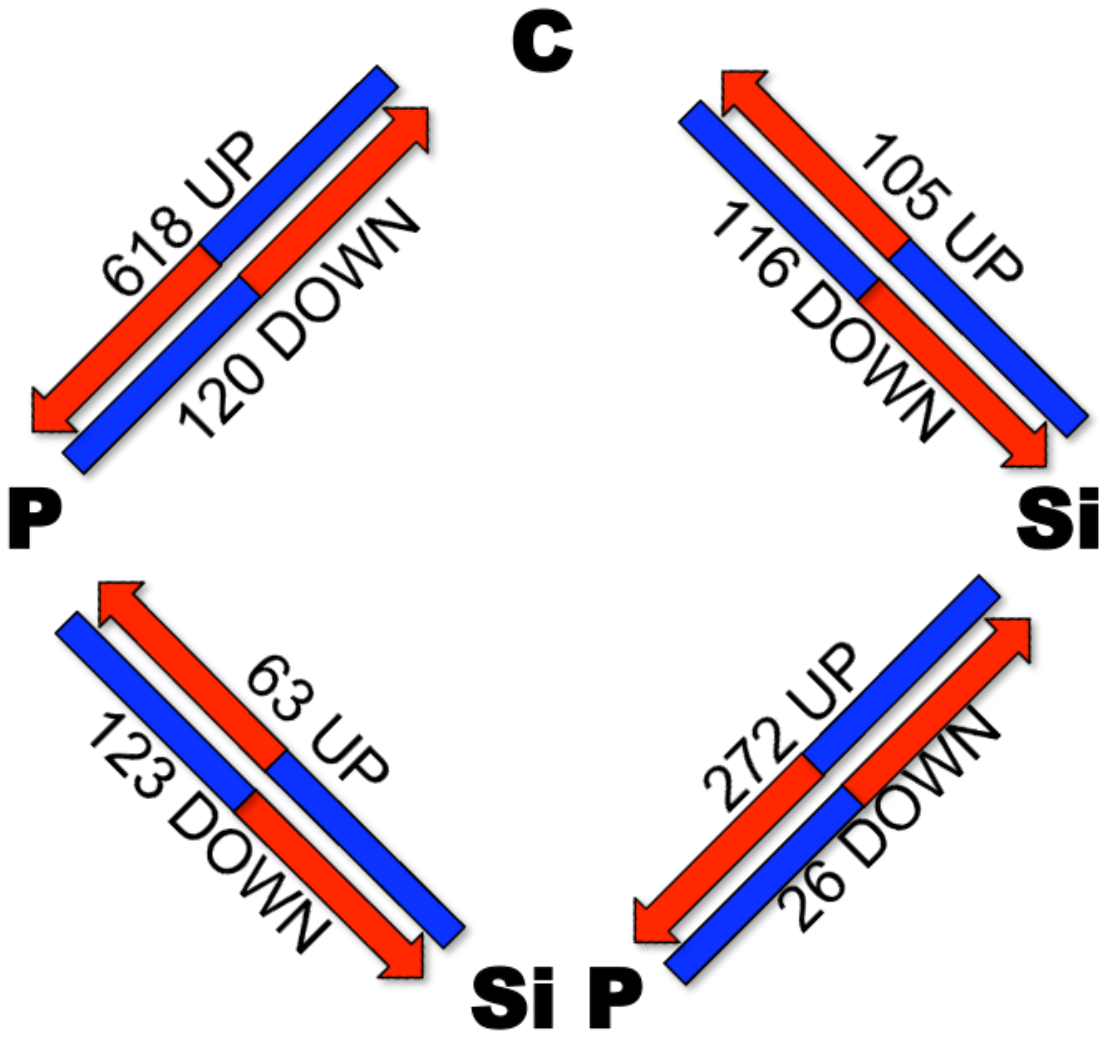


Figure 4-1 The number of differentially expressed genes for each treatment comparison.

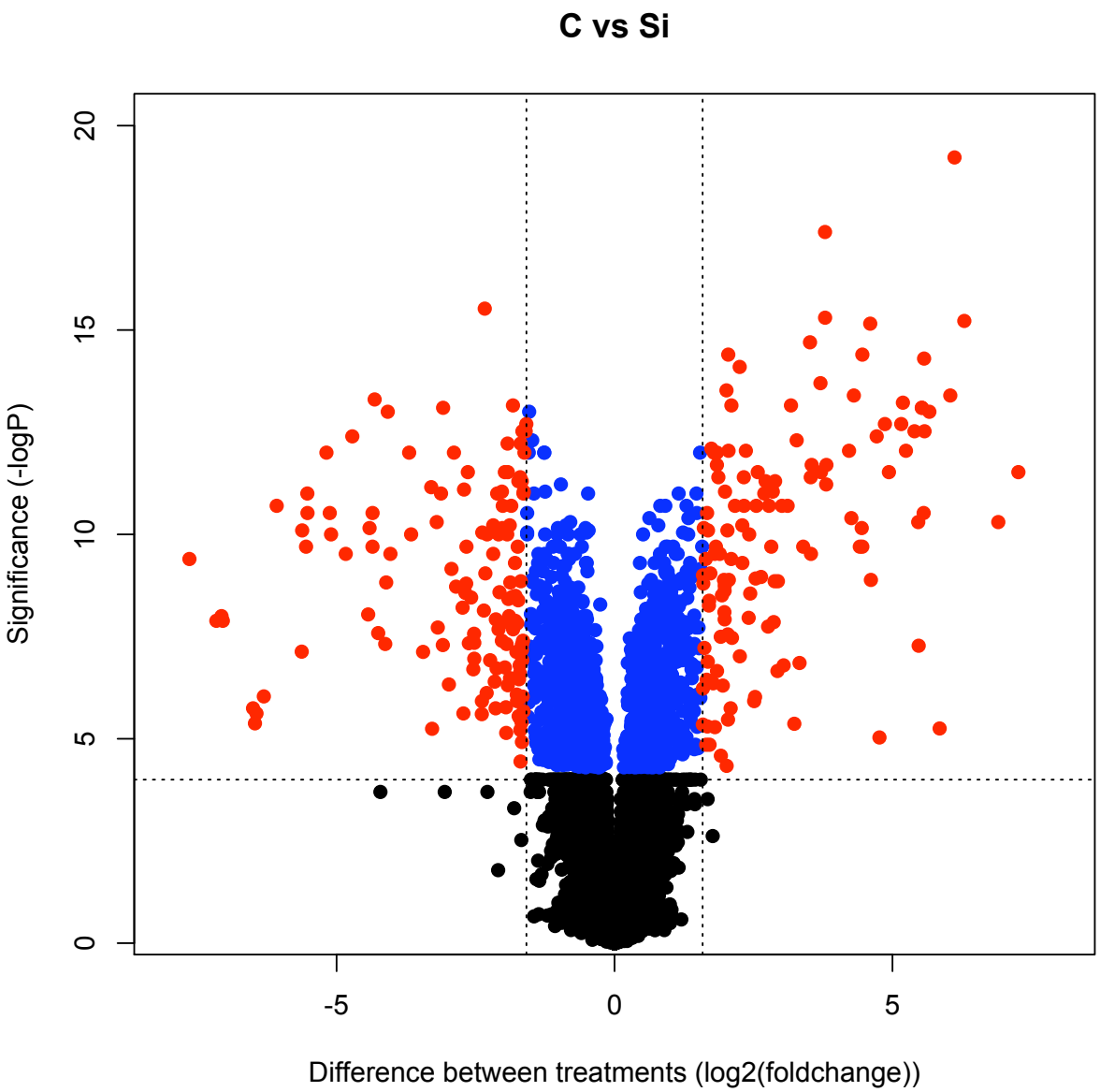


Figure 4-2 Volcanoplot C vs Si. The difference of expression levels of each array spot is represented by a dot. The level of expression is plotted on the x-axis as the log2 of the foldchange. Spots that have a higher expression level in silicon-amended plants compared to control plants have a positive difference, those with a lower expression level have a negative difference. The vertical lines represent 1/3 and 3-fold difference in expression. The horizontal line represents the cutoff point for declaring differences significant based on q, the adjusted P value for multiple tests ($q \geq 0.001$).

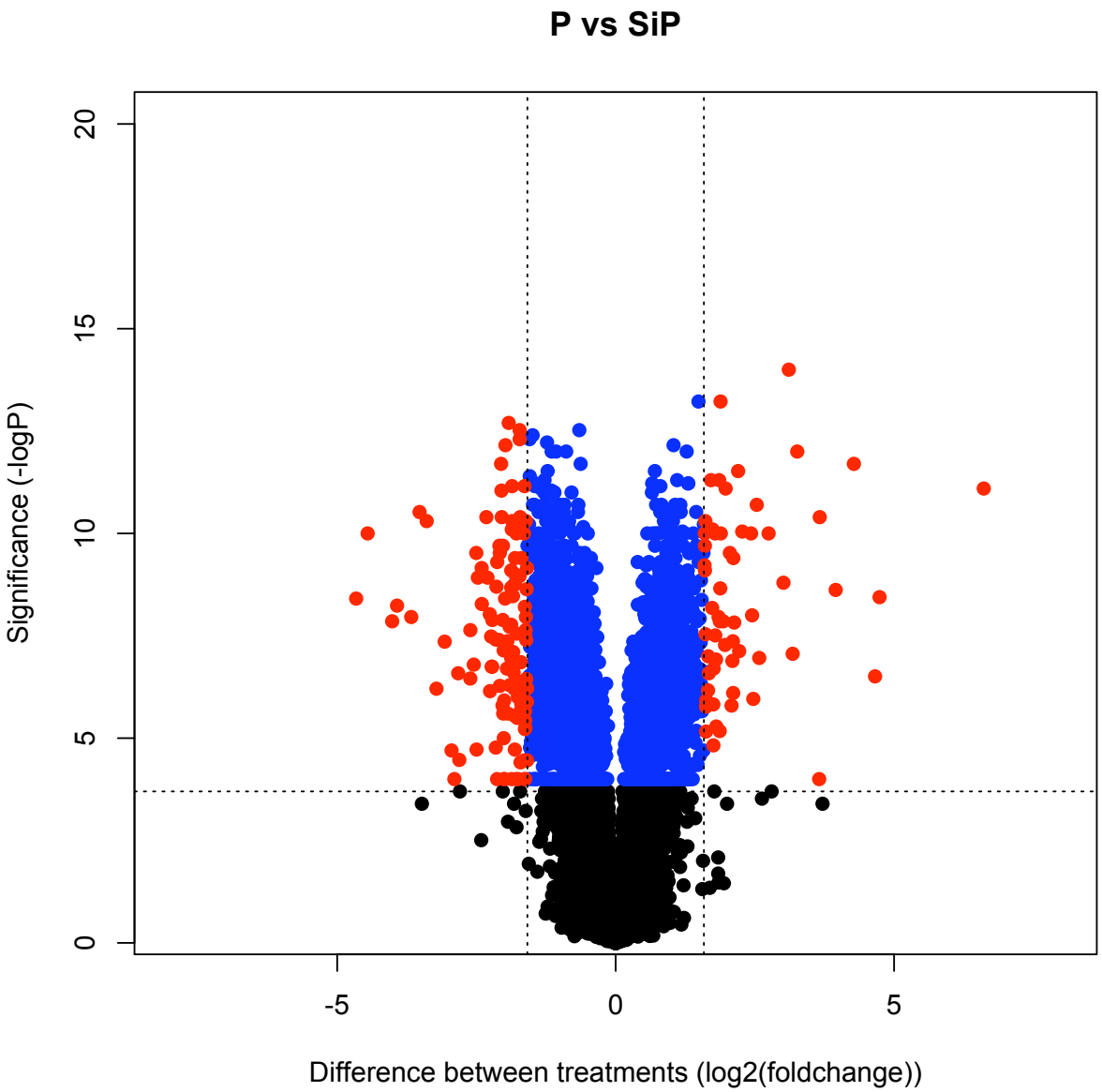


Figure 4-3 Volcanoplot P vs SiP. The difference of expression levels of each array spot is represented by a dot. The level of expression is plotted on the x-axis as the log2 of the foldchange. Spots that have a higher expression level in silicon-amended plants compared to control plants have a positive difference, those with a lower expression level have a negative difference. The vertical lines represent 1/3 and 3-fold difference in expression. The horizontal line represents the cutoff point for declaring differences significant based on q, the adjusted P value for multiple tests ($q \geq 0.001$).

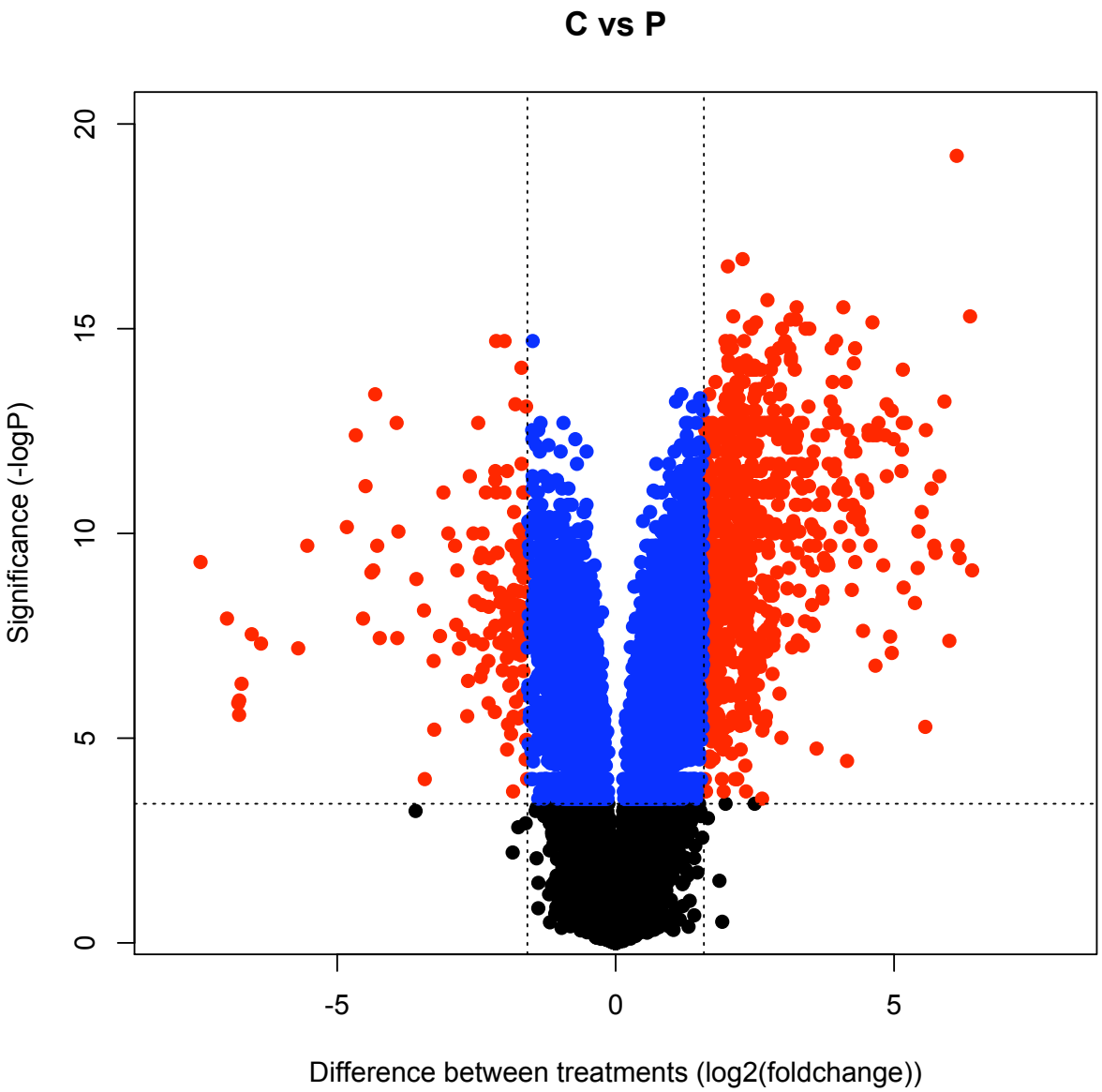


Figure 4-4 Volcanoplot C vs P. The difference of expression levels of each array spot is represented by a dot. The level of expression is plotted on the x-axis as the log2 of the foldchange. Spots that have a higher expression level in silicon-amended plants compared to control plants have a positive difference, those with a lower expression level have a negative difference. The vertical lines represent 1/3 and 3-fold difference in expression. The horizontal line represents the cutoff point for declaring differences significant based on q, the adjusted P value for multiple tests ($q \geq 0.001$).

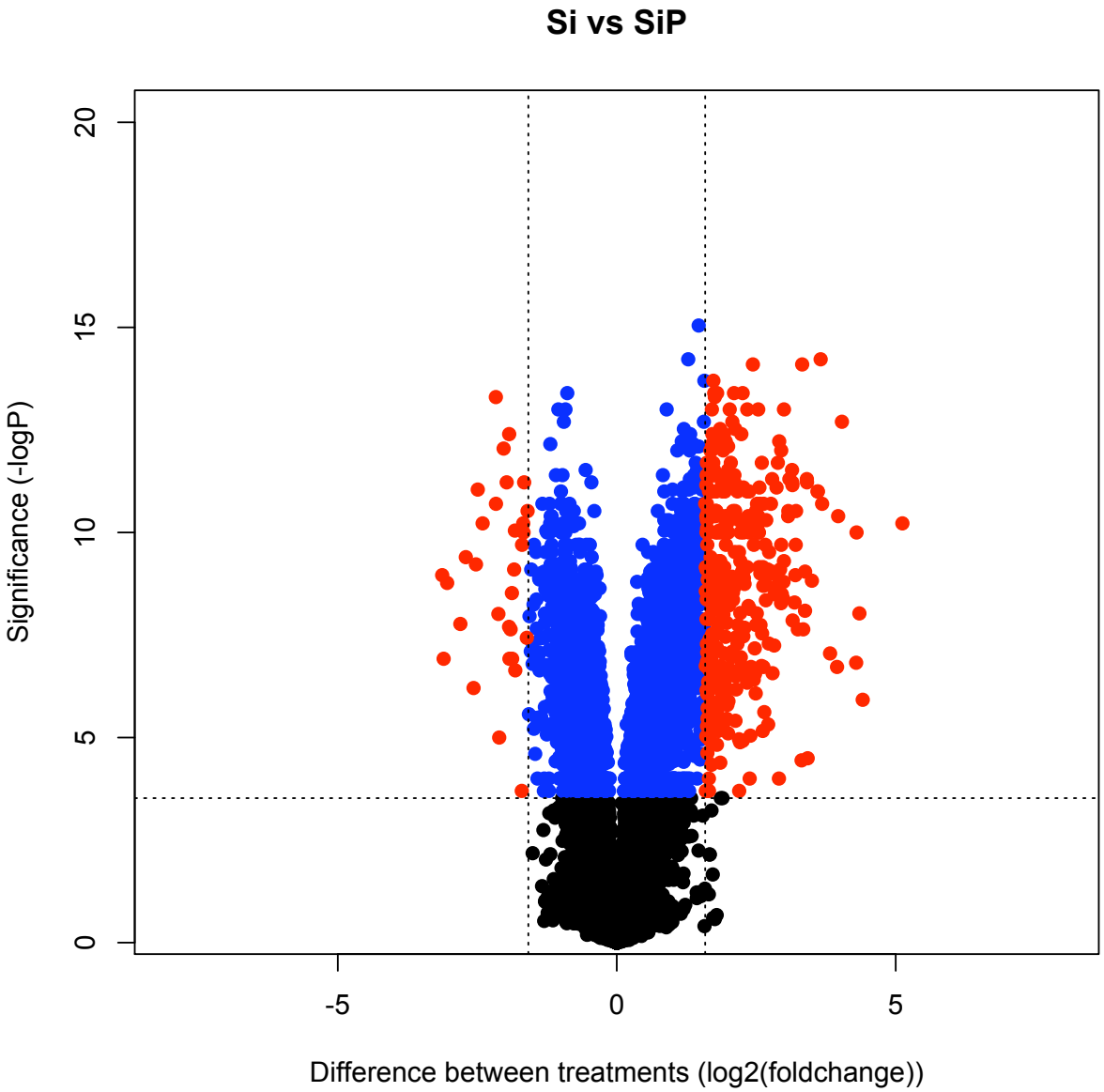


Figure 4-5 Volcanoplot Si vs SiP. The difference of expression levels of each array spot is represented by a dot. The level of expression is plotted on the x-axis as the log2 of the foldchange. Spots that have a higher expression level in silicon-amended plants compared to control plants have a positive difference, those with a lower expression level have a negative difference. The vertical lines represent 1/3 and 3-fold difference in expression. The horizontal line represents the cutoff point for declaring differences significant based on q, the adjusted P value for multiple tests ($q \geq 0.001$).

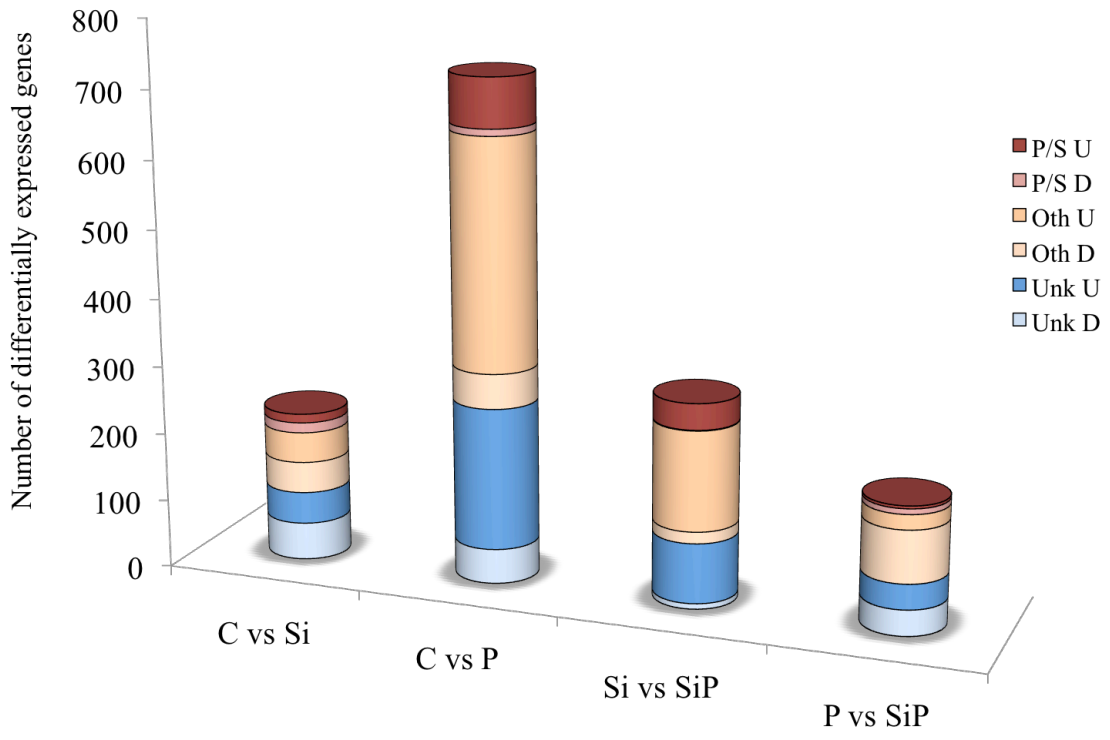


Figure 4-6 The distribution of differentially expressed genes for each of the treatment comparisons. P/S=pathogenicity/stress related genes, Oth=other genes, Unk=unknown genes, U=upregulated, D=downregulated.

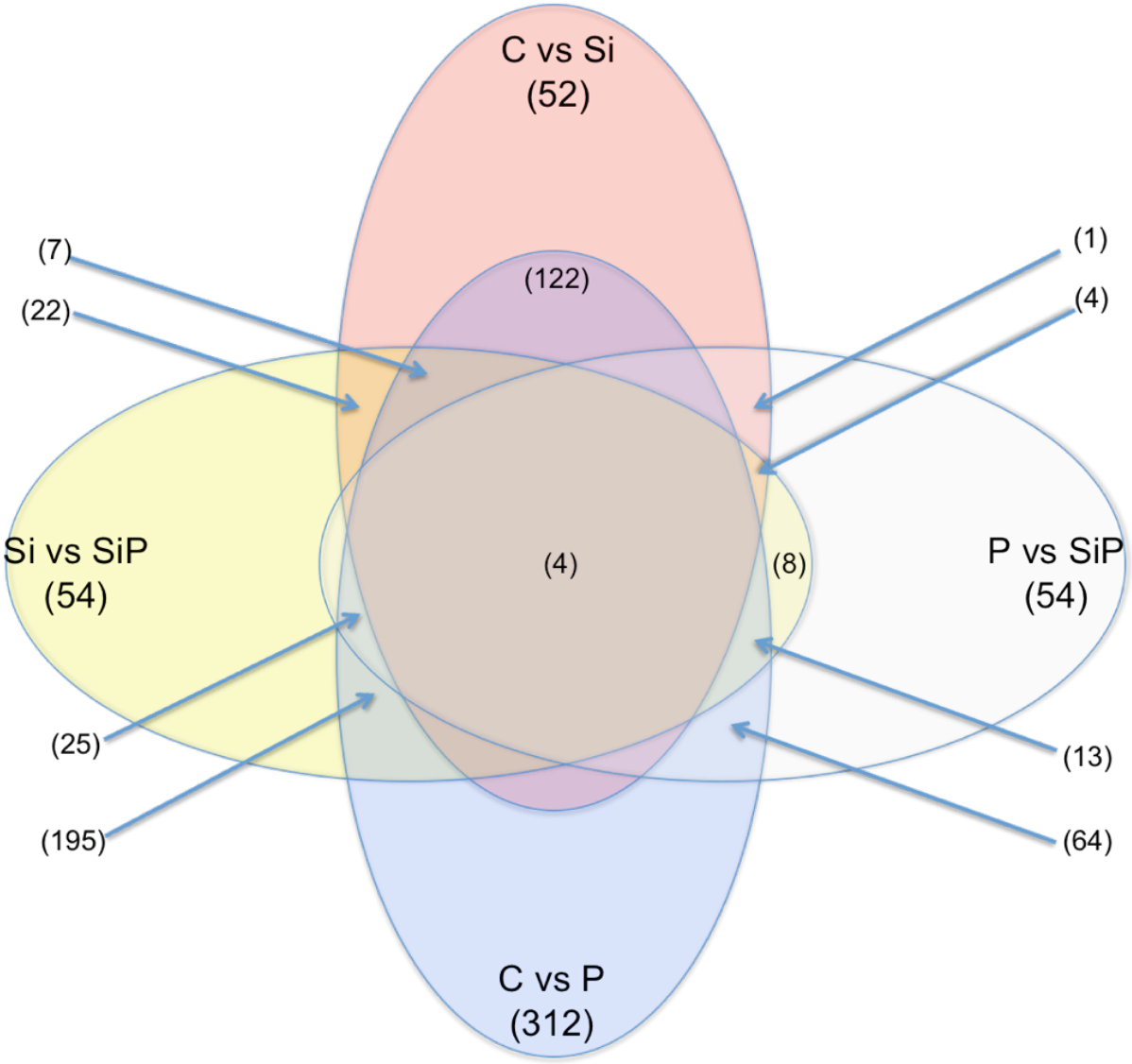


Figure 4-7 Number of unique and overlapping genes for each comparison combination.

# CHAPTER 5
# CONCLUSION

## Silicon in Ornamentals

A growing body of literature has reported on the beneficial effects of silicon in both the traditionally known accumulators of silicon, the monocotyledons (specifically graminaeceae), and dicotyledons. The research described herein was to measure the silicon concentration in plants grown in bedding or containerized systems that received silicon in the growing medium. Frantz et al. (2005) described silicon uptake by New Guinea impatiens in hydroponic systems. The results detailed in Chapter 2 however, imply that the uptake of silicon from nutrient solution in hydroponic systems is different from that in containerized systems with soilless medium. Locke et al. (2008) tested more than 30 floricultural crops, but found silicon accumulation only in zinnia, verbena, and sunflower.

The experiments described in this work did not show a difference in silicon concentration in begonia and marigold in silicon-amended plants compared to control plants; there was no evidence for a rate response. Although higher concentrations of silicon did occur when the plants were amended with AgSil25, from a practical point of view this is a not feasible technique. The high pH of AgSil25 increased the pH of the soilless medium to levels as high as 8.5, and the plants did not grow well, and often died. High pH may make micronutrients unavailable to plants by chemically converting the ions into plant-unavailable forms. The results of these studies suggested that there might be a limit in the practical use of AgSil25.

Application of AgSil25 would have advantages over the use of granular calcium silicate such as Excellerator, or the powdery source of calcium silicate Wollastonite. Both Excellerator and Wollastonite would need to be incorporated in the soilless medium before planting, while AgSil25 can be applied continuously through fertigation. Before AgSil25 can be used in such a

manner, studies would need to be performed that use smaller applications than the level of 1.40 kg elemental silicon/$m^3$. It is quite possible that the concentration of silicon needed to confer beneficial effects in ornamentals might be less if silicon is applied in a pre-dissolved form such as potassium silicate, which is instantly available to the plant, and can be applied as a drench together with liquid fertilizer. Therefore, lower concentrations of potassium silicate in the form of AgSil25 ought to be tested and confirmed by directly measuring silicon in the plant sap. In addition, the best way to buffer the medium needs to be studied. To offset the effects of AgSil25, including the potassium added, the formulation of the fertilizer would need to be adjusted.

The data support impatiens being an intermediate accumulator of silicon. The silicon concentration was higher in impatiens plants than in begonia and marigold and did show a small but statistically significant rate response. In addition, in an experiment where different sources of silicon were tested on impatiens, there was a 24% increase in silicon concentration. Additional experiments using impatiens with more replicates are necessary to confirm this conclusion.

The ornamental grass Tigergrass (*Thysanolaena maxima*) did accumulate silicon, and had a statistically significant rate response. In both experiments, the rate response fit a quadratic model. The rate-response curve for tigergrass was similar to those reported for turfgrasses (Datnoff and Nagata 1999; Datnoff and Rutherford 2003; Nanayakkara et al. 2008a,b). Tigergrass accumulated silicon to a level as high as 1.71 cg silicon/gm dry weight with silicon-amended at 1.87 kg elemental silicon/$m^3$. This is consistent with the fact that members of the family Poaceae such as barley (Williams and Vlamis 1957), rice (Datnoff et al. 2001), wheat (*Triticum aestivum* L.; Dietrich et al. 2003) and the turfgrasses mentioned above, accumulate silicon.

In conclusion, silicon amendment of begonia and marigold did not result in an increase in silicon concentration of the leaves in the context of resulting in marketable plants under the conditions used in this study. Impatiens did show a larger silicon concentration in silicon-amended plants, thus although the results were inconsistent among experiments, impatiens could still be considered an intermediate accumulator of silicon. Tigergrass is an accumulator of silicon. Based on the silicon concentration observed for tigergrass and the rate response measured in this study, support the hypothesis that tigergrass is a silicon accumulator according to the categories proposed by Ma et al. (2001).

### *Exserohilum rostratum* on Tigergrass

A fungal pathogen, which causes a leaf spot disease on tigergrass, was isolated, characterized, and identified as *Exserohilum rostratum*, a common pathogen of grasses, based on conidial morphology and the ITS1/4 sequence. *Exserohilum* belongs to what was formerly known as the Helminthosporium complex, a large genus that was separated into three genera (Sivanesan 1987). The three genera of anamorphs, largely coincide with three genera of teleomorps; all *Exserohilum* spp. for which a teleomorph has been identified, have one that belongs to the ascomycete genus *Setosphaeria* (Sivanesan 1987). Isolates of *E. rostratum* have been reported as diseases on bermuda grass (Pratt and Brink 2007), and on johnson grass, broadleaf signal grass, and yellow foxtail (all three are volunteer grasses found in bermuda grass pastures; Pratt 2006; Pratt and Brink 2007).

Increasing inoculum density resulted in increasing levels of disease severity, and a shortened incubation period. At the highest inoculum density tested ($10^5$ conidia/ml), symptoms were visible within 12 hours after inoculation.

The effect of silicon application to the growing medium of tigergrass in containers was investigated. Both disease severity and AUDPC (area under disease progress curve) were

significantly decreased with application of soluble silicon in the form of potassium silicate (AgSil25) as a drench to the containers twice a week at a rate equivalent to 700 kg elemental silicon/ha.

*Exserohilum rostratum* growth was completely inhibited *in vitro* when potassium silicate was added to V-8 juice agar at a rate of 277 mM silicon or higher. The inhibition was fungistatic, because after 2 weeks without any growth on the silicon-amended agar plates, all the plugs that were transferred to V-8 juice agar without potassium silicate grew normally. This might be an indication that soluble silicon in the plant plays a role in disease resistance of the host by affecting the ability of the pathogen to grow (fast). Samuels et al. (1991) found that soluble silicon was essential for the beneficial effect against powdery mildew on cucumber, even when the levels of accumulated insoluble silicon in the leaves were high. Recent information suggests that soluble and insoluble silicon both contribute to the resistance of rice against rice blast (Datnoff et al. 2008).

In the two experiments described in chapter 3, silicon did not affect the final disease severity. However, the control plants had significantly more disease for most of the experiments, resulting in a significantly decreased area under disease progress curve for the silicon treatments with respect to the control plants. In the first experiment, AgSil25 treated plants had an 87% lower AUDPC, while in the second experiment it was 46% lower. Excellerator-amended tigergrass had a 35% lower in AUDPC compared to control plants in the first experiment, and 34% in the second experiment. Wollastonite-amended tigergrass had a 56% and 32% decrease in AUDPC compared to the control in the first and second experiment, respectively. In the first experiment, only the AUDPC of AgSil-amended plants was significantly different than that of the control, while in the second experiment, all silicon-amended plants regardless of treatment

had a significantly decreased AUDPC compared to control plants. This leads to the conclusion that silicon indeed increases the resistance of tigergrass to *E. rostratum* infection.

Silicon can be as effective in controlling plant disease as fungicides, but the combination of both is most effective (Datnoff et al. 1997). For brown spot and blast of rice, the integration of silicon amendment and fungicide application resulted in the greatest reduction of disease severity and progress (Datnoff and Rodrigues 2005). The interaction of the two control measures resulted in lower disease development. The combined effect of fungicide treatments and silicon amendment might be considered for culture of tigergrass, particularly in the nursery. Although a control program using different fungicides was used on tigergrass supplied for this study, the plants often arrived with high levels of infection. More research is necessary to study the effectiveness of a spray program combined with silicon, but there is some indication that this might be useful based on the data from this study.

In conclusion, this part of the study confirmed that *E. rostratum* is the causal agent of tigergrass leaf spot. In addition, it was shown that silicon amendment decreases the area under disease progress curves compared to that of the control, so silicon amendment increases the resistance of tigergrass to *E. rostratum*.

**The Transcriptional Response of Rice to the Silicon-Pathogen Interaction**

The interaction of rice – *M. grisea* with silicon-amendment was investigated using 44k microarrays representing the entire rice genome. It was shown in this study that rice amended with silicon affects different levels of transcripts for 221 genes compared to non-amended rice. A number of genes are known or suspected to be involved in the plant pathogen response and imply that silicon-amendment may serve to prime the rice pathogen response pathways. This diffuses any arguments that silicon's only role in disease resistance is the deposition of an impenetrable layer in the epidermal cells as a mechanical barrier.

This finding contrast with the results of Fauteux et al. (2006) who found that silicon affected the transcript levels of only two *Arabidopsis thaliana* genes compared with unamended plants, and indicated that the role of silicon might be different between rice and *Arabidopsis*. This difference may be more general, and may for example be due to the difference of silicon's role between monocotyledons and dicotyledons. In the *Arabidopsis*-powdery mildew interaction, the number of genes differentially regulated in response to the pathogen when the plants were amended with silicon was also smaller than when non-amended plants were infected (Fauteux et al. 2006). The authors did not mention whether any of those differentially expressed genes were unique for the interaction. These genes might be of interest for future research.

Considering both theories of silicon's role in disease resistance, and the evidence that has been presented in the literature, it appears that silicon plays a role at different levels. There is evidence for the mechanical barrier hypothesis (Carver et al. 1987; Kim et al. 2002; Park et al. 2006), and for an active role of silicon in the molecular signaling pathways that leads to resistance (Rodrigues et al. 2003a, 2004, 2005, this work); for the necessity of both soluble and insoluble silicon (Datnoff et al. 2008; Samuels 1991; this work). None of these theories is mutually exclusive. It is quite possible that a mechanical barrier prevents and/or slows down fungal penetration. During the penetration process the germ tube can locally dissolve the silica gel layer, the locally higher concentration of silicon can inhibit growth of the fungus, and silicon can serve as a signaling compound for the timely activation of a plant defense response.

In conclusion, silicon amendment changes the basal level of gene of rice plants, and the rice response against rice blast infection by decreasing the number of differentially regulated gene. This confirms that silicon plays a role at the physiological level both in unchallenged and pathogen-challenged rice plants.

Table 5-1 Contributions to science for each chapter

| Chapter | Contributions to scientific knowledge |
|---|---|
| 2 | Begonia and marigold did not accumulate more silicon if the soilless growing medium was amended with calcium silicate. |
| | Potassium silicate raised the pH of the growing medium to levels so high that begonia and marigold no longer grew. |
| | The data on impatiens weakly supported a rate response. |
| | Tigergrass accumulated silicon in a rate dependent manner. |
| | Tigergrass tolerated the high medium pH better than begonia, impatiens, and marigold. |
| 3 | Tigergrass leaf spot is caused by *Exserohilum rostratum* (Drechsler) Leonard & Suggs. |
| | *In vitro* growth of *E. rostratum* is inhibited by silicon amendment of the medium. |
| | Spray-inoculation of E. rostratum resulted in symptoms as early as 12 hours after inoculation. |
| | Silicon amendment of the growing medium decreased the area under disease progress curve of *E. rostratum* on tigergrass. |
| 4 | Silicon amendment changes the physiology of rice, changing the basal level of gene expression of the unchallenged rice plant. |
| | Silicon amendment attenuated the response of rice gene expression to inoculation with the rice blast pathogen *Magnaporthe grisea*. |

# APPENDIX
# EXPRESSION PROFILE OF DIFFERENTIALLY EXPRESSED GENES

Table A-1 Expression profile of defense and/or stress-related genes

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os11g0692500 | Bacterial blight resistance protein. | U[1] | | | |
| Os01g0963200 | Peroxidase BP 1 precursor. | U | | | |
| Os01g0378100 | Peroxidase precursor (EC 1.11.1.7). | U | | | |
| Os11g0608300 | Barley stem rust resistance protein. | D | | | |
| Os11g0673600 | Disease resistance protein family protein. | D | | | |
| Os03g0266300 | Heat shock protein Hsp20 domain containing protein. | D | | | |
| Os03g0235000 | Peroxidase (EC 1.11.1.7). | D | | | |
| Os12g0491800 | Terpene synthase-like domain containing protein. | D | | | |
| Os10g0191300 | Type-1 pathogenesis-related protein. | D | | | |
| Os10g0562900 | Pathogenesis-related transcriptional factor and ERF domain containing protein. | | U | | |
| Os07g0677500 | Peroxidase POC1. | | U | | |
| Os12g0568900 | Thaumatin, pathogenesis-related family protein. | | U | | |
| Os03g0767000 | Allene oxide synthase (EC 4.2.1.92). | | | U | |
| Os09g0442100 | Avr9/Cf-9 rapidly elicited protein 264. | | | U | |
| Os03g0331700 | Avr9/Cf-9 rapidly elicited protein 31. | | | U | |
| Os05g0566400 | Blast and wounding induced mitogen-activated protein kinase. | | | U | |
| Os07g0583600 | Chitin-inducible gibberellin-responsive protein. | | | U | |
| Os02g0605900 | Chitinase (EC 3.2.1.14) A. | | | U | |
| Os01g0860500 | Chitinase (EC 3.2.1.14). | | | U | |
| Os10g0543400 | Chitinase (EC 3.2.1.14). | | | U | |
| Os06g0356800 | Class III chitinase homologue (OsChib3H-h) (Fragment). | | | U | |
| Os04g0514600 | Disease resistance protein family protein. | | | U | |
| Os03g0122300 | Flavanone 3-hydroxylase-like protein. | | | U | |
| Os04g0671200 | Flavin-containing amine oxidase family protein. | | | U | |
| Os04g0430600 | Harpin-induced 1 domain containing protein. | | | U | |
| Os12g0159000 | Harpin-induced 1 domain containing protein. | | | U | |
| Os04g0667600 | Heavy metal transport/detoxification protein domain containing protein. | | | U | |
| Os02g0592400 | Hypoxia induced protein conserved region family protein. | | | U | |
| Os01g0113200 | LRK14. | | | U | |
| Os09g0486500 | Multiple stress-responsive zinc-finger protein. | | | U | |
| Os06g0707800 | NBS-LRR disease resistance protein homologue. | | | U | |

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os04g0550200 | Pathogenesis-related transcriptional factor and ERF domain containing protein. | | | U | |
| Os06g0547400 | Peroxidase P7 (EC 1.11.1.7) (TP7). | | | U | |
| Os12g0520200 | Phenylalanine ammonia-lyase (EC 4.3.1.5) (Fragment). | | | U | |
| Os04g0490500 | Pto kinase interactor 1. | | | U | |
| Os07g0691300 | Wound-induced WI12 family protein. | | | U | |
| Os02g0125300 | Bax inhibitor-1 (BI-1) (OsBI-1). | | | U | |
| Os01g0855600 | Hs1pro-1 protein. | | | U | |
| Os11g0704800 | Membrane protein. | | | U | |
| Os11g0229400 | RPR1. | | | U | |
| Os03g0129100 | Seven transmembrane protein MLO2. | | | U | |
| Os01g0205900 | Peroxidase 52 precursor (EC 1.11.1.7) (Atperox P52) (ATP49). | | | D | |
| Os03g0347900 | Terpenoid synthase domain containing protein. | | | D | |
| Os01g0713200 | Beta-1,3-glucanase precursor. | | | | U |
| Os02g0584800 | Heavy metal transport/detoxification protein domain containing protein. | | | | U |
| Os02g0585100 | Heavy metal transport/detoxification protein domain containing protein. | | | | U |
| Os04g0469000 | Heavy metal transport/detoxification protein domain containing protein. | | | | U |
| Os04g0610400 | Pathogenesis-related transcriptional factor and ERF domain containing protein. | | | | U |
| Os07g0104100 | Peroxidase 27 precursor (EC 1.11.1.7) (Atperox P27) (PRXR7) (ATP12a). | | | | U |
| Os08g0539700 | PibH8 protein. | | | | U |
| Os07g0636600 | Plant disease resistance response protein family protein. | | | | D |
| Os04g0662600 | Flavanone-3-hydroxylase (Fragment). | U | D | U | D |
| Os11g0684100 | Disease resistance protein family protein. | U | | U | |
| Os11g0686400 | Disease resistance protein family protein. | U | | U | |
| Os11g0686500 | Disease resistance protein family protein. | U | | U | |
| Os01g0114300 | LRK14. | U | | U | |
| Os10g0135100 | NB-ARC domain containing protein. | U | | U | |
| Os05g0404200 | Methionine sulfoxide reductase B domain containing protein. | D | U | D | U |
| Os04g0493600 | Chitin-binding, type 1 domain containing protein. | D | | D | |

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os08g0411900 | Disease resistance protein family protein. | D | | D | |
| Os11g0605100 | Disease resistance protein family protein. | D | | D | |
| Os11g0606400 | Disease resistance protein family protein. | D | | D | |
| Os11g0639600 | Disease resistance protein family protein. | D | | D | |
| Os01g0114700 | LRK33. | D | | D | |
| Os01g0962700 | Peroxidase 12 precursor (EC 1.11.1.7) (Atperox P12) (PRXR6) (ATP4a). | D | | D | |
| Os07g0129200 | PR1a protein. | D | | D | |
| Os08g0168000 | Terpene synthase, metal-binding domain containing protein. | D | | D | |
| Os05g0375400 | (1,3;1,4) beta glucanase precursor (EC 3.2.1.73). | | U | D | |
| Os09g0319800 | Terpenoid cylases/protein prenyltransferase alpha-alpha toroid domain containing protein. | | U | | U |
| Os02g0626600 | Phenylalanine ammonia-lyase. | | D | U | U |
| Os07g0124900 | PR1a protein. | | D | U | U |
| Os07g0126100 | PR1a protein. | | D | U | U |
| Os07g0604300 | COBRA protein precursor (Cell expansion protein). | | D | U | U |
| Os01g0968800 | Avr9/Cf-9 rapidly elicited protein 111B. | | D | U | |
| Os01g0769700 | LpimPth4. | | D | U | |
| Os02g0121700 | Terpene synthase-like domain containing protein. | | D | U | |
| Os03g0225900 | Allene oxide synthase (EC 4.2.1.92). | | | U | U |
| Os07g0545800 | Chitin-inducible gibberellin-responsive protein. | | | U | U |
| Os03g0748500 | Flavodoxin/nitric oxide synthase domain containing protein. | | | U | U |
| Os01g0864300 | Harpin-induced 1 domain containing protein. | | | U | U |
| Os01g0864500 | Harpin-induced 1 domain containing protein. | | | U | U |
| Os07g0251200 | Harpin-induced 1 domain containing protein. | | | U | U |
| Os11g0130400 | Harpin-induced 1 domain containing protein. | | | U | U |
| Os12g0127200 | Harpin-induced 1 domain containing protein. | | | U | U |
| Os04g0464100 | Heavy metal transport/detoxification protein domain containing protein. | | | U | U |
| Os11g0229300 | NBS-LRR disease resistance protein homologue. | | | U | U |
| Os07g0129300 | Pathogenesis-related protein 1 precursor. | | | U | U |
| Os01g0868000 | Pathogenesis-related transcriptional factor and ERF domain containing protein. | | | U | U |
| Os01g0963000 | Peroxidase BP 1 precursor. | | | U | U |
| Os05g0427400 | Phenylalanine ammonia-lyase (EC 4.3.1.5). | | | U | U |

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os01g0136000 | 16.9 kDa class I heat shock protein. | | | U | U |
| Os03g0301200 | COBRA-like protein 7 precursor. | | | U | U |
| Os02g0562600 | Mlo-related protein family protein. | | | U | U |

[1]Treatment comparisons: C-Si=control vs silicon-amended, Si-SiP=silicon-amended vs silicon-amended and pathogen-inoculated, C-P=control vs pathogen inoculated, P-SiP=pathogen-inoculated vs silicon-amended and pathogen-inoculated. U=upregulated, D=downregulated. Differential regulation is reported with respect to the treatment on the left of the comparison.

Table A-2 Expression profile of transcription factor-encoding genes

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os03g0379300 | Basic helix-loop-helix dimerisation region bHLH domain containing protein. | U[1] | | | |
| Os01g0633400 | CBS domain containing protein. | U | | | |
| Os02g0731200 | Transcription factor MADS57. | U | | | |
| Os09g0417800 | DNA-binding WRKY domain containing protein. | D | | | |
| Os08g0332700 | Trans-acting transcriptional protein ICP0 (Immediate-early protein IE110). | D | | | |
| Os02g0695200 | P-type R2R3 Myb protein (Fragment). | D | | | |
| Os08g0414500 | Basic helix-loop-helix dimerisation region bHLH domain containing protein. | | D | | |
| Os11g0684000 | Myb, DNA-binding domain containing protein. | | D | | |
| Os04g0541700 | Homeobox domain containing protein. | | D | | |
| Os02g0673500 | Basic helix-loop-helix dimerisation region bHLH domain containing protein. | | | U | |
| Os04g0631600 | Basic helix-loop-helix dimerisation region bHLH domain containing protein. | | | U | |
| Os08g0490000 | Basic helix-loop-helix dimerisation region bHLH domain containing protein. | | | U | |
| Os07g0287000 | Cyclin-like F-box domain containing protein. | | | U | |
| Os03g0203800 | Cyclin, N-terminal domain containing protein. | | | U | |
| Os02g0618400 | MYB8 protein. | | | U | |
| Os10g0577600 | Transcription factor jumonji, jmjC domain containing protein. | | | U | |
| Os05g0442400 | Transcription factor MYBS3. | | | U | |
| Os01g0185900 | WRKY transcription factor 1 (Zn-dependent activator protein 1) (Transcription factor ZAP1). | | | U | |
| Os01g0246700 | WRKY transcription factor 1. | | | U | |
| Os06g0649000 | WRKY transcription factor 28. | | | U | |
| Os05g0583000 | WRKY transcription factor 34. | | | U | |
| Os04g0395800 | ZIM domain containing protein. | | | U | |
| Os09g0439200 | ZIM domain containing protein. | | | U | |
| Os01g0859100 | Zinc finger-like protein. | | | U | |
| Os03g0302200 | Zn-finger-like, PHD finger domain containing protein. | | | U | |
| Os02g0646200 | Zn-finger, B-box domain containing protein. | | | U | |
| Os03g0659400 | Zn-finger, CCHC type domain containing protein. | | | U | |
| Os08g0490100 | Zn-finger, Dof type domain containing protein. | | | U | |
| Os02g0559800 | Zn-finger, RING domain containing protein. | | | U | |
| Os03g0723000 | GRAS transcription factor domain containing protein. | | | U | |
| Os05g0560600 | Homeodomain-like containing protein. | | | U | |
| Os09g0483600 | Transcription factor jumonji, jmjC domain containing protein. | | | U | |

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os06g0637500 | Typical P-type R2R3 Myb protein (Fragment). | | | U | |
| Os01g0542700 | Basic-leucine zipper (bZIP) transcription factor domain containing protein. | | | D | |
| Os01g0647000 | Cyclin-like F-box domain containing protein. | | | D | |
| Os10g0133100 | Cyclin-like F-box domain containing protein. | | | D | |
| Os11g0246200 | Cyclin-like F-box domain containing protein. | | | D | |
| Os06g0140400 | DNA-binding protein (Homeodomain-leucine zipper transcription factor). | | | D | |
| Os04g0613000 | Zinc transporter 1 precursor (ZRT/IRT-like protein 1). | | | D | |
| Os05g0195200 | Zn-finger, C-x8-C-x5-C-x3-H type domain containing protein. | | | D | |
| Os09g0243200 | Zn-finger, RING domain containing protein. | | | D | |
| Os02g0732600 | Typical P-type R2R3 Myb protein (Fragment). | | | D | |
| Os04g0385600 | Cyclin-like F-box domain containing protein. | | | | U |
| Os11g0665600 | Helix-turn-helix, Fis-type domain containing protein. | | | | U |
| Os01g0952800 | Basic helix-loop-helix dimerisation region bHLH domain containing protein. | U | U | | |
| Os02g0677300 | CRT/DRE binding factor 1. | U | | U | U |
| Os04g0572400 | CRT/DRE binding factor 1. | U | | U | U |
| Os01g0824700 | Cyclin-like F-box domain containing protein. | U | | U | |
| Os11g0539600 | Cyclin-like F-box domain containing protein. | U | | U | |
| Os01g0127400 | Myb, DNA-binding domain containing protein. | U | | U | |
| Os11g0685600 | WRKY transcription factor 41. | U | | U | |
| Os11g0685700 | WRKY transcription factor 61. | U | | U | |
| Os01g0147700 | XS zinc finger domain containing protein. | U | | U | |
| Os08g0197500 | Cyclin-like F-box domain containing protein. | D | | D | |
| Os01g0871200 | Zn-finger, C2H2 type domain containing protein. | D | | D | |
| Os04g0301500 | Basic helix-loop-helix dimerisation region bHLH domain containing protein. | | D | U | U |
| Os01g0863300 | Myb, DNA-binding domain containing protein. | | D | U | U |
| Os01g0705700 | Transcription factor ICE1 (Inducer of CBF expression 1) (Basic HLH protein 116) | | D | U | U |
| Os03g0180800 | ZIM domain containing protein. | | D | U | U |
| Os03g0181100 | ZIM domain containing protein. | | D | U | U |
| Os02g0759400 | Zn-finger, RING domain containing protein. | | D | U | U |
| Os04g0493100 | Basic helix-loop-helix dimerisation region bHLH domain containing protein. | | D | U | |
| Os06g0164400 | Basic helix-loop-helix dimerisation region bHLH domain containing protein. | | D | U | |
| Os09g0417600 | DNA-binding WRKY domain containing protein. | | D | U | |
| Os02g0641300 | Myb, DNA-binding domain containing protein. | | D | U | |

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os05g0444200 | Zn-finger, C2H2 type domain containing protein. | | D | U | |
| Os03g0741100 | Basic helix-loop-helix dimerisation region bHLH domain containing protein. | | | U | U |
| Os07g0561300 | Cyclin-like F-box domain containing protein. | | | U | U |
| Os09g0341500 | Cyclin-like F-box domain containing protein. | | | U | U |
| Os01g0821600 | DNA-binding WRKY domain containing protein. | | | U | U |
| Os04g0517100 | Myb protein. | | | U | U |
| Os03g0315400 | MYB-related protein 340. | | | U | U |
| Os02g0624300 | MYB1 protein. | | | U | U |
| Os04g0416100 | Transcription factor E2F/dimerisation partner (TDP) family protein. | | | U | U |
| Os05g0473300 | Transcriptional factor TINY. | | | U | U |
| Os01g0826400 | WRKY transcription factor 24. | | | U | U |
| Os02g0462800 | WRKY transcription factor 42 (Transcription factor WRKY02). | | | U | U |
| Os02g0181300 | WRKY transcription factor 71 (Transcription factor WRKY09). | | | U | U |
| Os03g0402800 | ZIM domain containing protein. | | | U | U |
| Os10g0391400 | ZIM domain containing protein. | | | U | U |
| Os10g0392400 | ZIM domain containing protein. | | | U | U |
| Os09g0385700 | Zn-finger, AN1-like domain containing protein. | | | U | U |
| Os03g0437200 | Zn-finger, C2H2 type domain containing protein. | | | U | U |
| Os02g0682300 | Zn-finger, RING domain containing protein. | | | U | U |
| Os03g0820400 | ZPT2-13. | | | U | U |
| Os12g0139400 | Two-component response regulator ARR17. | | | U | U |
| Os11g0143300 | Type-A response regulator. | | | U | U |
| Os04g0673300 | ZmRR2 protein (Response regulator 2). | | | U | U |
| Os04g0583900 | Myb, DNA-binding domain containing protein. | | | D | D |
| Os07g0631200 | Zn-finger, RING domain containing protein. | | | D | D |

[1]Treatment comparisons: C-Si=control vs silicon-amended, Si-SiP=silicon-amended vs silicon-amended and pathogen-inoculated, C-P=control vs pathogen inoculated, P-SiP=pathogen-inoculated vs silicon-amended and pathogen-inoculated. U=upregulated, D=downregulated. Differential regulation is reported with respect to the treatment on the left of the comparison.

Table A-3 Expression profile of transporter-encoding genes

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os01g0871600 | TGF-beta receptor, type I/II extracellular region family protein. | U[1] | | | |
| Os02g0550800 | Ammonium transporter. | U | | | |
| Os05g0384600 | ABC transporter related domain containing protein. | | U | | |
| Os01g0930400 | K+ potassium transporter family protein. | | | U | |
| Os01g0759900 | Permease 1. | | | U | |
| Os09g0484900 | Sodium-dicarboxylate cotransporter-like. | | | U | |
| Os12g0231000 | TGF-beta receptor, type I/II extracellular region family protein. | | | | D |
| Os07g0258400 | Metal transporter Nramp6 (AtNramp6). | U | U | | |
| Os02g0528900 | PDR9 ABC transporter. | D | D | | |
| Os01g0189100 | TPR-like domain containing protein. | D | | D | |
| Os08g0384500 | PDR-like ABC transporter (PDR3 ABC transporter). | | | U | U |

[1]Treatment comparisons: C-Si=control vs silicon-amended, Si-SiP=silicon-amended vs silicon-amended and pathogen-inoculated, C-P=control vs pathogen inoculated, P-SiP=pathogen-inoculated vs silicon-amended and pathogen-inoculated. U=upregulated, D=downregulated. Differential regulation is reported with respect to the treatment on the left of the comparison.

Table A-4 Expression profile of hormone pathway-related genes

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os04g0511200 | EFA27 for EF hand, abscisic acid, 27kD. | | D[1] | | |
| Os07g0674800 | AP2 domain containing protein RAP2.2 (Fragment). | | | U | |
| Os06g0225300 | BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1 precursor (EC 2.7.1.37). | | | U | |
| Os07g0622000 | Abscisic acid-inducible protein kinase (EC 2.7.1.-) (Fragment). | | | U | |
| Os06g0543400 | CBL-interacting protein kinase 11 (Fragment). | | | D | |
| Os04g0669200 | Ethylene response factor 3. | | | | U |
| Os05g0497300 | Ethylene responsive element binding factor 5 (AtERF5). | | | | U |
| Os01g0675800 | No apical meristem (NAM) protein domain containing protein. | | | | U |
| Os02g0579000 | No apical meristem (NAM) protein domain containing protein. | U | U | | |
| Os03g0324200 | EIL3. | U | | U | |
| Os08g0474000 | AP2 domain containing protein RAP2.6 (Fragment). | | D | U | U |
| Os06g0127100 | CBF-like protein. | | D | U | U |
| Os01g0706900 | Auxin conjugate hydrolase (ILL5). | | D | U | |
| Os08g0452500 | Auxin responsive SAUR protein family protein. | | D | U | |
| Os01g0192900 | ACC synthase (EC 4.1.1.14) (Fragment). | | | U | U |
| Os04g0578000 | ACC synthase (EC 4.4.1.14). | | | U | U |
| Os09g0457900 | AP2 domain containing protein RAP2.6 (Fragment). | | | U | U |
| Os01g0141000 | AP2 domain containing protein RAP2.8 (Fragment). | | | U | U |
| Os05g0127300 | Cytokinin-regulated kinase 1. | | | U | U |
| Os01g0224100 | Ethylene-responsive element binding factor. | | | U | U |
| Os01g0862800 | No apical meristem (NAM) protein domain containing protein. | | | U | U |
| Os03g0133000 | No apical meristem (NAM) protein domain containing protein. | | | U | U |
| Os05g0442700 | No apical meristem (NAM) protein domain containing protein. | | | U | U |
| Os09g0522000 | CBF-like protein. | | | U | U |
| Os01g0206700 | CBL-interacting protein kinase 2. | | | D | D |

[1]Treatment comparisons: C-Si=control vs silicon-amended, Si-SiP=silicon-amended vs silicon-amended and pathogen-inoculated, C-P=control vs pathogen inoculated, P-SiP=pathogen-inoculated vs silicon-amended and pathogen-inoculated. U=upregulated, D=downregulated. Differential regulation is reported with respect to the treatment on the left of the comparison.

Table A-5 Expression profile of housekeeping genes

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os12g0227400 | Allyl alcohol dehydrogenase. | U[1] | | | |
| Os03g0226400 | Cation diffusion facilitator 8. | U | | | |
| Os01g0898500 | Patatin-like protein 2 (Fragment). | U | | | |
| Os09g0110300 | Putative cyclase family protein. | D | | | |
| Os08g0112300 | Transferase family protein. | D | | | |
| Os10g0154700 | Cyclophilin Dicyp-2. | D | | | |
| Os08g0155700 | DNA-directed RNA polymerase (EC 2.7.7.6) largest chain (Isoform B1)- like protein. | D | | | |
| Os11g0194800 | DNA-directed RNA polymerase II 7.6 kDa polypeptide. | D | | | |
| Os11g0106700 | Ferritin 1, chloroplast precursor (ZmFer1). | D | | | |
| Os12g0106000 | Ferritin 1, chloroplast precursor (ZmFer1). | D | | | |
| Os12g0258700 | Multicopper oxidase, type 1 domain containing protein. | D | | | |
| Os01g0770200 | Tyrosine decarboxylase 1 (EC 4.1.1.25) (ELI5) (Fragment). | D | | | |
| Os01g0814100 | Plant lipid transfer/seed storage/trypsin-alpha amylase inhibitor domain containing protein. | | U | | |
| Os01g0636400 | Alpha/beta hydrolase family protein. | | U | | |
| Os10g0532200 | Alpha/beta hydrolase family protein. | | U | | |
| Os03g0664400 | Fibroin. | | U | | |
| Os12g0630600 | Flagelliform silk protein (Fragment). | | U | | |
| Os05g0518300 | Lipolytic enzyme, G-D-S-L family protein. | | U | | |
| Os06g0257600 | Lipolytic enzyme, G-D-S-L family protein. | | U | | |
| Os07g0668300 | Lipolytic enzyme, G-D-S-L family protein. | | U | | |
| Os07g0119400 | Pectinesterase like protein. | | U | | |
| Os09g0548200 | Peptidoglycan-binding LysM domain containing protein. | | U | | |
| Os04g0561500 | Prolyl endopeptidase (EC 3.4.21.26) (Post-proline cleaving enzyme) (PE). | | U | | |
| Os04g0447700 | NAD(P)H dependent 6'-deoxychalcone synthase (EC 1.1.-.-). | | D | | |
| Os08g0409100 | Trehalose-6-phosphate phosphatase. | | D | | |
| Os05g0527000 | UDP-glucuronosyl/UDP-glucosyltransferase family protein. | | D | | |
| Os04g0604200 | Xyloglucan endotransglycosylase precursor. | | D | | |
| Os04g0182200 | 2OG-Fe(II) oxygenase domain containing protein. | | D | | |
| Os01g0945700 | Amino acid/polyamine transporter I family protein. | | D | | |
| Os03g0790500 | Esterase/lipase/thioesterase domain containing protein. | | D | | |

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os01g0858200 | DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 16. | | D | | |
| Os06g0725200 | Lipolytic enzyme, G-D-S-L family protein. | | D | | |
| Os10g0393800 | Lipolytic enzyme, G-D-S-L family protein. | | D | | |
| Os12g0617400 | Viviparous-14. | | D | | |
| Os02g0180700 | Cinnamoyl-CoA reductase (EC 1.2.1.44). | | | U | |
| Os03g0171600 | Galactose oxidase, central domain containing protein. | | | U | |
| Os08g0231400 | Germin family protein. | | | U | |
| Os08g0188900 | Germin-like protein precursor. | | | U | |
| Os08g0189100 | Germin-like protein precursor. | | | U | |
| Os07g0523400 | Glucose-6-phosphate/phosphate-translocator precursor. | | | U | |
| Os12g0283400 | Glutelin family protein. | | | U | |
| Os02g0588500 | Glycerophosphoryl diester phosphodiesterase family protein. | | | U | |
| Os08g0445700 | Glycosyl transferase, family 20 domain containing protein. | | | U | |
| Os03g0803600 | Glycosyl transferase, family 31 protein. | | | U | |
| Os09g0452900 | Glycosyl transferase, family 31 protein. | | | U | |
| Os06g0561000 | Myo-inositol oxygenase. | | | U | |
| Os02g0770800 | Nitrate reductase [NAD(P)H] (EC 1.7.1.2). | | | U | |
| Os06g0604200 | Phospholipase D. | | | U | |
| Os07g0290500 | Plant lipid transfer/seed storage/trypsin-alpha amylase inhibitor domain containing protein. | | | U | |
| Os03g0758500 | Plastocyanin-like domain containing protein. | | | U | |
| Os08g0482700 | Plastocyanin-like domain containing protein. | | | U | |
| Os11g0460700 | Protein prenyltransferase domain containing protein. | | | U | |
| Os02g0572400 | Riboflavin biosynthesis protein ribA, chloroplast precursor. | | | U | |
| Os04g0594400 | RNA-binding region RNP-1 (RNA recognition motif) domain containing protein. | | | U | |
| Os03g0241900 | Senescence-associated protein 12. | | | U | |
| Os07g0582400 | Sorbitol transporter. | | | U | |
| Os05g0449200 | Transferase family protein. | | | U | |
| Os05g0128900 | Trehalose-phosphatase domain containing protein. | | | U | |
| Os03g0280800 | UDP-glucuronic acid decarboxylase. | | | U | |
| Os06g0593200 | UDP-glucuronosyl/UDP-glucosyltransferase family protein. | | | U | |
| Os04g0683700 | 4-coumarate-CoA ligase-like protein (Adenosine monophosphate binding protein 3 AMPBP3). | | | U | |

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os03g0413100 | ACR4. | | | U | |
| Os06g0143400 | Acyl-ACP thioesterase. | | | U | |
| Os06g0556000 | Amino acid carrier (Fragment). | | | U | |
| Os12g0181600 | Amino acid/polyamine transporter II family protein. | | | U | |
| Os04g0463500 | Anthranilate synthase beta subunit (EC 4.1.3.27) (At1g25220). | | | U | |
| Os03g0154100 | Aromatic-ring hydroxylase family protein. | | | U | |
| Os01g0663400 | Aspartic protease (Fragment). | | | U | |
| Os04g0578400 | Beta-ring hydroxylase (Fragment). | | | U | |
| Os01g0270300 | Cationic peroxidase isozyme 40K precursor. | | | U | |
| Os04g0497200 | Cellulase precursor (Endo-1,4-beta-D- glucanase KORRIGAN) (EC 3.2.1.4). | | | U | |
| Os07g0621600 | Cytochrome c oxidase polypeptide VIb (Fragment). | | | U | |
| Os01g0760000 | Dynein 8 kDa light chain, flagellar outer arm. | | | U | |
| Os04g0462600 | Dynein light chain, type 1 family protein. | | | U | |
| Os06g0214300 | Esterase/lipase/thioesterase domain containing protein. | | | U | |
| Os07g0564500 | FAD-dependent pyridine nucleotide-disulphide oxidoreductase domain containing protein. | | | U | |
| Os05g0447700 | Ferritin/ribonucleotide reductase-like family protein. | | | U | |
| Os01g0600500 | HAD-superfamily subfamily IB hydrolase, hypothetical 1 protein. | | | U | |
| Os01g0908700 | Heterogeneous nuclear ribonucleoprotein A2 homolog 1 (hnRNP A2(A)). | | | U | |
| Os01g0127900 | Mannose-6-phosphate isomerase, type I family protein. | | | U | |
| Os11g0686000 | En/Spm-like transposon proteins family protein. | | | U | |
| Os03g0733800 | Endoplasmic oxidoreductin 1 precursor (EC 1.8.4.-). | | | U | |
| Os02g0803300 | Epsin, N-terminal domain containing protein. | | | U | |
| Os02g0526400 | ERD1 protein, chloroplast precursor. | | | U | |
| Os05g0473500 | Exo70 exocyst complex subunit family protein. | | | U | |
| Os06g0255900 | Exo70 exocyst complex subunit family protein. | | | U | |
| Os10g0542400 | Expansin/Lol pI family protein. | | | U | |
| Os03g0247900 | F5O11.14 (ACR8). | | | U | |
| Os03g0187600 | GRAM domain containing protein. | | | U | |
| Os04g0688300 | Haem peroxidase, plant/fungal/bacterial family protein. | | | U | |
| Os03g0114900 | Inner mitochondrial membrane protein | | | U | |

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os01g0314800 | Late embryogenesis abundant protein 3 family protein. | | | U | |
| Os11g0195500 | Lipase, class 3 family protein. | | | U | |
| Os03g0738600 | Lipoxygenase L-2 (EC 1.13.11.12). | | | U | |
| Os04g0447100 | Lipoxygenase. | | | U | |
| Os05g0557400 | Membrane attack complex component/perforin/complement C9 family protein. | | | U | |
| Os05g0392700 | Mitochondrial substrate carrier family protein. | | | U | |
| Os08g0520000 | Mitochondrial substrate carrier family protein. | | | U | |
| Os09g0524300 | Multi antimicrobial extrusion protein MatE family protein. | | | U | |
| Os01g0816100 | NAC-domain containing protein 2 (ANAC002). | | | U | |
| Os04g0458600 | Non-cell-autonomous protein pathway2. | | | U | |
| Os11g0183900 | Nucellin-like protein. | | | U | |
| Os02g0791500 | Nucleotide sugar epimerase-like protein (UDP-D-glucuronate 4- epimerase) (EC 5.1.3.6). | | | U | |
| Os06g0216000 | Oxo-phytodienoic acid reductase. | | | U | |
| Os03g0399000 | Pectinesterase family protein. | | | U | |
| Os10g0537800 | Peptidase A1, pepsin family protein. | | | U | |
| Os02g0136000 | Plant regulator RWP-RK domain containing protein. | | | U | |
| Os03g0203700 | Plasma membrane Ca2+-ATPase. | | | U | |
| Os07g0274700 | HvB12D protein (B12Dg1 protein). | | | U | |
| Os09g0536700 | Nodulin-like domain containing protein. | | | U | |
| Os06g0725000 | Ntdin. | | | U | |
| Os02g0757100 | Phi-1 protein. | | | U | |
| Os01g0168100 | Prefoldin family protein. | | | U | |
| Os01g0667600 | Ras-related protein Rab11B. | | | U | |
| Os05g0161500 | RelA/SpoT domain containing protein. | | | U | |
| Os07g0569100 | Remorin, C-terminal region domain containing protein. | | | U | |
| Os07g0695400 | Spectrin repeat containing protein. | | | U | |
| Os07g0154100 | Viviparous-14. | | | U | |
| Os07g0412100 | Granule-bound starch synthase I, chloroplast precursor (EC 2.4.1.21) (GBSS I). | | | D | |
| Os04g0517500 | Phosphoenolpyruvate carboxylase kinase. | | | D | |
| Os01g0855000 | Phospholipid/glycerol acyltransferase family protein. | | | D | |
| Os10g0558700 | 2OG-Fe(II) oxygenase domain containing protein. | | | D | |
| Os09g0344500 | O-methyltransferase ZRP4 (EC 2.1.1.-) (OMT). | | | D | |

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os07g0181100 | C4-dicarboxylate transporter/malic acid transport protein family protein. | | | | U |
| Os02g0807000 | Phosphoenolpyruvate carboxylase kinase. | | | | U |
| Os01g0554100 | RNA-directed DNA polymerase (Reverse transcriptase) domain containing protein. | | | | U |
| Os03g0803500 | 2OG-Fe(II) oxygenase domain containing protein. | | | | U |
| Os10g0559500 | 2OG-Fe(II) oxygenase domain containing protein. | | | | U |
| Os09g0432300 | AAA ATPase, central region domain containing protein. | | | | U |
| Os06g0676700 | High pI alpha-glucosidase. | | | | U |
| Os08g0190100 | Oxalate oxidase-like protein or germin-like protein (Germin-like 8) (Germin-like 12). | | | | U |
| Os05g0495600 | P-type ATPase (Fragment). | | | | U |
| Os03g0405500 | PDI-like protein. | | | | U |
| Os03g0757200 | UDP-glucuronosyl/UDP-glucosyltransferase family protein. | | | | D |
| Os01g0591000 | Cytosolic aldehyde dehydrogenase. | | | | D |
| Os07g0659400 | HAD-superfamily hydrolase, subfamily IA, variant 1 protein. | | | | D |
| Os02g0194700 | Plant lipoxygenase family protein. | | | | D |
| Os02g0270200 | Peptidase S8 and S53, subtilisin, kexin, sedolisin domain containing protein. | U | | U | U |
| Os10g0395400 | Glutathione S-transferase, N-terminal domain containing protein. | U | | U | D |
| Os01g0372500 | Leucoanthocyanidin dioxygenase 1. | U | | U | D |
| Os07g0529000 | Isocitrate lyase (EC 4.1.3.1) (Isocitrase) (Isocitratase) (ICL). | U | | | D |
| Os04g0474900 | Cyanogenic beta-glucosidase precursor (EC 3.2.1.21) (Linamarase) (Fragment). | U | | U | |
| Os12g0222500 | Glycoside hydrolase, family 19 protein. | U | | U | |
| Os08g0414700 | Glycosyl transferase, family 20 domain containing protein. | U | | U | |
| Os04g0156200 | Ribulose-phosphate binding barrel domain containing protein. | U | | U | |
| Os10g0122000 | UDP-glucuronosyl/UDP-glucosyltransferase family protein. | U | | U | |
| Os08g0560000 | 2OG-Fe(II) oxygenase domain containing protein. | U | | U | |
| Os10g0138100 | Aldehyde oxidase (EC 1.2.3.1). | U | | U | |
| Os11g0613900 | BED finger domain containing protein. | U | | U | |
| Os01g0738600 | Epsin, N-terminal domain containing protein. | U | | U | |

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os06g0215900 | Oxo-phytodienoic acid reductase. | U | | U | |
| Os01g0613500 | Peptidase C1A, papain family protein. | U | | U | |
| Os02g0271600 | Peptidase S8 and S53, subtilisin, kexin, sedolisin domain containing protein. | U | | U | |
| Os03g0109900 | Peptidase, trypsin-like serine and cysteine proteases domain containing protein. | U | | U | |
| Os08g0240000 | STF-1 (Fragment). | U | | U | |
| Os05g0592300 | RmlC-like cupin family protein. | U | U | | |
| Os04g0578600 | Ferric reductase-like transmembrane component family protein. | U | U | | |
| Os05g0246300 | Major pollen allergen Lol pI family protein. | U | U | | |
| Os03g0751100 | Oligopeptide transporter OPT superfamily protein. | U | U | | |
| Os11g0641500 | Multicopper oxidase, type 1 domain containing protein. | D | | U | U |
| Os04g0581100 | Isopenicillin N synthase family protein. | D | | | U |
| Os10g0113000 | NADPH-dependent codeinone reductase (EC 1.1.1.247). | D | D | D | |
| Os01g0176200 | UDP-glucuronosyl/UDP-glucosyltransferase family protein. | D | | D | |
| Os12g0202800 | 0-methyltransferase (EC 2.1.1.6) (Fragment). | D | | D | |
| Os12g0230100 | ATP-dependent Clp protease, chloroplast precursor (fragment). | D | | D | |
| Os12g0143800 | Meiotic recombination protein DMC1 homolog. | D | | D | |
| Os09g0472900 | Expansin-related protein 2 precursor (AtEXPR2) (Ath-ExpGamma-1.1). | D | | D | |
| Os09g0279300 | Mitochondrial import inner membrane translocase, subunit Tim17/22 family protein. | D | | D | |
| Os02g0568200 | Non-phototropic hypocotyl 3. | D | | D | |
| Os10g0118000 | O-methyltransferase ZRP4 (EC 2.1.1.-) (OMT). | D | | D | |
| Os11g0439600 | Nod factor binding lectin-nucleotide phosphohydrolase. | D | | D | |
| Os11g0440200 | Nod factor binding lectin-nucleotide phosphohydrolase. | D | | D | |
| Os07g0664300 | Glucose/ribitol dehydrogenase family protein. | D | D | | |
| Os05g0399300 | Glycoside hydrolase, family 19 protein. | D | D | | |
| Os04g0339400 | Aldo/keto reductase family protein. | D | D | | |
| Os04g0542200 | Oligopeptide transporter OPT superfamily protein. | D | D | | |
| Os04g0556400 | Cis-zeatin O-glucosyltransferase. | | | U | U |
| Os04g0556500 | Cis-zeatin O-glucosyltransferase. | | | U | U |
| Os01g0667900 | Glutaredoxin domain containing protein. | | | U | U |

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os03g0188500 | Glutelin family protein. | | | U | U |
| Os05g0495700 | Glycerol-3-phosphate dehydrogenase-like protein (Fragment). | | | U | U |
| Os04g0506800 | Glycosyl transferase, family 29 protein. | | | U | U |
| Os02g0205500 | Naringenin-chalcone synthase family protein. | | | U | U |
| Os10g0497700 | Phytochelatin synthetase-like conserved region family protein. | | | U | U |
| Os08g0137800 | Plastocyanin-like domain containing protein. | | | U | U |
| Os08g0482600 | Plastocyanin-like domain containing protein. | | | U | U |
| Os03g0432100 | Pyruvate, phosphate dikinase, chloroplast precursor (EC 2.7.9.1). | | | U | U |
| Os04g0684900 | Ribonuclease CAF1 family protein. | | | U | U |
| Os07g0550600 | Transferase family protein. | | | U | U |
| Os10g0380100 | Transferase family protein. | | | U | U |
| Os02g0661100 | Trehalose-6-phosphate phosphatase. | | | U | U |
| Os04g0497000 | (+)-pulegone reductase. | | D | U | U |
| Os08g0448000 | 4-coumarate--CoA ligase 1 (EC 6.2.1.12) (4CL 1) (Fragment). | | | U | U |
| Os12g0471100 | AAA ATPase, central region domain containing protein. | | | U | U |
| Os02g0739100 | Actin-binding FH2 domain containing protein. | | | U | U |
| Os08g0473900 | Alpha-amylase type B (Fragment). | | | U | U |
| Os01g0597600 | Amino acid/polyamine transporter II family protein. | | | U | U |
| Os04g0688100 | Anionic peroxidase precursor. | | | U | U |
| Os04g0688500 | Anionic peroxidase precursor. | | | U | U |
| Os03g0290300 | Fatty acid desaturase domain containing protein. | | | U | U |
| Os01g0905200 | Exo70 exocyst complex subunit family protein. | | | U | U |
| Os01g0326300 | Haem peroxidase, plant/fungal/bacterial family protein. | | | U | U |
| Os06g0521500 | Haem peroxidase, plant/fungal/bacterial family protein. | | | U | U |
| Os06g0652200 | Hly-III related proteins family protein. | | | U | U |
| Os06g0210900 | Lipase, class 3 family protein. | | | U | U |
| Os08g0508800 | Lipoxygenase, chloroplast precursor (EC 1.13.11.12). | | | U | U |
| Os08g0509100 | Lipoxygenase, chloroplast precursor (EC 1.13.11.12). | | | U | U |
| Os06g0218900 | LMBR1-like conserved region domain containing protein. | | | U | U |
| Os03g0226200 | Non-symbiotic hemoglobin 2 (rHb2) (ORYsa GLB1b). | | | U | U |
| Os08g0526100 | Nucleotide sugar epimerase family protein. | | | U | U |

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os04g0597800 | Oligopeptide transporter, kidney isoform (Peptide transporter 2). | | | U | U |
| Os08g0189900 | Oxalate oxidase-like protein or germin-like protein (Germin-like 8) (Germin-like 12). | | | U | U |
| Os02g0314600 | Peptidase A1, pepsin family protein. | | | U | U |
| Os02g0693400 | Peptidase C19, ubiquitin carboxyl-terminal hydrolase 2 family protein. | | | U | U |
| Os01g0892500 | Pectinacetylesterase family protein. | | | U | U |
| Os10g0521900 | Rhomboid-like protein family protein. | | | U | U |
| Os02g0242900 | UDP-glucuronosyl/UDP-glucosyltransferase family protein. | | U | | U |
| Os10g0409400 | BURP domain containing protein. | | U | | U |
| Os02g0630300 | 2OG-Fe(II) oxygenase domain containing protein. | | | D | D |
| Os05g0521300 | Kinesin 4 (Kinesin-like protein D). | | | D | D |
| Os01g0975900 | Tonoplast membrane integral protein ZmTIP1-2. | | D | | D |
| Os07g0523600 | Glucose-6-phosphate/phosphate-translocator precursor. | | D | U | |
| Os01g0241400 | Glutaredoxin domain containing protein. | | D | U | |
| Os04g0412300 | Glycoside hydrolase, family 17 protein. | | D | U | |
| Os01g0832600 | Isopenicillin N synthase family protein. | | D | U | |
| Os03g0289800 | Isopenicillin N synthase family protein. | | D | U | |
| Os02g0756800 | Phosphate-induced protein 1 conserved region family protein. | | D | U | |
| Os09g0538000 | RNase S-like protein. | | D | U | |
| Os06g0242000 | SAM dependent carboxyl methyltransferase family protein. | | D | U | |
| Os10g0178500 | UDP-glucuronosyl/UDP-glucosyltransferase family protein. | | D | U | |
| Os04g0604300 | Xyloglucan endotransglucosylase/hydrolase protein 24 precursor (EC 2.4.1.207) | | D | U | |
| Os04g0449200 | Xyloglucan fucosyltransferase family protein. | | D | U | |
| Os08g0391700 | 2OG-Fe(II) oxygenase domain containing protein. | | D | U | |
| Os02g0582900 | Conotoxin family protein. | | D | U | |
| Os07g0563400 | Cotton fibre expressed family protein. | | D | U | |
| Os01g0243000 | Esterase/lipase/thioesterase domain containing protein. | | D | U | |
| Os01g0716800 | Endonuclease/exonuclease/phosphatase family protein. | | D | U | |
| Os06g0216300 | Oxo-phytodienoic acid reductase (12-oxophytodienoic acid reductase). | | D | U | |
| Os06g0216200 | Oxo-phytodienoic acid reductase. | | D | U | |

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os01g0848700 | Ras-related protein Rab11C. | | D | U | |
| Os03g0273200 | Laccase (EC 1.10.3.2). | | U | D | |

[1]Treatment comparisons: C-Si=control vs silicon-amended, Si-SiP=silicon-amended vs silicon-amended and pathogen-inoculated, C-P=control vs pathogen inoculated, P-SiP=pathogen-inoculated vs silicon-amended and pathogen-inoculated. U=upregulated, D=downregulated. Differential regulation is reported with respect to the treatment on the left of the comparison.

Table A-6 Expression profile of cytochrome P450-encoding genes

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os01g0627800 | Cytochrome P450 monooxygenase CYP72A5 (Fragment). | D[1] | | | |
| Os04g0171800 | Cytochrome P450 79A1 (EC 1.14.13.41) (Tyrosine N-monooxygenase). | | | D | |
| Os01g0544200 | Cytochrome P450 family protein. | | | D | |
| Os02g0570500 | Cytochrome P450 family protein. | | | D | |
| Os04g0174100 | E-class P450, group I family protein. | | | D | |
| Os01g0227700 | Cytochrome P450 family protein. | | | | U |
| Os10g0144700 | Cytochrome P450 family protein. | D | | D | |
| Os10g0515900 | E-class P450, group I family protein. | D | | D | |
| Os09g0447300 | Cytochrome P450 family protein. | | U | | U |
| Os02g0703600 | Cytochrome P450 90C1 (EC 1.14.-.-) (ROTUNDIFOLIA3). | | D | U | U |
| Os11g0151400 | Cytochrome P450 family protein. | | D | U | U |
| Os12g0150200 | Cytochrome P450 family protein. | | D | U | U |
| Os05g0211100 | Cytochrome P450 51 (EC 1.14.13.70) (CYPLI) (P450-LIA1). | | D | U | |
| Os09g0457100 | Cytochrome P450 family protein. | | D | U | |
| Os11g0483000 | Cytochrome P450 family protein. | | D | U | |
| Os12g0582700 | Cytochrome P450 family protein. | | D | U | |
| Os06g0294600 | Cytochrome P450 family protein. | | | U | U |
| Os08g0507100 | E-class P450, group I family protein. | | | U | U |

[1]Treatment comparisons: C-Si=control vs silicon-amended, Si-SiP=silicon-amended vs silicon-amended and pathogen-inoculated, C-P=control vs pathogen inoculated, P-SiP=pathogen-inoculated vs silicon-amended and pathogen-inoculated. U=upregulated, D=downregulated. Differential regulation is reported with respect to the treatment on the left of the comparison.

Table A-7 Expression profile of genes encoding proteins involved in protein-protein interactions and/or protein turnover

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os02g0715000 | Cysteine proteinase GP-II (EC 3.4.22.-). | | U[1] | | |
| Os05g0393100 | Leucine-rich repeat, plant specific containing protein. | | | U | |
| Os12g0211500 | Leucine-rich repeat, plant specific containing protein. | | | U | |
| Os10g0469000 | Leucine-rich repeat, typical subtype containing protein. | | | U | |
| Os02g0540700 | U box domain containing protein. | | | U | |
| Os03g0240600 | U box domain containing protein. | | | U | |
| Os06g0248500 | U box domain containing protein. | | | U | |
| Os12g0222900 | Leucine-rich repeat, typical subtype containing protein. | U | | U | |
| Os09g0573100 | Ubiquitin domain containing protein. | D | D | D | |
| Os03g0667100 | BTB/POZ domain containing protein. | | | U | U |
| Os01g0158600 | Leucine-rich repeat, typical subtype containing protein. | | | U | U |
| Os03g0190300 | Leucine-rich repeat, typical subtype containing protein. | | | U | U |
| Os02g0269600 | Subtilase. | | | U | U |
| Os02g0539200 | U box domain containing protein. | | | U | U |
| Os04g0686000 | U box domain containing protein. | | | U | U |
| Os05g0476700 | U box domain containing protein. | | | U | U |

[1]Treatment comparisons: C-Si=control vs silicon-amended, Si-SiP=silicon-amended vs silicon-amended and pathogen-inoculated, C-P=control vs pathogen inoculated, P-SiP=pathogen-inoculated vs silicon-amended and pathogen-inoculated. U=upregulated, D=downregulated. Differential regulation is reported with respect to the treatment on the left of the comparison.

Table A-8 Expression profile of genes involved in calcium signaling/binding

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os01g0949300 | Calcium-binding EF-hand domain containing protein. | | | U[1] | |
| Os12g0603800 | Calmodulin NtCaM13. | | | U | |
| Os03g0319300 | Calmodulin TaCaM1-1. | | | U | |
| Os05g0223000 | Calmodulin-related protein 2, touch-induced. | | | U | |
| Os06g0683400 | EF-hand Ca2+-binding protein CCD1. | | | U | |
| Os09g0471800 | EGF-like calcium-binding domain containing protein. | | | U | |
| Os01g0743100 | IQ calmodulin-binding region domain containing protein. | | | U | |
| Os12g0228800 | Calmodulin-like protein. | U | | U | |
| Os01g0949500 | Calmodulin 2/3/5. | | D | U | U |
| Os07g0568600 | Calcium-dependent protein kinase. | | | U | U |
| Os02g0807200 | EGF-like calcium-binding domain containing protein. | | | U | U |
| Os09g0562600 | EGF-like calcium-binding domain containing protein. | | | U | U |
| Os01g0135700 | Flagellar calcium-binding protein (calflagin) family protein. | | | U | U |
| Os01g0604500 | Flagellar calcium-binding protein (calflagin) family protein. | | | U | U |
| Os05g0577500 | Flagellar calcium-binding protein (calflagin) family protein. | | | U | U |
| Os01g0570800 | IQ calmodulin-binding region domain containing protein. | | | U | U |
| Os05g0541100 | IQ calmodulin-binding region domain containing protein. | | | U | U |
| Os02g0733500 | Parvalbumin family protein. | | | U | U |

[1]Treatment comparisons: C-Si=control vs silicon-amended, Si-SiP=silicon-amended vs silicon-amended and pathogen-inoculated, C-P=control vs pathogen inoculated, P-SiP=pathogen-inoculated vs silicon-amended and pathogen-inoculated. U=upregulated, D=downregulated. Differential regulation is reported with respect to the treatment on the left of the comparison.

Table A-9 Expression profile of kinase/phosphatase-encoding genes

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os02g0241100 | Protein kinase domain containing protein. | D[1] | | | |
| Os01g0656200 | Protein phosphatase 2C family protein. | | D | | |
| Os03g0268600 | Protein phosphatase type 2C. | | D | | |
| Os04g0632100 | S-locus receptor-like kinase RLK13. | | D | | |
| Os01g0113500 | Protein kinase domain containing protein. | | | U | |
| Os01g0669100 | Protein kinase domain containing protein. | | | U | |
| Os01g0750600 | Protein kinase domain containing protein. | | | U | |
| Os01g0892800 | Protein kinase domain containing protein. | | | U | |
| Os02g0555900 | Protein kinase domain containing protein. | | | U | |
| Os03g0773300 | Protein kinase domain containing protein. | | | U | |
| Os05g0471000 | Protein kinase domain containing protein. | | | U | |
| Os05g0545300 | Protein kinase domain containing protein. | | | U | |
| Os10g0468500 | Protein kinase domain containing protein. | | | U | |
| Os06g0541600 | Protein kinase family protein. | | | U | |
| Os07g0186200 | Protein kinase family protein. | | | U | |
| Os09g0408900 | Protein kinase family protein. | | | U | |
| Os11g0208700 | Protein kinase family protein. | | | U | |
| Os11g0667600 | Protein kinase family protein. | | | U | |
| Os04g0340100 | Protein kinase-like domain containing protein. | | | U | |
| Os10g0134900 | Protein kinase-like domain containing protein. | | | U | |
| Os03g0104100 | Protein phosphatase 2C-like domain containing protein. | | | U | |
| Os07g0566200 | Protein phosphatase 2C-like domain containing protein. | | | U | |
| Os03g0761100 | Protein phosphatase 2C-like protein. | | | U | |
| Os08g0374600 | Receptor kinase-like protein. | | | U | |
| Os11g0570000 | Receptor kinase-like protein. | | | U | |
| Os11g0208800 | Receptor-like protein kinase. | | | U | |
| Os07g0680900 | Serine/threonine-protein kinase AtPK19 (EC 2.7.1.37). | | | U | |
| Os12g0224000 | Diacylglycerol kinase 2 (At5g63770) (EC 2.7.1.107). | | | U | |
| Os02g0787800 | Diacylglycerol kinase, catalytic region domain containing protein. | | | U | |

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os09g0533600 | Probable serine/threonine-protein kinase NAK (EC 2.7.1.37). | | | U | |
| Os01g0846300 | Protein phosphatase 2C (PP2C) (EC 3.1.3.16). | | | D | |
| Os02g0822900 | Protein kinase domain containing protein. | | | | U |
| Os09g0350900 | Protein kinase domain containing protein. | | | | U |
| Os07g0538300 | Serine/threonine kinase receptor-like protein. | | | | U |
| Os04g0634700 | Diacylglycerol kinase. | | | | U |
| Os01g0655500 | Protein kinase domain containing protein. | U | U | | |
| Os07g0541900 | Protein kinase domain containing protein. | U | | U | U |
| Os11g0691500 | Protein kinase domain containing protein. | U | | U | |
| Os11g0695800 | Protein kinase domain containing protein. | U | | U | |
| Os01g0114100 | Protein kinase family protein. | U | | U | |
| Os11g0625200 | Protein kinase family protein. | U | | U | |
| Os01g0124400 | Proteinase inhibitor I12, Bowman-Birk family protein. | D | D | U | |
| Os10g0142600 | Protein kinase domain containing protein. | D | D | | |
| Os01g0117600 | Protein kinase domain containing protein. | D | | D | |
| Os01g0690800 | Protein kinase domain containing protein. | D | | D | |
| Os11g0625900 | Protein kinase domain containing protein. | D | | D | |
| Os11g0694100 | Protein kinase domain containing protein. | D | | D | |
| Os03g0762000 | Casein kinase II alpha subunit. | D | | D | |
| Os01g0699100 | Protein kinase domain containing protein. | | D | U | |
| Os01g0699400 | Protein kinase domain containing protein. | | D | U | |
| Os04g0563900 | Protein kinase domain containing protein. | | D | U | |
| Os02g0126400 | Protein kinase CPK1. | | | U | U |
| Os01g0127700 | Protein kinase domain containing protein. | | | U | U |
| Os01g0699500 | Protein kinase domain containing protein. | | | U | U |
| Os01g0699600 | Protein kinase domain containing protein. | | | U | U |
| Os02g0165100 | Protein kinase domain containing protein. | | | U | U |
| Os02g0681700 | Protein kinase domain containing protein. | | | U | U |
| Os04g0371700 | Protein kinase domain containing protein. | | | U | U |
| Os04g0619400 | Protein kinase domain containing protein. | | | U | U |
| Os05g0165900 | Protein kinase domain containing protein. | | | U | U |
| Os05g0166300 | Protein kinase domain containing protein. | | | U | U |
| Os05g0486100 | Protein kinase domain containing protein. | | | U | U |

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os07g0537500 | Protein kinase domain containing protein. | | | U | U |
| Os08g0203100 | Protein kinase domain containing protein. | | | U | U |
| Os09g0400500 | Protein kinase domain containing protein. | | | U | U |
| Os09g0561600 | Protein kinase domain containing protein. | | | U | U |
| Os11g0666200 | Protein kinase domain containing protein. | | | U | U |
| Os04g0419900 | Protein kinase family protein. | | | U | U |
| Os03g0821300 | Protein phosphatase 2C-like domain containing protein. | | | U | U |
| Os07g0550900 | Receptor-like protein kinase 6. | | | U | U |
| Os02g0807900 | Serine threonine kinase. | | | U | U |
| Os01g0934100 | C2 domain containing protein. | | | U | U |
| Os05g0493100 | KI domain interacting kinase 1. | | | U | U |
| Os03g0285800 | MAP kinase 1 (MAP kinase MAPK5a). | | | U | U |
| Os01g0841700 | RPP17-1. | | | U | U |

[1]Treatment comparisons: C-Si=control vs silicon-amended, Si-SiP=silicon-amended vs silicon-amended and pathogen-inoculated, C-P=control vs pathogen inoculated, P-SiP=pathogen-inoculated vs silicon-amended and pathogen-inoculated. U=upregulated, D=downregulated. Differential regulation is reported with respect to the treatment on the left of the comparison.

Table A-10 Expression profile of genes with unknown function

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os03g0736900 | Conserved hypothetical protein. | U[1] | | | |
| Os02g0110500 | Hypothetical protein. | U | | | |
| Os11g0235400 | Hypothetical protein. | U | | | |
| Os11g0594800 | Protein of unknown function DUF538 family protein. | U | | | |
| Os05g0551000 | Protein of unknown function HHE domain containing protein. | U | | | |
| Os01g0793300 | (No Hit) | D | | | |
| Os09g0472700 | (No Hit) | D | | | |
| Os02g0259900 | Conserved hypothetical protein. | D | | | |
| Os05g0448700 | Conserved hypothetical protein. | D | | | |
| Os09g0304800 | Conserved hypothetical protein. | D | | | |
| Os02g0212300 | Hypothetical protein. | D | | | |
| Os06g0493100 | Hypothetical protein. | D | | | |
| Os10g0348900 | Hypothetical protein. | D | | | |
| Os10g0360600 | Hypothetical protein. | D | | | |
| Os10g0372800 | Hypothetical protein. | D | | | |
| Os11g0211800 | Hypothetical protein. | D | | | |
| Os12g0221400 | Hypothetical protein. | D | | | |
| Os12g0229100 | Non-protein coding transcript, uncharacterized transcript. | D | | | |
| Os11g0540600 | Plant protein of unknown function family protein. | D | | | |
| Os09g0396900 | Protein of unknown function DUF125 family protein. | D | | | |
| Os05g0409500 | MtN21 protein. | D | | | |
| Os01g0212000 | Conserved hypothetical protein. | | U | | |
| Os07g0142300 | Conserved hypothetical protein. | | U | | |
| Os07g0142500 | Conserved hypothetical protein. | | U | | |
| Os07g0565800 | Conserved hypothetical protein. | | U | | |
| Os07g0599900 | Conserved hypothetical protein. | | U | | |
| Os08g0478000 | Conserved hypothetical protein. | | U | | |
| Os12g0580600 | Conserved hypothetical protein. | | U | | |
| Os03g0107700 | EL2 protein. | | | U | |

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os03g0304100 | Hypothetical protein. | | U | | |
| Os05g0355900 | Hypothetical protein. | | U | | |
| Os12g0453500 | Hypothetical protein. | | U | | |
| Os03g0245200 | Protein of unknown function DUF1210 family protein. | | U | | |
| Os05g0454200 | Protein of unknown function DUF315 domain containing protein. | | U | | |
| Os08g0335600 | Protein of unknown function DUF568 domain containing protein. | | U | | |
| Os03g0746900 | Conserved hypothetical protein. | | D | | |
| Os05g0142900 | Conserved hypothetical protein. | | D | | |
| Os05g0421600 | Conserved hypothetical protein. | | D | | |
| Os04g0558700 | Hypothetical protein. | | D | | |
| Os03g0233000 | Protein of unknown function DUF607 family protein. | | D | | |
| Os05g0588900 | BCS1 protein-like protein. | | D | | |
| Os05g0214300 | MtN3 and saliva related transmembrane protein family protein. | | D | | |
| Os03g0431600 | Conserved hypothetical protein. | U | U | U | |
| Os07g0142100 | Conserved hypothetical protein. | U | U | U | |
| Os11g0262600 | Hypothetical protein. | U | U | U | |
| Os01g0647200 | Non-protein coding transcript, unclassifiable transcript. | U | U | U | |
| Os08g0466600 | (No Hit) | U | U | | |
| Os09g0130300 | Conserved hypothetical protein. | U | U | | |
| Os12g0236100 | Conserved hypothetical protein. | U | U | | |
| Os03g0725200 | Hypothetical protein. | U | U | | |
| Os10g0128000 | Hypothetical protein. | U | U | | |
| Os12g0282000 | Hypothetical protein. | U | U | | |
| Os01g0494300 | Non-protein coding transcript, putative npRNA. | U | U | | |
| Os03g0439700 | Protein of unknown function DUF1230 family protein. | U | U | | |
| Os01g0115100 | (No Hit) | U | | U | |
| Os01g0829300 | (No Hit) | U | | U | |

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os03g0228900 | (No Hit) | U | | U | |
| Os04g0136600 | (No Hit) | U | | U | |
| Os04g0197500 | (No Hit) | U | | U | |
| Os11g0695000 | (No Hit) | U | | U | |
| Os12g0468100 | (No Hit) | U | | U | |
| Os01g0363500 | Conserved hypothetical protein. | U | | U | |
| Os01g0567200 | Conserved hypothetical protein. | U | | U | |
| Os02g0310200 | Conserved hypothetical protein. | U | | U | |
| Os03g0629800 | Conserved hypothetical protein. | U | | U | |
| Os06g0189600 | Conserved hypothetical protein. | U | | U | |
| Os08g0338000 | Conserved hypothetical protein. | U | | U | |
| Os09g0269900 | Conserved hypothetical protein. | U | | U | |
| Os11g0692000 | Conserved hypothetical protein. | U | | U | |
| Os03g0247300 | Hypothetical protein. | U | | U | |
| Os05g0227100 | Hypothetical protein. | U | | U | |
| Os08g0240200 | Hypothetical protein. | U | | U | |
| Os08g0441800 | Hypothetical protein. | U | | U | |
| Os11g0235700 | Hypothetical protein. | U | | U | |
| Os11g0687200 | Hypothetical protein. | U | | U | |
| Os11g0422000 | Non-protein coding transcript, uncharacterized transcript. | U | | U | |
| Os11g0235300 | Non-protein coding transcript, unclassifiable transcript. | U | | U | |
| Os08g0351200 | Plant protein of unknown function family protein. | U | | U | |
| Os03g0269900 | Protein of unknown function DUF604 family protein. | U | | U | |
| Os04g0311300 | Viral coat and capsid protein family protein. | U | | U | |
| Os01g0155800 | Conserved hypothetical protein. | U | | | D |
| Os01g0360100 | Conserved hypothetical protein. | U | | | D |
| Os07g0688700 | Eggshell protein family protein. | U | | | D |
| Os08g0371200 | Conserved hypothetical protein. | D | U | D | U |
| Os08g0413500 | Protein of unknown function DUF1262 family protein. | D | U | D | U |
| Os10g0360700 | Conserved hypothetical protein. | D | D | D | |

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os01g0189700 | Hypothetical protein. | D | D | D | |
| Os01g0844000 | (No Hit) | D | | D | |
| Os04g0426700 | (No Hit) | D | | D | |
| Os06g0578700 | (No Hit) | D | | D | |
| Os08g0301000 | (No Hit) | D | | D | |
| Os12g0543800 | (No Hit) | D | | D | |
| Os08g0412500 | Brain protein 44-like protein. | D | | D | |
| Os01g0189800 | Conserved hypothetical protein. | D | | D | |
| Os01g0604100 | Conserved hypothetical protein. | D | | D | |
| Os01g0820800 | Conserved hypothetical protein. | D | | D | |
| Os01g0931100 | Conserved hypothetical protein. | D | | D | |
| Os01g0961600 | Conserved hypothetical protein. | D | | D | |
| Os08g0411500 | Conserved hypothetical protein. | D | | D | |
| Os08g0421400 | Conserved hypothetical protein. | D | | D | |
| Os10g0137300 | Conserved hypothetical protein. | D | | D | |
| Os10g0371600 | Conserved hypothetical protein. | D | | D | |
| Os12g0169300 | Conserved hypothetical protein. | D | | D | |
| Os01g0687700 | Eukaryotic protein of unknown function DUF292 domain containing protein. | D | | D | |
| Os01g0320700 | Hypothetical protein. | D | | D | |
| Os01g0598900 | Hypothetical protein. | D | | D | |
| Os02g0173300 | Hypothetical protein. | D | | D | |
| Os08g0184800 | Hypothetical protein. | D | | D | |
| Os08g0231100 | Hypothetical protein. | D | | D | |
| Os09g0496400 | Hypothetical protein. | D | | D | |
| Os10g0111800 | Hypothetical protein. | D | | D | |
| Os11g0626700 | Hypothetical protein. | D | | D | |
| Os11g0694000 | Hypothetical protein. | D | | D | |
| Os12g0218300 | Hypothetical protein. | D | | D | |
| Os12g0221600 | Hypothetical protein. | D | | D | |
| Os12g0222300 | Hypothetical protein. | D | | D | |
| Os12g0228500 | Hypothetical protein. | D | | D | |
| Os01g0962000 | Non-protein coding transcript, unclassifiable transcript. | D | | D | |
| Os12g0217800 | Non-protein coding transcript, unclassifiable | D | | D | |

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| | transcript. | | | | |
| Os12g0218100 | Non-protein coding transcript, unclassifiable transcript. | D | | D | |
| Os08g0412700 | Protein of unknown function DUF1262 family protein. | D | | D | |
| Os02g0513800 | Ovarian tumour, otubain domain containing protein. | D | | D | |
| Os01g0251400 | Hypothetical protein. | | U | D | |
| Os06g0188400 | Hypothetical protein. | | U | D | |
| Os10g0150300 | Protein of unknown function DUF1210 family protein. | | U | D | |
| Os04g0280500 | Conserved hypothetical protein. | | U | | U |
| Os01g0392600 | Hypothetical protein. | | U | | U |
| Os05g0394000 | (No Hit) | | D | U | U |
| Os09g0259400 | (No Hit) | | D | U | U |
| Os01g0952900 | Conserved hypothetical protein. | | D | U | U |
| Os02g0733900 | Conserved hypothetical protein. | | D | U | U |
| Os05g0516700 | Conserved hypothetical protein. | | D | U | U |
| Os03g0183500 | Protein of unknown function DUF581 family protein. | | D | U | U |
| Os01g0180200 | Protein of unknown function DUF635 family protein. | | D | U | U |
| Os02g0748300 | VMP3 protein. | | D | U | U |
| Os01g0821300 | (No Hit) | | D | U | |
| Os02g0676800 | (No Hit) | | D | U | |
| Os05g0162800 | (No Hit) | | D | U | |
| Os05g0545700 | (No Hit) | | D | U | |
| Os07g0125500 | (No Hit) | | D | U | |
| Os07g0126700 | (No Hit) | | D | U | |
| Os01g0130900 | Conserved hypothetical protein. | | D | U | |
| Os01g0306400 | Conserved hypothetical protein. | | D | U | |
| Os02g0527200 | Conserved hypothetical protein. | | D | U | |
| Os02g0787700 | Conserved hypothetical protein. | | D | U | |
| Os03g0126900 | Conserved hypothetical protein. | | D | U | |
| Os03g0740200 | Conserved hypothetical protein. | | D | U | |

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os07g0661400 | Conserved hypothetical protein. | | D | U | |
| Os09g0380600 | Conserved hypothetical protein. | | D | U | |
| Os10g0580900 | Conserved hypothetical protein. | | D | U | |
| Os02g0597300 | Hypothetical protein. | | D | U | |
| Os03g0187400 | Hypothetical protein. | | D | U | |
| Os03g0342100 | Hypothetical protein. | | D | U | |
| Os05g0420000 | Hypothetical protein. | | D | U | |
| Os05g0130300 | Non-protein coding transcript, putative npRNA. | | D | U | |
| Os03g0277700 | Protein of unknown function DUF26 domain containing protein. | | D | U | |
| Os12g0548700 | CI2C. | | D | U | |
| Os07g0686600 | VQ domain containing protein. | | D | U | |
| Os12g0242100 | Eggshell protein family protein. | | D | | D |
| Os01g0136700 | (No Hit) | | | U | |
| Os01g0512900 | (No Hit) | | | U | |
| Os01g0554800 | (No Hit) | | | U | |
| Os01g0705000 | (No Hit) | | | U | |
| Os01g0725000 | (No Hit) | | | U | |
| Os01g0970100 | (No Hit) | | | U | |
| Os02g0206200 | (No Hit) | | | U | |
| Os02g0560200 | (No Hit) | | | U | |
| Os02g0561000 | (No Hit) | | | U | |
| Os02g0561400 | (No Hit) | | | U | |
| Os02g0561800 | (No Hit) | | | U | |
| Os02g0834600 | (No Hit) | | | U | |
| Os03g0155000 | (No Hit) | | | U | |
| Os03g0285200 | (No Hit) | | | U | |
| Os03g0342400 | (No Hit) | | | U | |
| Os03g0655100 | (No Hit) | | | U | |
| Os04g0258700 | (No Hit) | | | U | |
| Os05g0369100 | (No Hit) | | | U | |
| Os05g0468900 | (No Hit) | | | U | |
| Os05g0468900 | (No Hit) | | | U | |
| Os06g0208200 | (No Hit) | | | U | |

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os06g0572600 | (No Hit) | | | U | |
| Os07g0560100 | (No Hit) | | | U | |
| Os07g0587300 | (No Hit) | | | U | |
| Os08g0181800 | (No Hit) | | | U | |
| Os11g0242200 | (No Hit) | | | U | |
| Os11g0557500 | (No Hit) | | | U | |
| Os12g0505800 | (No Hit) | | | U | |
| Os08g0478700 | Brain mitochondrial carrier protein-1 (BMCP-1) (Mitochondrial uncoupling protein 5). | | | U | |
| Os01g0194000 | Conserved hypothetical protein. | | | U | |
| Os01g0551000 | Conserved hypothetical protein. | | | U | |
| Os01g0799000 | Conserved hypothetical protein. | | | U | |
| Os01g0833400 | Conserved hypothetical protein. | | | U | |
| Os01g0850100 | Conserved hypothetical protein. | | | U | |
| Os01g0864200 | Conserved hypothetical protein. | | | U | |
| Os01g0888800 | Conserved hypothetical protein. | | | U | |
| Os01g0934400 | Conserved hypothetical protein. | | | U | |
| Os02g0162600 | Conserved hypothetical protein. | | | U | |
| Os02g0324700 | Conserved hypothetical protein. | | | U | |
| Os02g0566300 | Conserved hypothetical protein. | | | U | |
| Os03g0148400 | Conserved hypothetical protein. | | | U | |
| Os04g0640900 | Conserved hypothetical protein. | | | U | |
| Os05g0546300 | Conserved hypothetical protein. | | | U | |
| Os06g0713100 | Conserved hypothetical protein. | | | U | |
| Os07g0194800 | Conserved hypothetical protein. | | | U | |
| Os07g0580200 | Conserved hypothetical protein. | | | U | |
| Os07g0589600 | Conserved hypothetical protein. | | | U | |
| Os07g0659300 | Conserved hypothetical protein. | | | U | |
| Os09g0416600 | Conserved hypothetical protein. | | | U | |
| Os10g0358100 | Conserved hypothetical protein. | | | U | |
| Os10g0536400 | Conserved hypothetical protein. | | | U | |
| Os11g0207300 | Conserved hypothetical protein. | | | U | |
| Os11g0471200 | Conserved hypothetical protein. | | | U | |
| Os02g0814000 | Eukaryotic protein of unknown function DUF862 family protein. | | | U | |

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os01g0121500 | Hypothetical protein. | | | U | |
| Os01g0814400 | Hypothetical protein. | | | U | |
| Os01g0834900 | Hypothetical protein. | | | U | |
| Os03g0305200 | Hypothetical protein. | | | U | |
| Os03g0334200 | Hypothetical protein. | | | U | |
| Os04g0414500 | Hypothetical protein. | | | U | |
| Os04g0621900 | Hypothetical protein. | | | U | |
| Os12g0113600 | Hypothetical protein. | | | U | |
| Os02g0792100 | Non-protein coding transcript, putative npRNA. | | | U | |
| Os04g0634600 | Non-protein coding transcript, uncharacterized transcript. | | | U | |
| Os04g0639000 | Non-protein coding transcript, uncharacterized transcript. | | | U | |
| Os05g0563900 | Non-protein coding transcript, uncharacterized transcript. | | | U | |
| Os08g0452900 | Non-protein coding transcript, uncharacterized transcript. | | | U | |
| Os02g0209300 | Non-protein coding transcript, unclassifiable transcript. | | | U | |
| Os03g0716900 | Non-protein coding transcript, unclassifiable transcript. | | | U | |
| Os03g0740900 | Non-protein coding transcript, unclassifiable transcript. | | | U | |
| Os09g0425500 | Non-protein coding transcript, unclassifiable transcript. | | | U | |
| Os03g0773000 | Protein of unknown function DUF1005 family protein. | | | U | |
| Os03g0739700 | Protein of unknown function DUF1334 family protein. | | | U | |
| Os04g0659300 | Protein of unknown function DUF26 domain containing protein. | | | U | |
| Os07g0530600 | Protein of unknown function DUF299 family protein. | | | U | |
| Os01g0956200 | Protein of unknown function DUF563 family | | | U | |

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| | protein. | | | | |
| Os11g0575500 | Protein of unknown function DUF563 family protein. | | | U | |
| Os01g0727500 | Protein of unknown function DUF584 family protein. | | | U | |
| Os01g0798800 | Protein of unknown function DUF594 family protein. | | | U | |
| Os11g0136300 | Protein of unknown function DUF6 domain containing protein. | | | U | |
| Os12g0518200 | Protein of unknown function DUF6 domain containing protein. | | | U | |
| Os01g0817000 | Protein of unknown function DUF607 family protein. | | | U | |
| Os02g0654600 | Protein of unknown function DUF630 domain containing protein. | | | U | |
| Os12g0164600 | Protein of unknown function DUF793 family protein. | | | U | |
| Os01g0948500 | ARM repeat fold domain containing protein. | | | U | |
| Os03g0297600 | Bet v I allergen family protein. | | | U | |
| Os10g0499400 | CBS domain containing protein. | | | U | |
| Os01g0695800 | Multidrug resistance protein 1 homolog. | | | U | |
| Os04g0461600 | PGPS/D12. | | | U | |
| Os10g0343100 | t-snare domain containing protein. | | | U | |
| Os01g0796000 | (No Hit) | | | U | U |
| Os02g0584700 | (No Hit) | | | U | U |
| Os02g0807800 | (No Hit) | | | U | U |
| Os07g0208300 | (No Hit) | | | U | U |
| Os08g0137900 | (No Hit) | | | U | U |
| Os08g0173300 | (No Hit) | | | U | U |
| Os08g0516400 | (No Hit) | | | U | U |
| Os12g0111800 | (No Hit) | | | U | U |
| Os01g0134700 | Conserved hypothetical protein. | | | U | U |
| Os01g0714600 | Conserved hypothetical protein. | | | U | U |
| Os02g0601000 | Conserved hypothetical protein. | | | U | U |
| Os02g0736900 | Conserved hypothetical protein. | | | U | U |

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os03g0170100 | Conserved hypothetical protein. | | | U | U |
| Os03g0734500 | Conserved hypothetical protein. | | | U | U |
| Os04g0586500 | Conserved hypothetical protein. | | | U | U |
| Os05g0546400 | Conserved hypothetical protein. | | | U | U |
| Os05g0552800 | Conserved hypothetical protein. | | | U | U |
| Os06g0133300 | Conserved hypothetical protein. | | | U | U |
| Os06g0133500 | Conserved hypothetical protein. | | | U | U |
| Os06g0201200 | Conserved hypothetical protein. | | | U | U |
| Os06g0203600 | Conserved hypothetical protein. | | | U | U |
| Os07g0680600 | Conserved hypothetical protein. | | | U | U |
| Os08g0402500 | Conserved hypothetical protein. | | | U | U |
| Os08g0448100 | Conserved hypothetical protein. | | | U | U |
| Os10g0563800 | Conserved hypothetical protein. | | | U | U |
| Os03g0710000 | Eukaryotic protein of unknown function DUF292 domain containing protein. | | | U | U |
| Os01g0186900 | Hypothetical protein. | | | U | U |
| Os01g0768100 | Hypothetical protein. | | | U | U |
| Os01g0833600 | Hypothetical protein. | | | U | U |
| Os01g0905300 | Hypothetical protein. | | | U | U |
| Os02g0597200 | Hypothetical protein. | | | U | U |
| Os03g0255900 | Hypothetical protein. | | | U | U |
| Os03g0723700 | Hypothetical protein. | | | U | U |
| Os05g0181700 | Hypothetical protein. | | | U | U |
| Os06g0318800 | Hypothetical protein. | | | U | U |
| Os07g0561800 | Hypothetical protein. | | | U | U |
| Os11g0572200 | Hypothetical protein. | | | U | U |
| Os12g0499200 | Hypothetical protein. | | | U | U |
| Os12g0556200 | Hypothetical protein. | | | U | U |
| Os12g0592900 | Hypothetical protein. | | | U | U |
| Os09g0246300 | Non-protein coding transcript, uncharacterized transcript. | | | U | U |
| Os01g0137800 | Non-protein coding transcript, unclassifiable transcript. | | | U | U |
| Os05g0131000 | Plant protein of unknown function family protein. | | | U | U |

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os03g0187800 | Protein of unknown function DUF250 domain containing protein. | | | U | U |
| Os03g0277600 | Protein of unknown function DUF26 domain containing protein. | | | U | U |
| Os04g0316200 | Protein of unknown function DUF26 domain containing protein. | | | U | U |
| Os04g0322100 | Protein of unknown function DUF26 domain containing protein. | | | U | U |
| Os01g0498300 | Protein of unknown function DUF563 family protein. | | | U | U |
| Os03g0816800 | Protein of unknown function DUF567 family protein. | | | U | U |
| Os01g0845100 | Protein of unknown function DUF668 family protein. | | | U | U |
| Os01g0389200 | Protein of unknown function DUF679 family protein. | | | U | U |
| Os01g0389700 | Protein of unknown function DUF679 family protein. | | | U | U |
| Os01g0975000 | Protein of unknown function DUF966 family protein. | | | U | U |
| Os02g0327500 | Arabidopsis protein of unknown function DUF266 family protein. | | | U | U |
| Os10g0555100 | DNA chromosome 4, ESSA I CONTIG fragment NO. 6 (Glucosyltransferase like protein). | | | U | U |
| Os01g0575200 | Rb (Fragment). | | | U | U |
| Os02g0437200 | SNAP-34. | | | U | U |
| Os02g0251900 | VQ domain containing protein. | | | U | U |
| Os03g0676400 | VQ domain containing protein. | | | U | U |
| Os05g0463300 | (No Hit) | | | D | |
| Os01g0798200 | Conserved hypothetical protein. | | | D | |
| Os02g0191800 | Conserved hypothetical protein. | | | D | |
| Os02g0193100 | Conserved hypothetical protein. | | | D | |
| Os02g0485000 | Conserved hypothetical protein. | | | D | |
| Os02g0684400 | Conserved hypothetical protein. | | | D | |

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os03g0746700 | Conserved hypothetical protein. | | | D | |
| Os06g0147100 | Conserved hypothetical protein. | | | D | |
| Os06g0523300 | Conserved hypothetical protein. | | | D | |
| Os08g0420700 | Conserved hypothetical protein. | | | D | |
| Os02g0160500 | Hypothetical protein. | | | D | |
| Os10g0364900 | Hypothetical protein. | | | D | |
| Os12g0603900 | Non-protein coding transcript, uncharacterized transcript. | | | D | |
| Os01g0592500 | Protein of unknown function DUF1070 family protein. | | | D | |
| Os10g0150600 | Protein of unknown function DUF1210 family protein. | | | D | |
| Os10g0150700 | Protein of unknown function DUF1210 family protein. | | | D | |
| Os06g0714800 | Protein of unknown function DUF581 family protein. | | | D | |
| Os01g0778600 | (No Hit) | | | | U |
| Os06g0174000 | (No Hit) | | | | U |
| Os08g0219800 | (No Hit) | | | | U |
| Os11g0156600 | (No Hit) | | | | U |
| Os01g0731100 | Conserved hypothetical protein. | | | | U |
| Os02g0134200 | Conserved hypothetical protein. | | | | U |
| Os06g0133400 | Conserved hypothetical protein. | | | | U |
| Os09g0498200 | Conserved hypothetical protein. | | | | U |
| Os11g0115600 | Conserved hypothetical protein. | | | | U |
| Os12g0186600 | Conserved hypothetical protein. | | | | U |
| Os03g0114100 | Hypothetical protein. | | | | U |
| Os11g0291500 | Hypothetical protein. | | | | U |
| Os11g0482200 | Hypothetical protein. | | | | U |
| Os12g0141000 | Hypothetical protein. | | | | U |
| Os01g0972000 | Protein of unknown function DUF1117 domain containing protein. | | | | U |
| Os01g0973100 | (No Hit) | | | | D |
| Os03g0168300 | Conserved hypothetical protein. | | | | D |
| Os04g0282400 | Conserved hypothetical protein. | | | | D |

| Gene | Description | C-Si | P-SiP | C-P | Si-SiP |
|---|---|---|---|---|---|
| Os06g0278000 | Conserved hypothetical protein. | | | | D |
| Os01g0550800 | Protein of unknown function DUF239 domain containing protein. | | | | D |
| Os06g0277900 | Protein of unknown function DUF239 domain containing protein. | | | | D |

[1]Treatment comparisons: C-Si=control vs silicon-amended, Si-SiP=silicon-amended vs silicon-amended and pathogen-inoculated, C-P=control vs pathogen inoculated, P-SiP=pathogen-inoculated vs silicon-amended and pathogen-inoculated. U=upregulated, D=downregulated. Differential regulation is reported with respect to the treatment on the left of the comparison.

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# BIOGRAPHICAL SKETCH

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