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EVALUATION OF ABSORPTION AND UPTAKE OF SOIL- AND FOLIAR-APPLIED SILICON IN RICE AND ITS ACCUMULATION UNDER DIFFERENT PHOSPHORUS RATES

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The School of Plant, Environmental, and Soil Sciences

by
Flávia B. Agostinho
B.S., University of Uberlandia, 2011
May 2016

To my father (in memory), who will always be my greatest strength.

To my mother and brother, for their support, prayers and never ending love.

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Abstract

Silicon (Si) fertilization has gained attention in rice (*Oryza sativa*) production. However, the common soil-applied sources are amended at high rates, whereas the efficacy of foliar Si application is yet to be proven. A series of pot experiments were conducted to (1) elucidate the effects of different Si sources on grain yield and Si accumulation of rice supplied with varying P rates, and 2) evaluate Si absorption and uptake by rice via foliar- and soil-application of Si fertilizers. First, three phosphorus (P) rates (0, 112, and 224 kg P ha^{-1}) and three Si sources: two soil-applied (wollastonite and silicate slag) and a liquid Si formulation applied as foliar spray at rates of 20, 40, and 80 mg Si L^{-1} were set as treatments. Silicon applied to soil (wollastonite and silicate slag) and leaves (Si solution) did not result in significant increase in rice P content and uptake in straw and grain. However, a corresponding increase in soil P content was observed with wollastonite application. Across all rice stages, wollastonite application consistently increased biomass Si content ($P<0.05$), but no significant increase in rice yield was observed with Si fertilization. For the second objective, two greenhouse experiments were conducted to determine if Si in solution can be absorbed through leaf surface and translocated within the plant. Three application rates of Si solution (20, 40, and 80 mg Si L^{-1}) were sprayed to either whole rice plants or leaves of the primary third tiller of each plant, whereas for the second experiment, Si solution (80 mg Si L^{-1}) was strictly applied to adaxial side of rice leaves, including two soil-sources and a check. The experiments were conducted in a randomized complete block design with at least four replications. There was no significant effect observed on rice growth and yield with Si fertilization. Foliar application of Si solution did not increase Si content of leaves, whereas wollastonite-treated rice attained the highest Si content ($P<0.01$). The outcomes of this

series of greenhouse studies suggest that Si absorption on leaf surface did not take place as well as the translocation of Si within the plant.

Chapter 1. Introduction

Rice (*Oryza sativa*) is a staple food that accounts for more than 22% of world's population calorie intake, with Asia and Africa as the largest consuming regions (Wailes et al., 1997). In 2014, the global rice production reached 497 million tons and 83% of it was consumed for food intake (FAO, 2014). For the third consecutive year, rice consumption was reported to exceed production, and ending stocks in 2015/2016 are expected to decline 15% from a year earlier, the lowest global ending stocks since 2007/2008 (USDA-ERS, 2015). The world population has been growing at an exponential rate and both the population increase and the rise in healthy lifestyles, which demands for more gluten-free foods, are intensifying the global consumption of rice (USDA-ERS, 2015). During the 20th century, the world population supported an increment of 4.6 billion people (Haub, 2011), whereas the expectation for the 21st century is an addition of another roughly 3 billion people by the mid-century (Fedoroff et al., 2010).

Climate changes such as extreme weather, unexpected temperature and rainfall fluctuations have affected crop productivity (Georgescu et al., 2011; Lobell et al., 2011). The global warming impact on rice production has been highlighted by several authors (Masud et al., 2014). Abdullah (2007) reported that a 1°C increase in daily average temperature in the peninsular nation of Malaysia might reduce rice yield by 10%. In addition, according to Tao et al. (2008), rice yield reduction would range from 6 to 19%, 14 to 32%, and 24 to 40% for global mean temperature increase of 1, 2, and 3°C, respectively. Other negative effects were also noted for atmospheric carbon dioxide (CO_2) concentration of 400–800 ppm and precipitation fluctuations of ±14% (Masud et al., 2014).

An effective soil nutrient management is an essential component of crop production, responsible for increasing and sustaining crop yield at high levels (Gruhn et al., 2000). All plant-essential nutrients already have established fertilization programs for rice, except the micronutrients chloride (Cl), manganese (Mn), molybdenum (Mo), and nickel (Ni) that might be supplied through the impurities or composition of common-applied fertilizers (Dobermann and Fairhurst, 2000). Interestingly, the only non-essential nutrient that is included in the guidelines for rice fertilization is silicon (Si) (Dobermann and Fairhurst, 2000). Early studies indicate that monocots species contain higher Si concentration than non-monocots (Jones and Handreck, 1967). Depending on the plant species, plant Si concentrations can range from 0.1–10% of dry matter (Epstein, 1999). Plant species are classified as high-Si accumulators when Si concentration is greater than 1% of dry leaf matter (Epstein, 1994). Since rice accumulates Si levels at 5% or higher on a dry matter basis (Epstein, 1994), it is considered as a high-Si accumulating plant (Takahashi et al., 1990). There were reports that the amount of Si taken up by rice sometimes is higher than some plant-essential nutrients, such as nitrogen (N) (Cassman et al., 1995).

Silicon comprises 28% of the earth's crust but most of it is unavailable for plant uptake (Epstein, 1994). The three general forms of Si found in soil are monosilicic acid (H_4SiO_4), polysilicic acid $[Si(OH)_4]_x$, and amorphous silica (SiO_2) (Bauer et al., 2011). Whereas the most abundant form is presented as SiO_2, it is a non-soluble mineral unavailable for plant uptake (Bauer et al., 2011). On the other hand, the soluble polysilicic acid has a high molecular weight; hence it is not available for uptake by the plant (Casey et al., 2004). The only plant-available form is monosilicic acid, H_4SiO_4 (Raven, 1983). Once H_4SiO_4 is in soil solution, it is taken up by roots through transpiration stream (Sangster et al., 2001) or active transport (Ma et al., 2007). In rice, Si uptake is mediated by specific Si transporters in the roots (Tamai and Ma, 2003; Ma et al., 2006). These transporters were identified and code by low-Si genes (Lsi1 and Lsi2), which transport Si from the soil solution to the root cells (influx, Lsi1) and from inside to outside of the root cells (efflux, Lsi2) (Ma et al., 2006; 2007). Following its absorption, H_4SiO_4 is transported through xylem to stem and leaves of plants where it is polymerized and deposited as solid amorphous silica ($SiO_2.nH_2O$) (Yoshida et al., 1962). Amorphous silica (silica body) is accumulated in several cell types, but particularly in bulliform cells of rice leaves (Motomura et al., 2000).

Silicon's known beneficial roles to plants include enhancing plant defense response against disease (Rodrigues and Datnoff, 2015), protecting plants against insects attacks (Hunt et al., 2008), increasing plant photosynthesis and growth (Gong et al., 2005), preventing lodging (Epstein, 1991), alleviating water (Agarie, 1998) and mineral toxicity stresses (Horiguchi, 1988; Savant et al., 1997), and improving fertilizer use efficiency (Friesen et al., 1994). Enhanced Si nutrition has been associated with improved resistance of rice to diseases, such as brown spot (*Cochliobolus miyabeanus*) (Savant et al., 1997) and leaf blast (*Magnaporthe oryzae*) (Datnoff et al., 1997). Silicon protects plants against diseases by acting as physical barrier on leaf surface, and stimulating defense reactions and biochemical mechanisms of host (Rodrigues and Datnoff, 2015). Erect position of leaves was also related to plants grown under Si fertilization, which results in greater light interception, hence greater photosynthetic rate (Epstein, 1994). There were studies indicating that Si fertilization enhanced plant phosphorus (P) utilization by increasing both P content of rice (IRRI, 1966) and phosphate fertilizer efficiency (IARI, 1988). Ma and Takahashi (1990) observed that rice shoots from plants cultivated in solutions of Si had twice the inorganic P content than shoots without Si treatment. Furthermore, when superphosphate was applied along with a Si fertilizer, an increase of 8% in P absorption by rice was reported (IARI, 1988).

Early on, the effect of Si on P availability was thought to be related to Si influences on soil pH, when it was applied as calcium (Ca) and sodium (Na) silicate (Noda and Komai, 1958; Roy et al., 1971; Syouji, 1981). Later, the chemical similarity between phosphate ($H_2PO_4^-$) and silicate ($H_3SiO_4^-$) ions was believed to govern this interaction (Brown and Mahler, 1987). Brown and Mahler (1987) found a strong competition between $H_2PO_4^-$ and $H_3SiO_4^-$ ions for specific soil sorption sites, as both ions are adsorbed by iron (Fe) and aluminum (Al) oxides of clay fractions. It has been suggested that previously adsorbed $H_2PO_4^-$ are displaced by $H_3SiO_4^-$ and became available for plant uptake (Bastisse, 1947; Reifenberg and Buckwold, 1954; Silva, 1971; Hingston et al., 1972; Carvalho et al., 2001; Lima, 2011). However, in an experiment involving acid soils, the effect of Si fertilization on plant P availability was not observed (Ma and Takahashi, 1990). Even so, an indirect improvement in P utilization was noticed owing to higher shoot growth provided by Si (Ma and Takahashi, 1990).

Fertilizers containing P are produced from phosphate rocks (Smil, 2000). As these rocks are finite and non-renewable P source, it may completely be exhausted within the foreseeable future (Smil, 2000; Udo de Haes et al., 2009; van Kauwenbergh, 2010; Cordell and White, 2011; Koppelaar and Weikard, 2013). Different types of models have been used to investigate the potential depletion of P reserves (Walan et al., 2014). The most alarming estimation predicted that P reserves would be depleted in 80 years (Smil, 2000) and 75 years (Udo de Haes et al., 2009). On the other hand, a steady depletion rate was also presented by van Kauwenbergh (2010) showing that P reserves may only last over the next 300-400 years. So far, the prediction that phosphate rocks can still last for a few more hundred years cannot neglect the fact that global food production is relying on a single source of P (Walan et al., 2014).

Geologically old soils and those undergoing accelerated weathering processes have high inorganic P fixation capacity, which reduces plant P availability (Novais and Smyth, 1999). Low Si content in soil was also associated with high weathering processes (Foy, 1992). Moreover, it is common to find depletion of plant-available Si in soils where rice is cultivated for a long time (Savant et al., 1997). In some countries, such as the United States and Japan, the practice of Si fertilization is already common in rice fields (Datnoff et al., 2001). Silicon fertilization is done as broadcast application through soil-applied sources (Chiu and Huang, 1971). Native calcium metasilicate (Wollastonite, $CaSiO_3$) has been considered as standard treatment (100% efficacy in supplying Si) for Si studies (Sousa and Korndorfer, 2010; Haynes et al., 2013). However, its agronomical use is limited by the high cost of the material and transportation to agriculture areas (Haynes et al., 2013). Different types of slags from Fe, ferronickel, and Mn ore smelter are used as Si fertilizer (IRRI, 1978). Slags are produced during iron and steel processing, where calcium (Ca) and magnesium (Mg) oxides (CaO, MgO) bind to Si (present in the ore) and forms Ca and Mg silicates (Sousa and Korndorfer, 2010). Industrial waste materials (slags) are abundant and an inexpensive Si source (Sousa and Korndorfer, 2010). However, the common rate of slag application in rice production ranges from 2 ton ha^{-1} (Ma and Takahashi, 2002) to 4.5 ton ha^{-1} (Korndorfer et al., 2001). High amounts per area translate to high transportation costs; thus there is a need to identify alternative Si sources that are effective even when applied in small amounts (Datnoff et al., 2005).

The development of Si-containing solutions has offered another mean of supplying Si to plants (Guével et al., 2007). Reports were made on the positive effect of foliar-Si application on diseases control in different crops, such as rice (Cacique et al., 2013), wheat (*Triticum aestivum*) (Guével et al., 2007), grape (*Vitis vinifera)* (Bowen et al., 1992), cucumber (*Cucumis sativus*), zucchini (*Cucurbita pepo*), and muskmelon (*Cucumis melo*) (Menzies et al., 1992). Pathogen-inoculated wheat treated with foliar Si-containing solution was taller over the control, but a similar result was not observed for Si solution applied foliarly to non-inoculated wheat (Guével et al., 2007). It was observed that Si solution applied directly to the roots controlled *Podosphaera xanthii* in cucumber via activation of defense enzymes; however this was not detected for cucumber which received Si foliarly (Liang et al., 2005). Whereas root uptake is an established mechanism of Si absorption by rice (Takahashi and Hino, 1978; Ma et al., 2006), transporter genes have not been reported to exist in rice leaves and there is no strong evidence showing that Si can be absorbed through the leaves (Bowen et al., 1992; Menzies et al., 1992; Liang et al., 2005; Rodrigues and Datnoff, 2015).

The effect of foliar application of Si on disease control has been explained by the deposition of dried solution on the leaf surface (Bowen et al., 1992; Menzies et al., 1992; Liang et al., 2005; Rodrigues and Datnoff, 2015). This deposition was suggested to change the pH and/or osmotic potential of leaf surface and also acts as a physical barrier against diseases development (Rodrigues and Datnoff, 2015). Bowen et al. (1992) observed the formation of whitish spots on leaf surface, suggested as dried solution which coats the leaves and protect plants against pathogen penetration. Liang et al. (2005) detected that foliar applied potassium silicate effectively controlled infection by *Podosphaera xanthii* in cucumber, but only via physical barrier and osmotic effects of the silicate applied; though, no Si absorption was observed. According to Rezende et al. (2009), Si sprayed leaves had higher Si deposit on the adaxial (upper) leaf surface, which is more exposed to Si solution spray, than abaxial (lower) leaf surface. On the other hand, some reports indicate that Si content increased in plants under foliar application of Si in comparison to the check (Guével et al., 2007; Crusciol et al., 2013a; Crusciol et al., 2013b). It has been reported that foliar applied Si provides benefits to plants; however, the mechanism by which this happen is still unclear (Liang et al., 2005).

Several studies have been done on the effect of foliar Si application in rice. If proven effective, foliar application of Si may provide a manageable means of boosting Si uptake in rice production (Bowen et al., 1992). While there were studies conducted which evaluated the effect of soil-applied Si fertilizer on soil and plant nutrient content, specifically on P availability, foliar spray as Si source to rice and its interaction with P has not been evaluated. Thus, this study was conducted to: 1) elucidate the effects of different Si sources on grain yield and Si accumulation of rice supplied with varying P rates, and 2) evaluate Si absorption and uptake by rice via foliar- and soil-application of Si fertilizers.

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Chapter 2. Effect of Silicon Sources on Grain Yield and Silicon Accumulation of Rice Grown Under Different Phosphorus Rate

2.1.Introduction

Rice (*Oryza sativa*) is a staple food that accounts for more than 22% of world's population calorie intake (Wailes et al., 1997). In 2014, the global rice production reached 497 million tons and 83% of it was consumed for food intake (FAO, 2014). For the third consecutive year, rice consumption was reported to exceed production, and ending stocks in 2015/16 are projected to decline 15% from a year earlier (USDA-ERS, 2015). The world population has grown at an exponential rate and increasing awareness on healthy lifestyles, which demands for more gluten-free foods, are intensifying the global consumption of rice (USDA-ERS, 2015). Along with higher consumption of rice, climate changes such as extreme weather, unexpected temperature and rainfall fluctuations have affected crop productivity, and strategies to increase yield have been studied (Georgescu et al., 2011; Lobell et al., 2011).

An effective soil nutrient management is an essential component of crop production, responsible for increasing and sustaining crop yields at high levels (Gruhn et al., 2000). Most plant-essential nutrients already have established fertilization programs for rice (Dobermann and Fairhurst, 2000). Interestingly, the only non-essential nutrient that is included in the guidelines for rice fertilization is silicon (Si) (Dobermann and Fairhurst, 2000). Plant species are classified as high-Si accumulators when Si concentration is greater than 1% of dry leaf matter (Epstein, 1994). Since rice accumulates Si levels at 5% or higher on a dry matter basis (Epstein, 1994), it is considered as a high-Si accumulating plant (Takahashi et al., 1990).

Silicon comprises 28% of the earth's crust but most of it is unavailable for plant uptake (Epstein, 1994). The only plant-available form is monosilicic acid (H_4SiO_4) (Raven, 1983). Once H_4SiO_4 is in soil solution, it is taken up by roots through transpiration stream (Sangster et al., 2001) or active transport (Ma et al., 2007). In rice, Si uptake is mediated by specific Si transporters in the roots (Tamai and Ma, 2003; Ma et al., 2006). These transporters were identified and code by low-Si genes (Lsi1 and Lsi2), which transport Si from the soil solution to the root cells (influx, Lsi1) and from inside to outside of the root cells (efflux, Lsi2) (Ma et al., 2006; 2007). Following its absorption, H_4SiO_4 is transported through xylem to stem and leaves of plants where it is polymerized and deposited as solid amorphous silica ($SiO_2.nH_2O$) (Yoshida et al., 1962). Amorphous silica (silica body) is accumulated in several cell types, but particularly in bulliform cells of rice leaves (Motomura et al., 2000).

Silicon's known beneficial roles to plants include enhancing plant defense response against disease (Rodrigues and Datnoff, 2015), protecting plants against insects attacks (Hunt et al., 2008), increasing plant photosynthesis and growth (Gong et al., 2005), preventing lodging (Epstein, 1991), alleviating water (Agarie, 1998) and mineral toxicity stresses (Horiguchi, 1988; Savant et al., 1997), and improving fertilizer use efficiency (Friesen et al., 1994). There were studies indicating that Si fertilization increased both P content of rice (IRRI, 1966) and phosphate fertilizer efficiency (IARI, 1988). Ma and Takahashi (1990) observed that rice shoots from plants cultivated in solutions of Si had twice the inorganic P content than shoots without Si treatment. Furthermore, when superphosphate was applied along with a Si fertilizer, an increase of 8% in P absorption by rice was reported (IARI, 1988).

Early on, the effect of Si on P availability was thought to be related to Si influences on soil pH, when it was applied as calcium (Ca) and sodium (Na) silicate (Noda and Komai, 1958;

Roy et al., 1971; Syouji, 1981). Later, the chemical similarity between phosphate ($H_2PO_4^-$) and silicate ($H_3SiO_4^-$) ions was believed to govern this interaction (Brown and Mahler, 1987). Brown and Mahler (1987) found a strong competition between $H_2PO_4^-$ and $H_3SiO_4^-$ ions for specific soil sorption sites, as both ions are adsorbed by iron (Fe) and aluminum (Al) oxides of clay fractions. It was suggested that previously adsorbed $H_2PO_4^-$ are displaced by $H_3SiO_4^-$ and became available for plant uptake (Bastisse, 1947; Reifenberg and Buckwold, 1954; Silva, 1971; Hingston et al., 1972; Carvalho et al., 2001; Lima, 2011). However, in an experiment involving acid soils, the effect of Si fertilization on plant P availability was not observed (Ma and Takahashi, 1990). Even so, an indirect improvement in P utilization was noticed owing to higher shoot growth provided by Si (Ma and Takahashi, 1990).

Fertilizers containing P are produced from phosphate rocks (Smil, 2000). As these rocks are finite and a non-renewable P source, it may completely be exhausted within the foreseeable future (Smil, 2000; Udo de Haes et al., 2009; van Kauwenbergh, 2010; Cordell and White, 2011; Koppelaar and Weikard, 2013). Different types of models have been used to investigate the potential depletion of P reserves (Walan et al., 2014). The most alarming estimation predicted that P reserves would be depleted in 80 years (Smil, 2000) and 75 years (Udo de Haes et al., 2009). On the other hand, a steady depletion rate was also presented by van Kauwenbergh (2010) showing that P reserves may only last over the next 300-400 years. So far, the prediction that phosphate rocks can still last for a few more hundred years cannot neglect the fact that global food production is relying on a single source of P (Walan et al., 2014).

Geologically old soils and those undergoing accelerated weathering processes have high inorganic P fixation capacity, which reduces plant P availability (Novais and Smyth, 1999). Low Si content in soil was also associated with high weathering processes (Foy, 1992). In some countries, such as the United States and Japan, producers have been applied Si fertilizers in rice fields (Datnoff et al., 2001). Silicon fertilization is done as broadcast application through soil-applied sources (Chiu and Huang, 1971). Native calcium metasilicate (Wollastonite, $CaSiO_3$) is widely used on Si studies, but its agronomical use is limited by the high cost of material and transportation to agriculture areas (Haynes et al., 2013). The most common source of fertilizing Si is slags from Fe, ferronickel, and manganese ore smelter (IRRI, 1978). Industrial waste materials (slags) are abundant and an inexpensive Si source (Sousa and Korndorfer, 2010), but it is applied in high amounts which limits adoption of Si fertilization by producers (Korndorfer et al., 2001). The application of Si-containing solution as foliar spray is done at manageable rates and has proposed another option of supplying Si to plants (Guével et al., 2007).

Several studies have been done to evaluate the effect of foliar Si application in rice diseases. While there were studies conducted which evaluated the effect of soil-applied Si fertilizer on soil and plant nutrient content, specifically on P availability, foliar spray as Si source to rice and its interaction with P has not been evaluated. Thus, this study was conducted to elucidate the effects of different Si sources (soil- and foliar-applied) on grain yield and Si accumulation of rice supplied with varying P rates.

2.2.Materials and Methods

2.2.1. Soil Sampling and Analysis

Bulk soil samples were collected in a rice field in Eunice (Evangeline Parish), Louisiana (30.54808 N, 92.50907 W). The soil was Crowley-Vidrine complex (CV) classified as a fine, smectitic, thermic, and typic albaqualfs soil (SSURGO-USDA, 2015). The soil texture was silt loam to silt clay, with poor drainage. The soil was air-dried for a week in a greenhouse facility at Louisiana State University campus in Baton Rouge. Daily mixing was made to break massive aggregates and to facilitate processing. Unwanted materials such as dry roots and weeds were removed by hand, and soil was sieved through a 6.5 mm stainless-steel mesh. Composite soil samples were collected, oven-dried at 65°C, and analyzed for soil characterization. The soil has a silt loam texture, slightly acidic 1:1 pH in water (6.14) and low Mehlich-3 soil test P (16 mg kg^{-1}), Si (37 mg kg^{-1}), and K (39 mg kg^{-1}) levels. Calcium, Mg, Na, sulfur (S), copper (Cu) and zinc (Zn) content were 756, 112, 20, 14, 1 and 2 mg kg^{-1}, respectively. Soil organic matter content was 18 g kg^{-1} with cation exchange capacity (CEC) of 12 $cmol_c$ kg^{-1} and sum of bases of 5 $cmol_c$ dm^{-3}.

2.2.2. Treatment Structure and Experimental Design

The experiment was established in a randomized complete block design with four replications. The treatment structure was a two-way factorial with six Si sources and three P rates (Table 2.1). Phosphorus rates were 0, 112, and 224 kg of P ha^{-1} and the Si sources were two soil-applied at 4.5 ton ha^{-1} and one foliar-applied at 0, 2, 4, and 8 L ha^{-1} of concentrated solution containing 6000 mg Si L^{-1} and diluted to final application volume of 600 L ha^{-1}; the resulting concentration were 20, 40 and 80 mg Si L^{-1}, respectively. Wollastonite (Vansil®) and silicate slag (Plant Tuff®) were used as the two soil Si sources and were applied only once before planting. On the other hand, foliar Si solutions (Taminco®) were applied to the whole plants three times: at early tillering, booting, and early flowering stages. Early tillering was considered when plants have the second tiller emerged from the main stem, booting when a bulging of leaf stem that conceals the developing panicle is formed, and early flowering when panicle is completely out of the leaf with visible flowers at the tip (10% flowering).

Silicate slag contains by 14% Si, 23% Ca, and 7% Mg, but may contain Al, Fe, Mn and S as well. Wollastonite contains by 24% Si, and 31% Ca, with lower impurities than slag. With these concentrations, soil amends at 4.5 ton ha^{-1} delivered about 690 and 1190 kg of Si ha^{-1} for silicate slag and wollastonite, respectively. The liquid Si formulation delivered to the plants for every application 12, 24, and 48 g of Si ha^{-1} at 2, 4, and 8 L ha^{-1}, respectively.

Table 2.1. Description of the treatment structure.

P Rate, kg ha^{-1}	Si Treatments
0	0
0	Foliar spray at 20 mg Si L^{-1}
0	Foliar spray at 40 mg Si L^{-1}
0	Foliar spray at 80 mg Si L^{-1}
0	Wollastonite at 1190 kg Si ha^{-1}
0	$CaSiO_3$ Slag at 690 kg Si ha^{-1}
112	0
112	Foliar spray at 20 mg Si L^{-1}
112	Foliar spray at 40 mg Si L^{-1}
112	Foliar spray at 80 mg Si L^{-1}
112	Wollastonite at 1190 kg Si ha^{-1}
112	$CaSiO_3$ Slag at 690 kg Si ha^{-1}
224	0
224	Foliar spray at 20 mg Si L^{-1}
224	Foliar spray at 40 mg Si L^{-1}
224	Foliar spray at 80 mg Si L^{-1}
224	Wollastonite at 1190 kg Si ha^{-1}
224	$CaSiO_3$ Slag at 690 kg Si ha^{-1}

Note: Foliar spray application was done three times at early tillering, booting and early flowering stages.

2.2.3. Experiment Establishment

Plastic pots (Encore Plastics®) with 13 L capacity were filled with 11 kg of air-dried and sieved soil. Phosphorus (triple superphosphate, 46% P) and soil-applied Si (wollastonite and silicate slag) treatments were established before sowing by spreading and incorporating the fertilizers into the soil by hand. At the same time, pre-planting fertilization was done with potassium chloride (KCl, 60% K) and zinc sulfate ($ZnSO_4$, 23% Zn) at rates of 90 and 6 kg ha^{-1}, respectively.

Seeds of rice variety CL151 were sown at rate of ten seeds per pot and, at four-leaf growth stage, thinned to six plants per pot. The first N fertilization (urea-45% N) was applied right after sowing at 115 kg ha^{-1}. Two weeks after sowing, pots were flooded and a 2.5 cm-water column was maintained till two-three week before harvesting. After flooding the pots, N and K fertilizations were conducted as solutions applications to water. Potassium and N second applications were done 5 and 20 days after flooding at 56 and 68 kg ha^{-1}, respectively. Foliar solution of Si was sprayed to the whole plant using a pressurized handheld sprayer (Stihl® SG 10). During the application, the soil was covered with plastic sheet to prevent the Si solution dripping into the soil.

2.2.4. Biomass Sampling

One week after each Si foliar application (early tillering, booting and early flowering), plant biomass was collected from all treatments. Two plants per pot were selected for each sampling and tillers were cut with a sickle as close as possible to soil surface. Tiller number was recorded and separated into two groups: one was washed thoroughly with deionized (DI) water before oven-drying and the other group was oven-dried without washing. The washing was done by soaking the plants three times into a container filled with DI water for 1 minute with final washing using running DI water. Washing biomass samples prior to analysis was done to remove foliar solution that may have dried on the surface of sprayed leaves and stems. Biomass samples were oven-dried at 65°C for 72 hours, weighed, ground, and analyzed for Si and elemental composition.

2.2.5. Harvesting, Yield Components, and Soil Sampling

At maturity, panicles were separated from tillers by cutting them with a pair of scissors. The remaining aboveground portion of the rice plant (straw) was cut with a sickle as close as possible to soil surface. Tiller and panicle number were noted before placing them into separated bags. Both straw and panicle were oven-dried at 65ºC for 72 hours and weighed. Rice grains were detached from panicle by hand and unfilled grains were separated from filled grains (true grains) using Almaco® Air Blast Seed Cleaner. Weights of filled and unfilled grains per pot as well as the 1000-grain weight were determined. Rice grains were ground (Cyclone Sample® Mill) as well as straw (Wiley® Mill no. 3) for further nutrient analysis.

After harvest, composite soil samples were collected and left to dry in a greenhouse for two days. Soil samples were taken for further oven drying at 40°C followed by grinding at Humboldt® (5DPJ3) soil grinder. Processed soil samples were analyzed for Si and extractable nutrient content.

2.2.6. Plant Analysis

Silicon content in plant tissue samples was determined by Oven-Induced Digestion procedure (OID) (Kraska and Breitenbeck, 2010) followed by Molybdenum Blue Colorimetric (MBC) procedure (Hallmark et al., 1982). For digestion, 100 mg of ground tissue sample was weighed into a 50-mL polyethylene centrifuge tubes and oven-dried for 15 minutes at 60°C in order to take out any remaining moisture from the samples. Five drops of octyl alcohol and 2 mL of hydrogen peroxide (H_2O_2) were added to the tubes before placing it back to the oven at 95°C for 30 minutes. Samples were then taken and 4 mL of 50% sodium hydroxide (NaOH) was added. Tubes were loosely capped and placed back into the oven. Every 15 minutes for 4 hours, tubes were taken out of the oven and gently mixed using a vortex mixer. After 4 hours, 1 mL of ammonium fluoride (NH_4F) was added to the digested samples, mixed, and diluted to 50 mL with DI water. Soybean and sugarcane known Si references samples as well as blanks were also digested for quality assurance.

For MBC procedure, 2 mL aliquot of plant digest solution was obtained and placed into 30-mL centrifuge tube. The 20% acetic acid at 10 mL and 0.26 M ammonium molybdate [$(NH_4)_6Mo_7O_2$] at 2 mL was added. Samples then were allowed to stand for 5 minutes before adding 2 mL of 20% tartaric acid. The sample solution was mixed and allowed to sit for 2 minutes before adding 2 mL of ANSA (reducing agent composed by 0.5 mg of 1-amino-2-naphthol-4-sulphonic acid, 1.0 g of sodium sulfite and 30.0 g of sodium bisulfite). The samples were diluted with 20% acetic acid to a final volume of 30 mL, and absorbance readings were measured at 630 nm using UV-Visible Spectrophotometer (Hach DR 500). Standard series at rates of 0, 0.4, 0.8, 1.6, 3.2, 4.8 and 6.4 ug mL^{-1} of Si, as well as references and blanks samples were also included. Silicon content (μg g^{-1}) of plants was determined using this formula:

$$\text{Si content} = \frac{\lfloor(\text{Abs}_{\text{samp}} - \text{Abs}_{\text{blk}}) - \text{Cfi}\rfloor}{\text{Cfs}} * \left\lfloor\left(\frac{V_d}{S_{wt}} * \frac{V_c}{V_a}\right)\right\rfloor$$

Where:

Abs_{samp} = absorbance reading of sample

Abs_{blk} = absorbance reading of reagent blank

C_{fi} = µg Si g^{-1} when absorbance is zero (derived from standard curve or intercept)

C_{fs} = µg Si g^{-1} per unit of absorbance (derived from standard curve or slope of the curve)

V_d = final digest volume (mL)

S_{wt} = oven-dry equivalent weight of digested sample (g)

V_c = final colorimetric volume (mL)

V_a = volume of aliquot used for colorimetric analysis (mL)

For essential nutrient and heavy metal contents, plant tissue samples were digested with concentrated nitric acid (HNO_3) and 30% H_2O_2 at 152°C, and analyzed using inductively coupled plasma (ICP) – Optical Emission Spectroscopy (OEM). Five hundred milligrams of ground plant tissue samples was weighed and placed into a 125-mL digestion tube. Five milliliters of concentrated HNO_3 was added. Each sample was mixed using a vortex mixer, and after 50 minutes the tubes were set on the heating block for five minutes at 152°C to initiate vigorous boiling. The tubes were removed from digestion block and allowed to cool down for 15 minutes before adding 3 mL of 30% H_2O_2. Small glass funnels were placed on each tube to prevent excessive evaporation and drying of solution while digesting. Samples were returned to the heating block and allowed to digest for 2 hours and 45 minutes. Digested samples were allowed to cool down overnight, then were mixed, transferred to centrifuge tubes and diluted with DI water to 12.5 mL. Samples were filtered using Whatman® no. 1 filter paper and analyzed through ICP–OEM. For every batch, reference material (soybean) and blanks were included.

2.2.7. Soil Analysis

Silicon content was determined by 0.5 M acetic-acid extraction procedure followed by MBC (Korndorfer et al., 2001), whereas analysis of extractable nutrient content was based on Mehlich-3 procedure followed by ICP atomic spectrometry (Mehlich, 1984).

For soil Si content analysis, 2 g of soil was weighed into a polyethylene centrifuge tube and added with 20 mL of 0.5 M acetic acid. The tubes were shaken using reciprocal shaker (Eberbach; model number E6010.00) set at high speed for 1 hour. Soil suspension was filtered using Whatman® no. 1 filter paper. A 0.5 mL aliquot was transferred to a centrifuge tube for MBC analysis. Ten milliliters of DI water, 0.5 mL of 1:1 HCl:water solution, and 1 mL of 10% ammonium molybdate (adjusted for pH 7.5) were successively added to the samples. Samples were allowed to stand for 5 minutes before adding 1 mL of 20% tartaric acid. Samples were gently swirled for 10 seconds, allowed to sit for 2 minutes, added with 1 ml of ANSA and then with DI water to make 25 mL final volume. Absorbance reading was measured after 5 minutes at 630 nm using UV visible spectrophotometer (Hach DR 5000). Standard series prepared with the same background (0.5 M acetic acid) at rates of 0, 0.2, 0.4, 0.8, 1.2, 1.6 and 2.0 ug mL^{-1} of Si, blanks, and reference samples (sharkey and commerce) were also included.

The plant-essential nutrients contents in the soil and selected heavy metals were measured by weighting 2 g of soil in a 125 mL plastic bottle, and adding 20 mL of Mehlich-3 solution (dilute acid-fluoride-EDTA solution corrected to pH 2.5). Samples were shaken for 5 minutes using a reciprocal shaker at high speed and filtered using Whatman® filter paper no. 42. Clear filtrates were transferred to 10-mL plastic tubes and analyzed by ICP atomic spectrometry. Two blanks reagents and two repetitions of each reference (sharkey and commerce) were also included.

2.2.8. Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray (EDX) Analysis

For biomass samples collected at flowering stage, twelve small sections of washed and unwashed leaves were cut before oven drying the whole biomass samples. These small cuts sections of leaves were stored in the refrigerator for later microscopic characterization of Si deposition. Scanning Electron Microscopy and EDX microanalysis and mapping were used to determine Si deposition in the adaxial and abaxial leaf surface of rice. Two replications for each treatment were analyzed. Under SEM, the magnification of samples' images was set to 400 times, and system operation at voltage of 20 kV. Focus and brightness were also adjusted to obtain clear and good quality images.

The SEM is a microscopic technology that beams electrons into leaf sample and generate images of its topography by interacting with sample atoms (McMullan, 2006). After generation of SEM images, EDX was set to scan samples. The EDX machine relies on high-energy beam of charged particles, such as electrons or protons, into the sample which results in atomic excitation and generation of a unique set of peaks that is read on emission spectrum. Each peak corresponds to a specific nutrient, as each element has unique atomic structure. Elements quantifications are done by proportionality of the produced peaks, and liquid N is required for EDX analysis (Goldstein, 2003). Silicon picks were proportionally quantified according to leaf carbon (C), oxygen (O), chlorine (Cl) and K contents. Mapping analysis was also obtained with EDX, where sample number of pixels was multiplied by corresponding scale value and summed to give the overall silicon content. Silicon content was associated with a blue color in this study.

2.2.9. Statistical Analysis

The data were subjected to analysis of variance (ANOVA) using SAS 9.4 (SAS Institute, 2012). Using PROC MIXED, P rates, Si source, and their interaction were assigned as fixed effects whereas replication was set as random effect. Treatment means were compared using Tukey test for any significant effect detected at $P<0.05$.

2.3.Results and Discussion

In general, there was no significant interaction between Si and P observed for plant measured variables across all growth stages and at harvest (Table 2.2). The main effect of P was not observed as well. Significant effect of Si treatment was observed for most measured variables at booting and flowering growing stage, and at harvest.

Table 2.2. Analysis of variance for tiller and panicle number, total biomass, filled, unfilled and thousand grains weight under different phosphorus rates and silicon sources and at different stages of rice.

Stage	Sources of Variation	Tiller Number	Total Biomass	Panicle Number	Filled Grain Weight	Unfilled Grain Weight	1000 Grains Weight
Tillering	P	NS	NS	-	-	-	-
	Si	NS	NS	-	-	-	-
	P x Si	NS	NS	-	-	-	-
Booting	P	NS	NS	-	-	-	-
	Si	<0.1	<0.1	-	-	-	-
	P x Si	NS	NS	-	-	-	-
Flowering	P	NS	NS	-	-	-	-
	Si	<0.05	<0.05	-	-	-	-
	P x Si	NS	NS	-	-	-	-
Harvest	P	NS	NS	NS	NS	NS	NS
	Si	NS	<0.05	NS	<0.05	<0.05	NS
	P x Si	NS	NS	NS	NS	NS	NS

NS = non-significant.

At booting stage, the application of foliar Si solution spray at 40 mg Si L^{-1} increased tiller number on average from 7.7 to 9.9 compared to the check (Table 2.3). Plants which received 40 mg Si L^{-1} of foliar application resulted in 36% higher biomass at booting than wollastonite-treated plants, whereas at flowering the foliar application of 20 and 80 mg Si L^{-1} enhanced rice biomass by 42% compared to wollastonite (Table 2.3). At this stage, plants applied with 80 mg Si L^{-1} of foliar solution produced on average 2 tillers more than plants applied with wollastonite (Table 2.3). The improvement in production of tillers was also noted at booting for foliar application at 40 mg Si L^{-1} over all treatments, except silicate slag. Prakash et al. (2011) reported that foliar spray of silicic acid at 2 and 4 mL L^{-1} increased the number of tillers of rice. In a similar study by Guevel et al. (2007) wheat plants treated with foliar Si were taller than the check. While there were observable positive effects of Si solution applied as foliar spray on plant growth and yield, excessive or extremely high concentration of Si in solution was found to reduce these parameters in wheat and rice crops as well (Abro et al., 2009; Prakash et al., 2011).

Table 2.3. Effect of silicon treatments across phosphorus rates on rice tiller number and biomass at booting and flowering stages.

Si Treatments	Booting		Flowering	
	Tiller Number	Biomass (g)	Tiller Number	Biomass (g)
Check	7.7 e	17.2 ab	6.0 ab	17.5 ab
Foliar 20 mg Si L^{-1}	9.1 bc	19.5 ab	6.7 ab	21.0 a
Foliar 40 mg Si L^{-1}	9.9 a	20.9 a	5.9 ab	19.0 ab
Foliar 80 mg Si L^{-1}	8.3 de	16.9 ab	7.0 a	21.0 a
Silicate Slag at 690 kg Si ha^{-1}	9.4 ab	17.3 ab	5.9 ab	19.0 ab
Wollastonite at 1190 kg Si ha^{-1}	8.8 cd	15.3 b	5.3 b	14.8 b
P-value	<0.1	<0.1	<0.05	<0.05

Means followed by the same letter within columns are not significantly different according to Tukey's test.

At harvest, no difference for tiller number and number of panicle was observed across Si treatments (Table 2.4). While soil- and foliar-applied Si did not affect biomass production compared to the check, application of foliar solution at 80 mg Si L^{-1} produced 19% higher biomass at harvest than soil-applied silicate slag treatment (Table 2.4). Singh and Singh (2005) also did not observed significant increase in plant growth with Si fertilization under greenhouse conditions. According to Epstein (2001), plants treated with Si increased growth and performance under environmental and biological stress. Sousa and Korndorfer (2010) noted that in greenhouse the environment is controlled and plants experience minimal or no stress condition, which could partially explained the lack of plant response in this experiment across Si fertilization sources.

Table 2.4. Effect of silicon treatments across phosphorus rates on rice tiller number, number of panicle, total biomass, and yield at harvest.

Si Treatments	Tiller number	Panicle number	Biomass (g)	Yield (g pot^{-1})
Check	17.4 a	16.0 a	74.6 ab	43.2 ab
Foliar 20 mg Si L^{-1}	16.8 a	16.5 a	73.6 ab	42.8 ab
Foliar 40 mg Si L^{-1}	17.6 a	16.8 a	74.6 ab	43.6 ab
Foliar 80 mg Si L^{-1}	18.1 a	18.3 a	79.4 a	45.8 a
Silicate Slag at 690 kg Si ha^{-1}	16.3 a	15.9 a	64.6 b	37.2 b
Wollastonite at 1190 kg Si ha^{-1}	16.1 a	15.3 a	70.2 ab	40.7 ab
P-value	NS	NS	<0.05	<0.05

NS = non-significant. Means followed by the same letter within columns are not significantly different according to Tukey's test.

There was no significant increase in rice yield (Table 2.4) and filled grains (Figure 2.1) with Si fertilization, but the highest foliar Si solution (80 mg Si L^{-1}) resulted in 19% higher yield and filled grains than silicate slag-treated rice. The result of this study is in agreement with results obtained by Deren et al. (1994), Liang et al. (1994), and Korndorfer et al. (1999) wherein there was no difference in yield of plants with and without application of Si. Incorrect soil pH compromises availability of plant-essential nutrient resulting in reduction in nutrient uptake which ultimately limits plant growth and yield (Steenbjerg and Jakobsen, 1962). The negative effect of silicate slag on rice may be attributed to its effect on soil pH (Nanayakkara et al., 2008). Unlike with the present study, foliar application was reported to increase yield of rice, corn (*Zea mays*) (Crusciol et al., 2013a), soybean (*Glycine max*), common bean (*Phaseolus vulgaris*), and peanut (*Arachis hypogaea*) crops (Crusciol et al., 2013b). However, the increased yield reported in these experiments was correlated to plant drought stress (Crusciol et al., 2013a, b), as Si enhances production and accumulation of total sugars and proline under stress condition (Crusciol et al., 2009). In addition, these studies were conducted under field condition wherein soils were not protected from runoff of foliar Si solution.

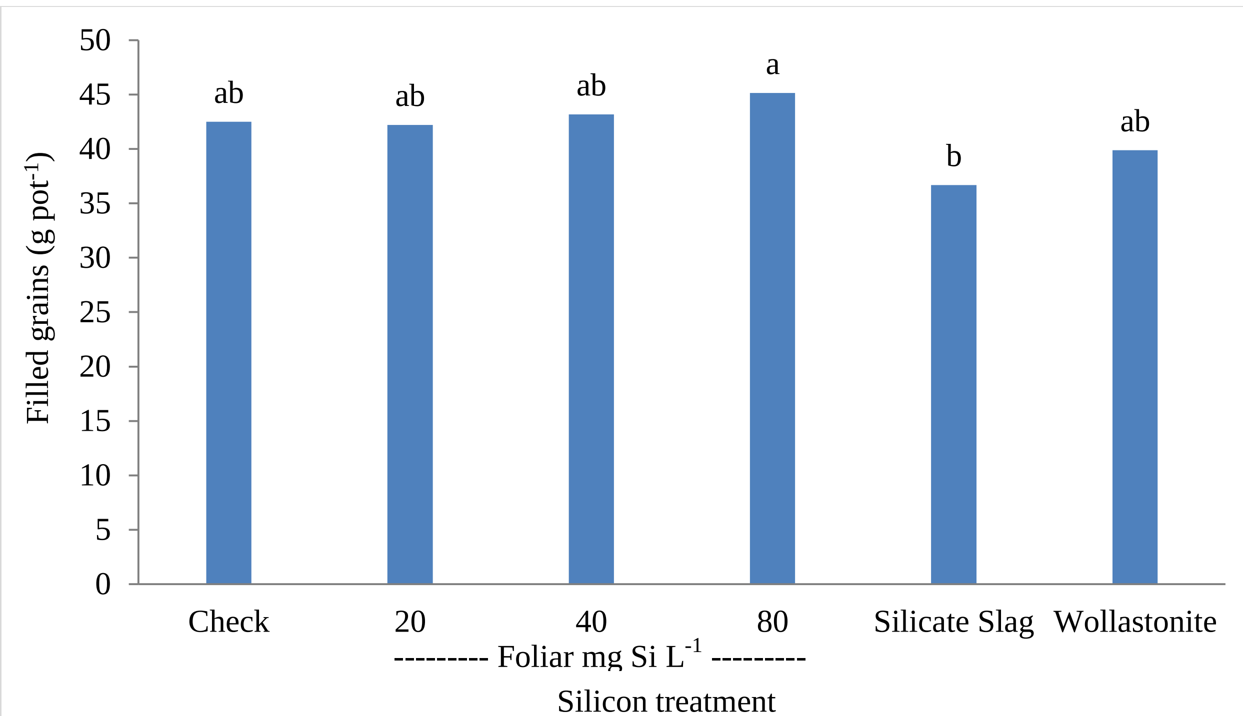

Figure 2.1. Effect of silicon treatments across phosphorus rates on rice filled grains at harvest. Bars labeled with the same letter are not significantly different at $P<0.05$ according to Tukey's test. Silicate slag and wollastonite at 690 and 1190 kg Si ha^{-1}, respectively.

Across all growth stages and at harvest, wollastonite application consistently increased biomass Si content (*P*<0.05, Figure 2.2). At tiller, booting and flowering stages, wollastonite increased it by 12, 10 and 23%, respectively, compared with the check, whereas at harvest Si content was increased from 4.46% (check) to 5.38% (wollastonite). Wollastonite treated rice obtained the highest grain Si content among the treatments, except for foliar application at 40 mg Si L^{-1} (*P*<0.05; Figure 2.3). Pereira et al. (2004) reported higher biomass Si content in plants which received wollastonite compared to plants with slag application and the check. Sousa and Korndorfer (2010) also observed greater Si straw content for wollastonite-treated plants compared to three different slags materials. Increasing rates of wollastonite was related to a linear increase in biomass Si content in rice (Pereira et al., 2004). The total Si uptake by rice for each growth stage was not affected by Si source (Table 2.5).

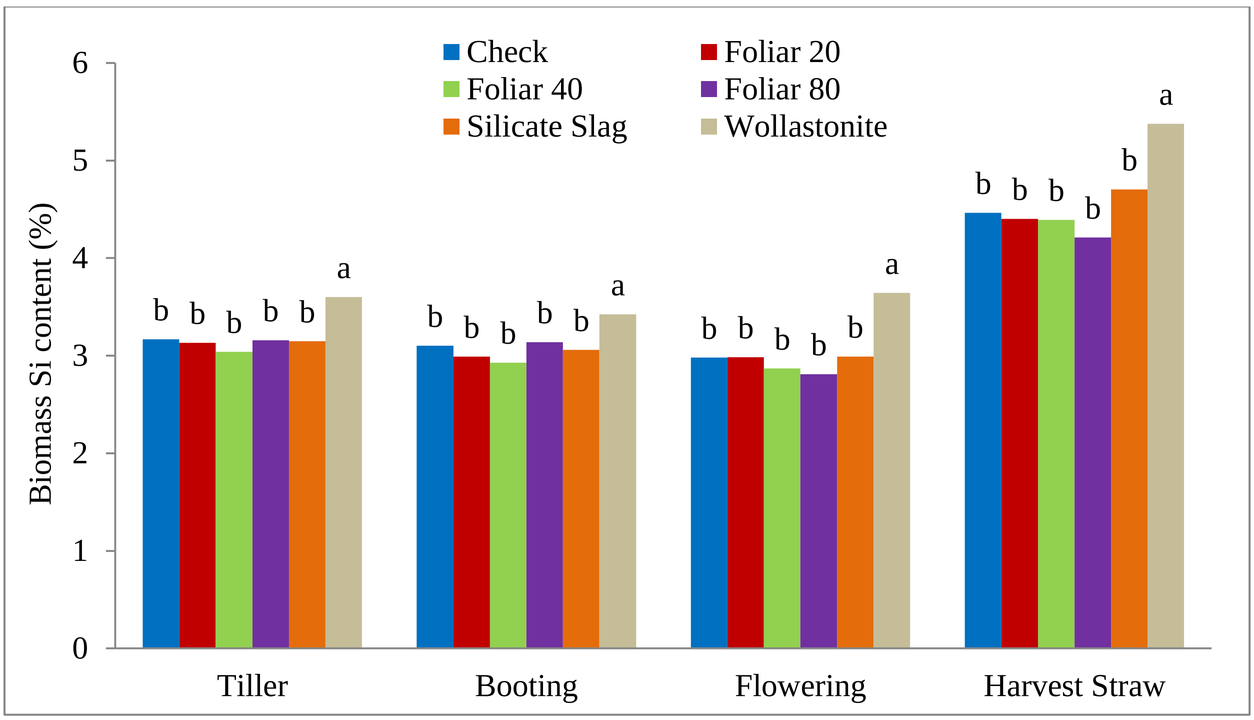

Figure 2.2. Effect of silicon treatments across phosphorus rates on silicon content of rice biomass at tiller, booting, flowering and harvest straw. Bars labeled with the same letter within sampling time are not significantly different at *P*<0.05 according to Tukey's test. Silicate slag and wollastonite rates: 690 and 1190 kg Si ha^{-1}, respectively.

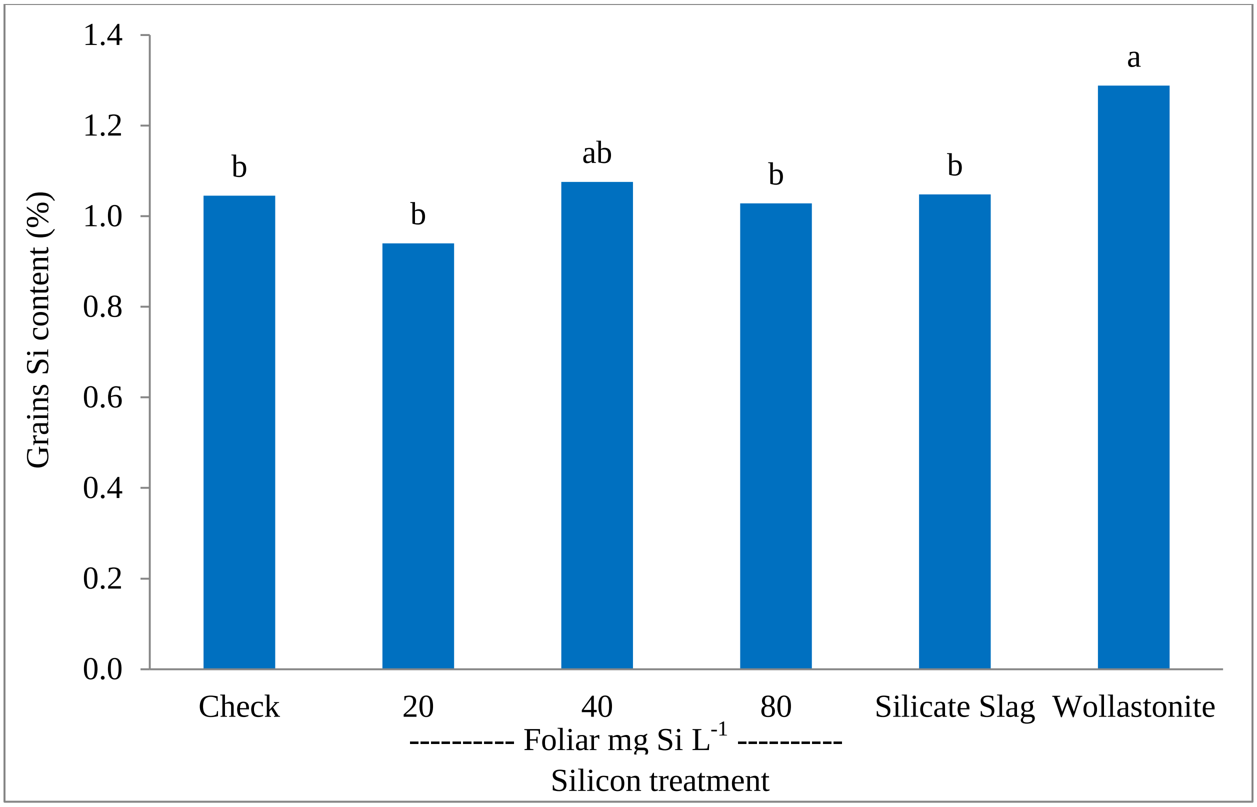

Figure 2.3. Effect of silicon treatments across phosphorus rates on silicon content of rice grains. Bars labeled with the same letter are not significantly different at $P<0.05$ according to Tukey's test. Silicate slag and wollastonite rates: 690 and 1190 kg Si ha^{-1}, respectively.

Table 2.5. Effect of silicon treatments across phosphorus rates on silicon uptake by rice.

Si treatments	Si uptake (mg plant^{-1})							
	Tiller		Booting		Flowering		Harvest Straw	
Check	67	a	267	a	261	a	397	a
Foliar 20 mg Si L^{-1}	66	a	293	a	317	a	395	a
Foliar 40 mg Si L^{-1}	68	a	306	a	286	a	392	a
Foliar 80 mg Si L^{-1}	72	a	268	a	313	a	406	a
Silicate Slag at 690 kg Si ha^{-1}	59	a	271	a	287	a	373	a
Wollastonite at 1190 kg Si ha^{-1}	60	a	262	a	277	a	458	a
P-value	NS		NS		NS		NS	

NS = non-significant. Means followed by the same letter within columns are not significantly different at $P<0.05$ according to Tukey's test.

Leaves sprayed with Si solution presented whitish spots on its surface, which was suggested by Bowen et al. (1992) and Rezende et al. (2009) as possible accumulation of dehydrated Si solution on leaf surface. However, no differences in Si content were detected between washed and unwashed biomass samples (Figure 2.4). Moreover, there was no clear evidence showing an increased in biomass Si content due to foliar Si application (Figure 2.2). The washing of leaves with DI water was not effective in removing this possible surface deposition of Si, but this result does not confirm that foliar Si absorption took place since the leaf Si content was comparable between foliar Si treatments and the check.

Under SEM-EDX analysis, both foliar treatment at 80 mg Si L^{-1} and wollastonite tended to have higher Si foliar content than the check, but this increase in Si content was statistically the same (Figure 2.5). Unlike plant Si determined by OID-MBC procedure, SEM-EDX technique may not detect minor quantitative difference for Si content between treated samples (Bowen et al., 1992), which could explain the contradicting results observed between these two procedures. Perhaps, a better evaluation could have been made if more replications of small sections of leaves were subject to SEM-EDX analysis. Guével et al. (2007) found different Si concentration in different leaf's spots under EDX, but overall Si content was similar for foliar-treated and untreated plants. In general, the adaxial leaf surface tended to have higher Si content than the abaxial leaf surface for all treatments including the check; however, significant difference was only observed for the highest rate of foliar application (80 mg Si L^{-1}) (Figure 2.6). These results are in agreement with the findings of Rezende et al. (2009) wherein the X-ray microanalysis showed higher deposition of Si bodies on the adaxial than the abaxial leaf surface of rice. Mapping of select samples visually showed greater distribution of silica bodies on the adaxial leaf surface of rice applied with wollastonite and foliar Si spray in comparison to the check (Figure 2.7). A different pattern of Si deposition on the leaf surface among plants with and without Si application was observed by Guével et al. (2007) who noted Si concentration along specific lines on foliar and soil Si treatments while the check exhibited a very faint deposition.

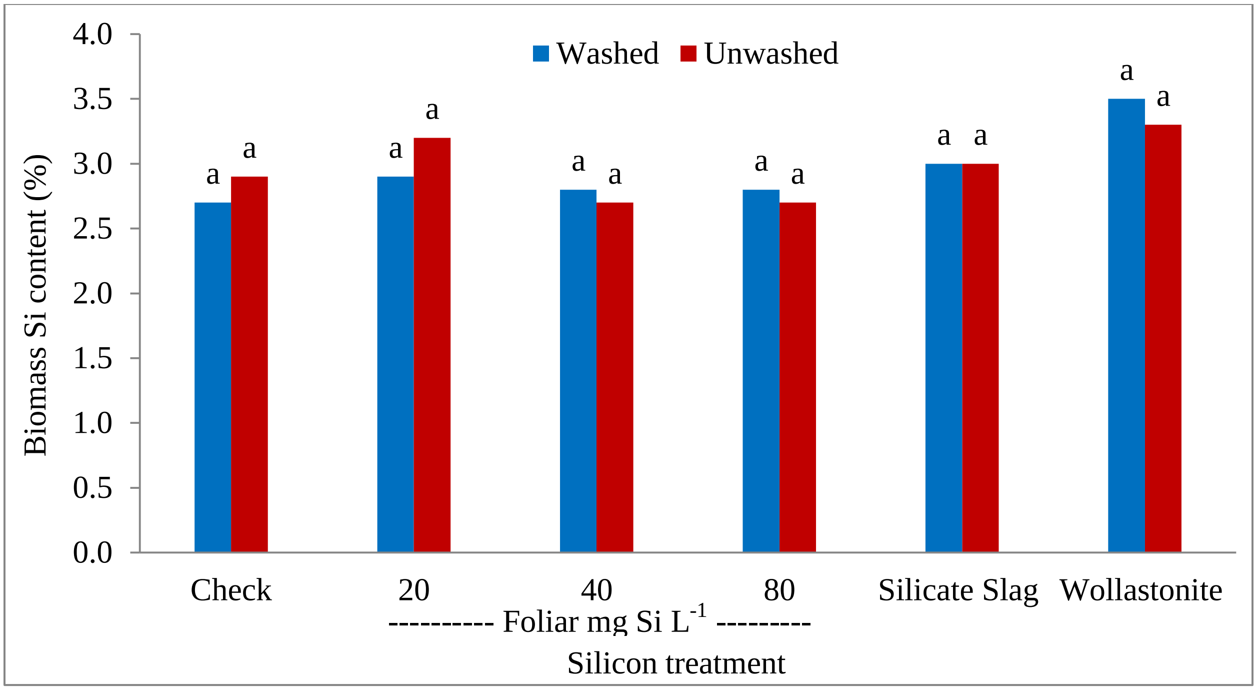

Figure 2.4. Silicon content of washed and unwashed rice biomass at flowering with different silicon treatments. Bars labeled with the same letter are not significantly different at *P*<0.05 according to Tukey's test. Silicate slag and wollastonite rates: 690 and 1190 kg Si ha^{-1}, respectively.

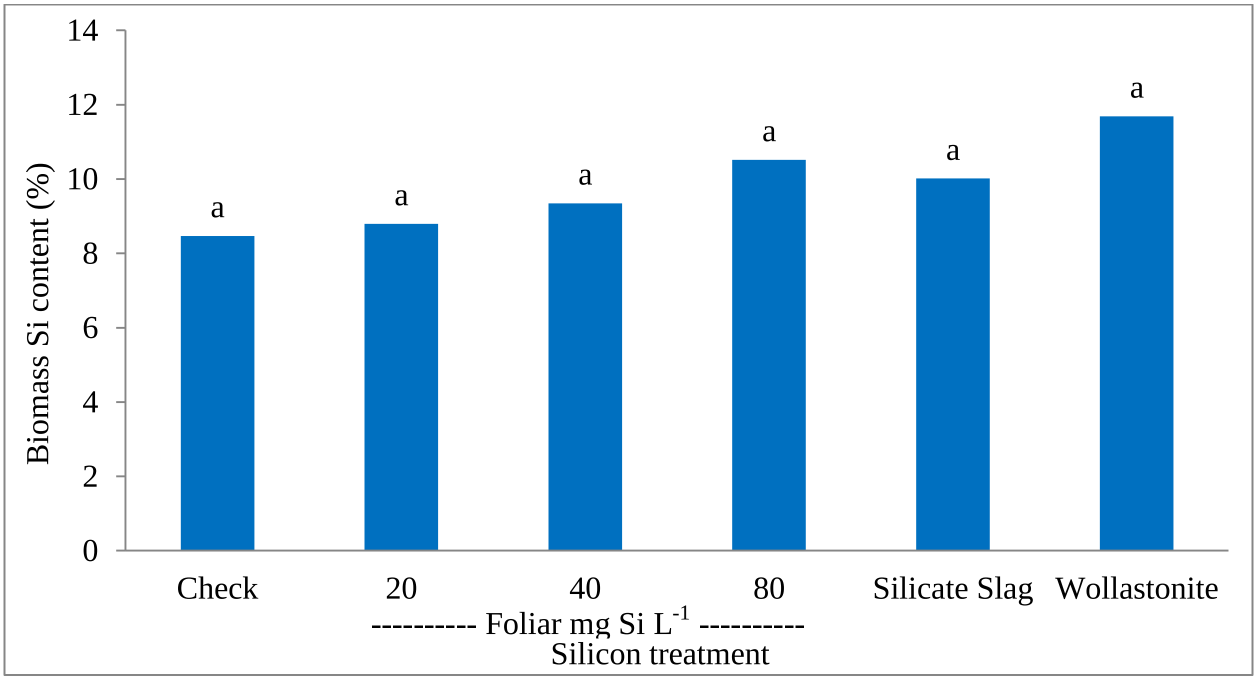

Figure 2.5. Silicon content of rice biomass at early flowering under SEM and EDX with different silicon treatments. Bars labeled with the same letter are not significantly different at *P*<0.05 according to Tukey's test. Silicate slag and wollastonite rates: 690 and 1190 kg Si ha^{-1}, respectively.

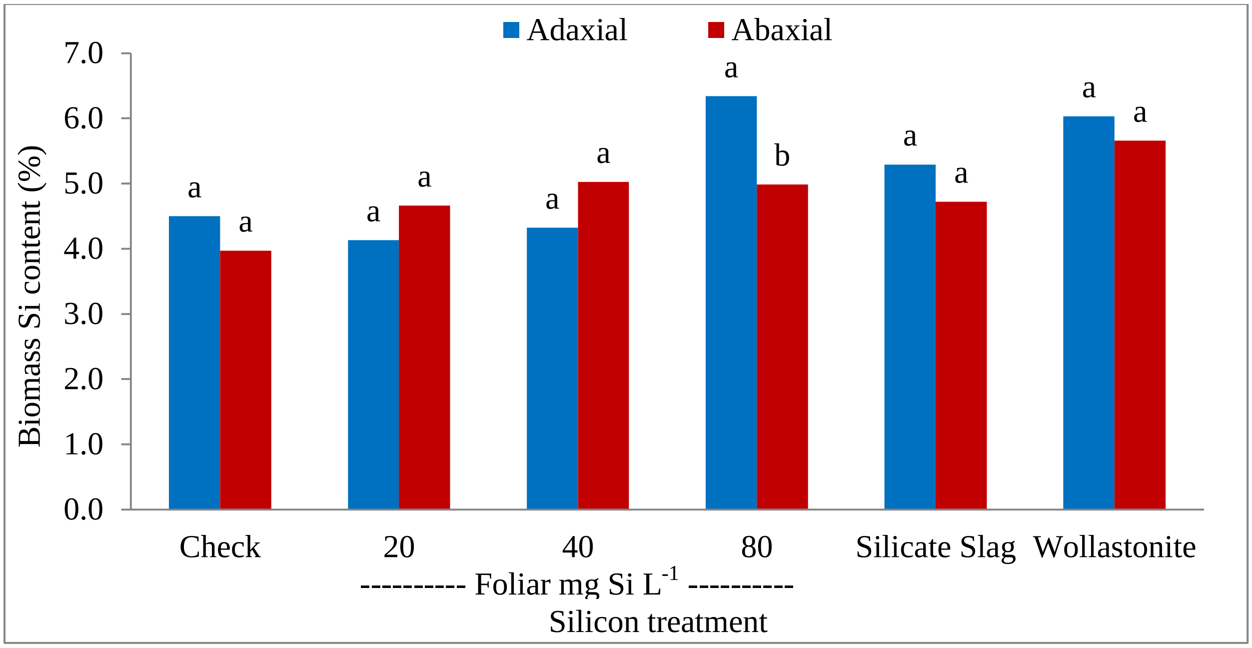

Figure 2.6. Silicon content of rice adaxial and abaxial leaf surface under SEM and EDX with different silicon treatments. Bars labeled with the same letter within Si treatment are not significantly different at $P<0.05$ according to Tukey's test. Silicate slag and wollastonite rates: 690 and 1190 kg Si ha^{-1}, respectively.

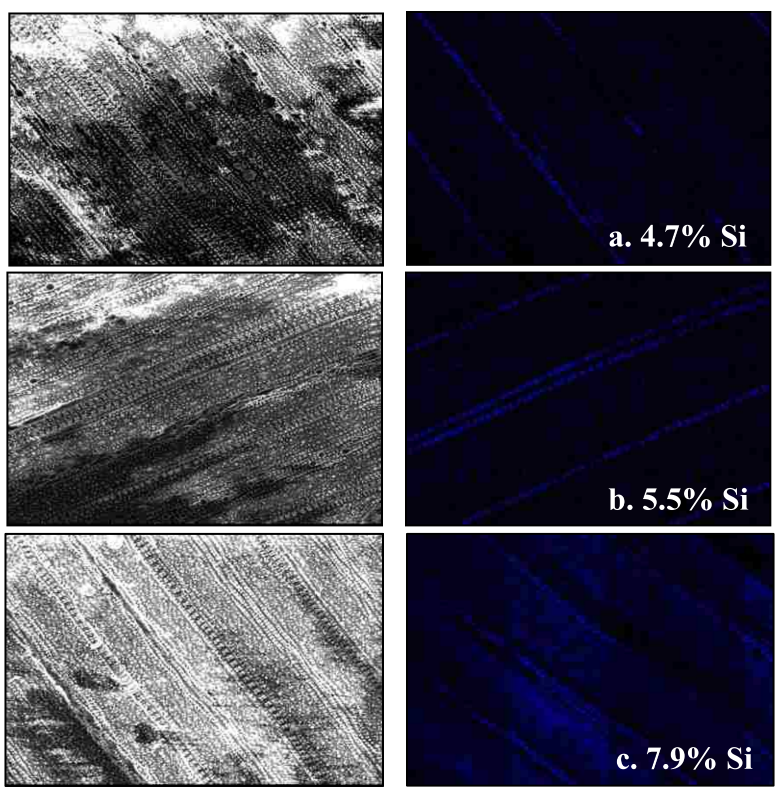

Figure 2.7. SEM and EDAX of adaxial leaf surface at flowering for check (a) foliar application at 40 mg Si L^{-1} (b) and wollastonite (c).

Silicon treatment had no effect on P content of plants (Table 2.6), and neither P rates on plant Si content (Table 2.7). Foliar applied Si did not show any significant effect on plant elemental composition, except for Ca content in straw and K in grains (Table 2.6). There was a significant reduction in Mn content of plants treated with silicate slag and wollastonite (Table 2.6). Wollastonite reduced Mn content both in the straw and grain while silicate slag application lowered Mn content of grain. Williams and Vlamis (1957) noted that Mn toxicity was alleviated by addition of Si, and further studies confirmed that the concentration of Si in shoot significantly reduced Mn content of rice (Okuda and Takahashi, 1962; Ma and Takahashi, 1990; Rogalla and Romheld, 2002). Rice which received 224 kg P ha^{-1} had lower Mn straw content than the check (417 to 358 mg kg^{-1}); whereas lower Fe content in the grain was observed as well (Table 2.7). Reports were made that the high affinity of P for metals, such as Mn and Fe, might reduce its plant content alleviating metal toxicity (Ma, 2004). The K concentration in straw of plants which received Si via soil application was 0.2% higher than untreated plants (Table 2.6), and a 26% increase in K straw content with P fertilization was also observed (Table 2.7). Silicon fertilization through soil amendments increased soil pH, on average, from 7 to 7.8 (Table 2.8), and at pH levels higher than 7.5, K and Ca are abundant for uptake by the plants (Londo et al., 2006). On the other hand, only silicate slag application increased Mg straw content (Table 2.6), which could be explained by the increased soil content of Mg (Table 2.8). Silicon applied as wollastonite reduced Al concentration in the soil compared to check (Table 2.8). Also, there was a significant reduction on soil Al concentration by application of different P rates (Table 2.9). Phosphate and silicates were reported to be adsorbed by the Al and Fe oxides of clay fractions (Brown and Mahler, 1987). The competition of Si and P for Al bound might explain the reduction in soil Al content in the presence of both nutrients.

Table 2.6. Effect of silicon treatments across phosphorus rates on nutrient content of rice straw and grains.

	Si Treatments	Nutrient Content P		K		Ca		Mg		S		Mn		Fe		As	
		----		----		%		----		----		----		mg kg^{-1}		----	
Straw	Check	0.052	a	0.220	b	0.193	d	0.074	bc	0.019	a	423	a	180	a	2.066	a
	Foliar 20 mg Si L^{-1}	0.052	a	0.242	b	0.219	b	0.089	b	0.020	a	442	a	143	a	2.116	a
	Foliar 40 mg Si L^{-1}	0.050	a	0.245	b	0.199	d	0.079	bc	0.017	ab	403	a	148	a	2.242	a
	Foliar 80 mg Si L^{-1}	0.053	a	0.223	b	0.192	cd	0.082	bc	0.018	ab	426	a	144	a	1.889	a
	Silicate Slag at 690 kg Si ha^{-1}	0.046	a	0.323	a	0.240	a	0.113	a	0.014	ab	393	a	133	a	1.106	b
	Wollastonite at 1190 kg Si ha^{-1}	0.042	a	0.368	a	0.210	bc	0.070	c	0.012	b	300	b	145	a	0.455	c
Grains	Check	0.234	a	0.100	c	0.021	ab	0.079	a	0.036	a	38	a	121	a	-	
	Foliar 20 mg Si L^{-1}	0.263	a	0.139	ab	0.021	ab	0.088	a	0.037	a	38	a	128	a	-	
	Foliar 40 mg Si L^{-1}	0.263	a	0.173	a	0.021	ab	0.090	a	0.038	a	40	a	106	a	-	
	Foliar 80 mg Si L^{-1}	0.239	a	0.161	ab	0.021	a	0.080	a	0.034	a	40	a	126	a	-	
	Silicate Slag at 690 kg Si ha^{-1}	0.250	a	0.126	bc	0.020	b	0.090	a	0.037	a	31	b	139	a	-	
	Wollastonite at 1190 kg Si ha^{-1}	0.250	a	0.148	ab	0.021	ab	0.088	a	0.036	a	31	b	148	a	-	

Means followed by the same letter within columns are not significantly different at *P*<0.05 according to Tukey's test.

Table 2.7. Effect of phosphorus rates across silicon sources on nutrient content of rice straw and grains.

	P kg ha^{-1}	Nutrient Content Si		P		K		Ca		Mg		S		Mn		Fe	
		mg kg^{-1}		----		----		%		----		----		---- mg kg^{-1}		----	
Straw	0	4.62	a	0.049	a	0.218	b	0.209	a	0.086	a	0.018	a	417	a	156	a
	112	4.64	a	0.048	a	0.298	a	0.216	a	0.087	a	0.017	a	416	a	144	a
	224	4.51	a	0.050	a	0.294	a	0.201	a	0.081	a	0.016	a	358	b	147	a
Grains	0	1.12	a	0.254	a	0.100	b	0.022	a	0.087	a	0.034	b	36.9	a	147	a
	112	1.05	a	0.244	a	0.108	b	0.021	ab	0.084	a	0.035	b	36.3	a	122	ab
	224	1.04	a	0.250	a	0.215	a	0.020	b	0.086	a	0.039	a	35.5	a	114	b

Means followed by the same letter within columns are not significantly different at *P*<0.05 according to Tukey's test.

Table 2.8. Effect of silicon treatments across phosphorus rates on soil pH, electrical conductivity, 0.5 M acetic acid extractable-Si, Mehlich-3 extractable nutrients, and heavy metals.

Si Treatments	pH 1:1 Water		EC µs cm^{-1}		Nutrients Content, mg kg^{-1}															
					Si		P		K		Ca		Mg		Al		Mn		As	
Check	6.97	b	380	b	39	c	37	b	61	ab	628	c	120	b	651	a	140	a	0.398	bc
Foliar 20 mg Si L^{-1}	7.01	b	406	ab	36	c	38	ab	62	ab	611	c	119	b	634	a	126	a	0.399	abc
Foliar 40 mg Si L^{-1}	7.13	b	408	ab	37	c	38	ab	64	ab	649	c	125	b	637	a	133	a	0.406	ab
Foliar 80 mg Si L^{-1}	7.08	b	401	ab	37	c	45	ab	60	b	665	c	128	b	631	a	142	a	0.411	ab
Silicate Slag at 690 kg Si ha^{-1}	7.79	a	414	ab	77	b	39	ab	64	ab	1005	b	197	a	635	a	143	a	0.433	a
Wollastonite at 1190 kg Si ha^{-1}	7.94	a	459	b	106	a	48	a	71	a	1779	a	109	b	460	b	140	a	0.370	c

Means followed by the same letter within columns are not significantly different at *P*<0.05 according to Tukey's test.

Table 2.9. Effect of phosphorus rates across silicon sources on soil pH, electrical conductivity, 0.5 M acetic acid extractable-Si, Mehlich-3 extractable nutrients, and heavy metals.

P Kg ha^{-1}	pH 1:1 Water		EC µs cm^{-1}		Nutrients Content, mg kg^{-1}																	
					Si		P		K		Ca		Mg		S		Al		Mn		As	
0	7.33	a	413	a	57	a	24	c	67	a	1001	a	138	a	9.2	a	643	a	149	a	0.424	a
112	7.35	a	413	a	53	a	39	b	64	ab	816	a	131	b	8.2	a	605	b	134	b	0.399	b
224	7.28	a	410	a	58	a	59	a	60	b	853	a	131	b	8.3	a	575	b	130	b	0.385	b

Means followed by the same letter within columns are not significantly different at *P*<0.05 according to Tukey's test.

The Si application through soil amendments decreased As content of straw (Table 2.6). Silicic acid transporters also have been reported to mediate the arsenite uptake in rice (Guo et al., 2007; Ma et al., 2008); thus the high concentration of Si decreased the availability of these transporters and reduced As uptake. For As content of the grains, there was an interaction between Si and P treatments (P<0.01). Between the check and wollastonite treatments, rice treated with increasing P showed a reduction in As grain content (Figure 2.8). Without P, wollastonite significantly reduced As in grain whereas in the presence of P (112 and 224 kg ha^{-1}), grain As content of both check and wollastonite treatment was similar. There was no clear effect of both foliar Si and P on As content of rice grain, whereas the combined application of silicate slag and P application exacerbated grain quality by raising As content.

As expected, Si soil-sources significantly increased soil Si content compared to check and foliar application at different rates (Table 2.8). Wollastonite resulted in the highest soil Si content having 67 and 29 µg Si g^{-1} higher Si content than the check and silicate slag treatment, respectively. Whereas wollastonite increased soil P content from 37 to 48 ug g^{-1} compared to the check, no effect was observed for silicate slag and foliarly applied Si treatments (Table 2.8). The concentration of Si in wollastonite (23%) is higher than slag materials (14%) and the rate of application was the same for both sources, which may have caused the difference on soil Si content and, consequently, adsorption of P. The decrease in P adsorption by Si treated-soil and further increased on soil P content was reported by several authors (Noda and Komai, 1958; Roy et al., 1971 and Syouji, 1981). Lima (2011) observed that Si application via soil lead not only to reduction in P fixation but also to increased uptake of P by the plant. In contrast, Ma and Takahashi (1990) study showed that the addition of Si was not accompanied by increased P concentration in shoots. Soil pH above 7 was reported to cause precipitation of the phosphates presented in soil solution (Ferguson et al., 1973). Soil-applied Si treatments also increased soil pH to higher than 7, which could have resulted in phosphate precipitation and its unavailability for plant uptake.

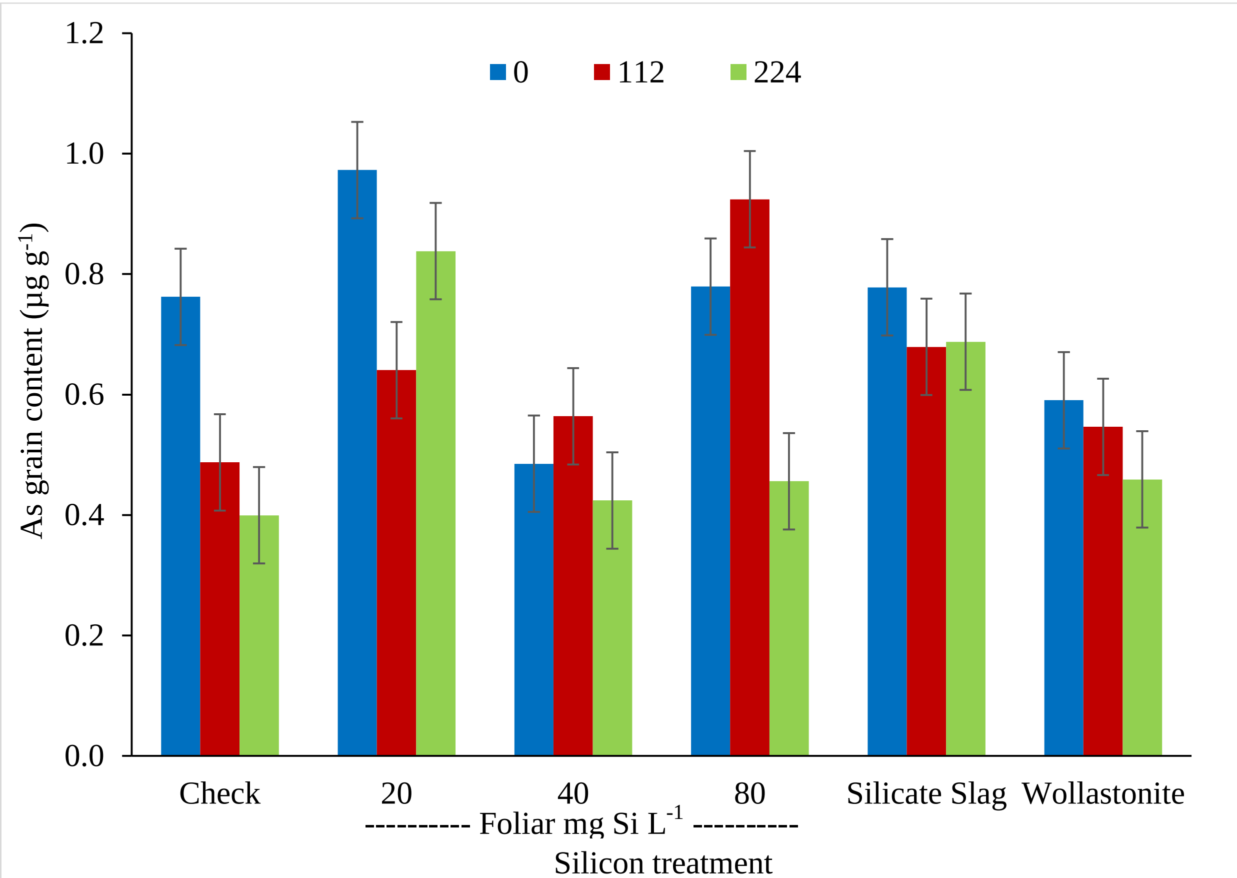

Figure 2.8. Effect of silicon treatments across phosphorus rates on arsenic content of rice grains. The overlap of standard error (SE=0.08) bars within Si treatment means no significant difference at $P<0.05$ according to Tukey's test. Silicate slag and wollastonite rates: 690 and 1190 kg Si ha^{-1}, respectively.

Phosphorus application at different rates did not affect Si soil content (Table 2.9). Regardless of rate, P reduced some soil nutrient concentrations, such as Mn, Mg, and As (Table 2.9). Phosphate is chemically analogous to arsenate and will compete for binding sites; thus the application of P reduces arsenate availability to plants (Smith et al., 2002). There was a significant increase in soil pH with soil application of Si (Table 2.8). Electrical conductivity was not affected by Si sources in comparison to the check treatments, but wollastonite lead to higher soil conductivity than slag. Increase in exchangeable Ca levels was observed for soil-applied Si (Table 2.8). The elevation of pH and Ca content is explained by the increase in hydroxyls (OH^-) and Ca^{2+} as wollastonite (composed by $CaSiO_3$) reacting to water in soil and releases Ca^{2+}, SiO_3 and two OH^- (Haynes et al., 2013). Elevated Mg content in comparison to check treatment was detected in soils applied with silicate slag (Table 2.8). Magnesium (7%) is also present in slag materials which may explain the elevated level of Mg observed in soil-grown rice applied with slag (Takijima et al., 1970).

2.4.Conclusions

The relationship between P and Si was not clearly demonstrated in the present study. Silicon applied to soil and leaves did not result in significant increase in P content and uptake in straw and grains of rice. However, wollastonite application enhanced soil P content from 37 to 48 ug g^{-1}, suggesting that high levels of Si (compared to silicate slag) may displace some phosphates from the binding sites. Perhaps, the lack of plant P uptake was due to the precipitation of phosphates as a result of increased pH from wollastonite application. The application of P did not affect soil and plant Si content.

There was no clear evidence showing that foliar Si application enhanced Si content of rice. The possible accumulation of dehydrated Si solution on leaf surface was not completely washed off by DI water. Even so, the Si contained in dried solution was too low to raise rice biomass Si. Wollastonite consistently enhanced soil and rice Si content, whereas the lower rate of silicate slag raised the soil Si content but did not increase rice Si content. There were no changes detected on nutrients in soil and plants due to foliar application. The outcomes of this study suggest that soil-applied Si is effective in improving Si uptake by rice.

2.5.References

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Chapter 3. Evaluation of absorption and uptake of soil- and foliar-applied silicon fertilizer in rice

3.1. Introduction

Silicon (Si) is the second most abundant element in the earth's crust, found in different forms in soil (Savant et al., 1997). The three general forms are monosilicic acid (H_4SiO_4), polysilicic acid $[Si(OH)_4]_x$, and amorphous silica (SiO_2) (Bauer et al., 2011). Whereas SiO_2 is the most abundant form, it is a non-soluble mineral unavailable for plant uptake (Bauer et al., 2011). The soluble forms are polysilicic and monosilicic acid but since polysilicic acid has a high molecular weight it is not available for taken up by the plants (Casey et al., 2004). The only plant-available form is monosilicic acid, which is taken up by roots when present in soil solution (Raven, 1983). Once in the plant, H_4SiO_4 is transported along with water to shoots, and is deposited as hydrated amorphous silica in leaves, stem, and hulls (Yoshida, 1965; Casey et al., 2004). In rice (*Oryza sativa*), the deposition of Si occurs in epidermal bulliform cells, middle lamellae, and intercellular spaces (Motomura et al., 2000; Kim et al., 2002). Silicon was also reported to form a double layer underneath leaf cuticle, which provides physical strengthening to the plants (Yoshida, 1965).

Although the essentiality of Si has not been established, it is recognized as a beneficial element for many terrestrial plant species (Epstein, 1994; Epstein and Bloom, 2005). There are reports that the accumulation of Si in monocot species is higher than in dicots species (Jones and Handreck, 1967). Among the monocots, rice is the most Si accumulating plant (Takahashi et al., 1990), accumulating amounts of Si sometimes higher than some plant-essential nutrients, such as nitrogen (N) (Cassman et al., 1995). Based on these findings, Si was classified by Ma et al. (2001) as “agronomic essential nutrient” for rice cultivation.

Plants supplied with Si show alleviation of biotic and abiotic stresses (Epstein, 2001). There were reports that water use efficiency (Agarie, 1998), light interception, photosynthetic rate and plant growth are reduced in Si-deficient plants (Savant et al., 1997). Enhanced Si nutrition has been associated with improved resistance of rice to diseases, such as brown spot (*Cochliobolus miyabeanus*) (Savant et al., 1997) and leaf blast (*Magnaporthe oryzae*) (Datnoff et al., 1997). Substantial increase in yield was also observed due to Si application in rice fields (Snyder et al., 1986).

Since the annual removal of soil Si by rice ranges from 210 to 224 million tons kg ha^{-1} (CRRI, 1976), it is common to find depletion of plant-available Si in soils where rice is cultivated for a long time (Savant et al., 1997). Silicon fertilization has become a practice in rice fields (Datnoff et al., 2001) and it is normally done as soil application of Si sources before planting (Chiu and Huang, 1971). The most common source of Si is slags produced during iron and steel processing (Sousa and Korndorfer, 2010). In this process, calcium (Ca) and magnesium (Mg) oxides (CaO, MgO) bind to Si (present in the ore) and forms Ca and Mg silicates (Sousa and Korndorfer, 2010). For the industry this material is considered as waste, but for agriculture it has a high use value: an inexpensive source of Si and liming material (Prado and Fernandes, 2000). Although it is inexpensive, the common rate of silicate slag application in rice field is 2 to 4.5 ton ha^{-1} (Korndorfer et al., 2001; Ma and Takahashi, 2002), which translates high transportation costs.

The use of foliar spray of Si-containing solutions was proposed as an alternative and a more feasible way of supplying Si to plants (Guével et al., 2007). Reports were made on the positive effect of foliar-Si application for disease control of rice (Cacique et al., 2013), wheat (*Triticum aestivum*) (Guével et al., 2007), grape (*Vitis vinifera)* (Bowen et al., 1992), cucumber (*Cucumis sativus*), zucchini (*Cucurbita pepo*), and muskmelon (*Cucumis melo*) (Menzies et al., 1992). Pathogen-inoculated wheat treated with foliar Si-containing solution was taller than the control, but this positive effect of Si application was not observed on non-inoculated wheat (Guével et al., 2007). It was observed that Si solution applied directly to the roots controlled *Podosphaera xanthii* in cucumber via activation of defense enzymes; however this was not detected for cucumber which received Si foliarly (Liang et al., 2005). Whereas root uptake is an established mechanism of Si absorption by rice (Takahashi and Hino, 1978; Ma et al., 2006), transporter genes have not been reported to exist in rice leaves and there is no strong evidence showing that Si can be absorbed through the leaves (Bowen et al., 1992; Menzies et al., 1992; Liang et al., 2005; Rodrigues and Datnoff, 2015).

The effect of foliar application of Si on disease control has been explained by the deposition of dried solution on the leaf surface (Bowen et al., 1992; Menzies et al., 1992; Liang et al., 2005; Rodrigues and Datnoff, 2015). This deposition was suggested to change the pH and/or osmotic potential of leaf surface and/or acts as a physical barrier against diseases infection (Rodrigues and Datnoff, 2015). Bowen et al. (1992) observed the formation of whitish spots on leaf surface, suggested as dried solution which coats the leaves and protect plants against pathogen infection. Liang et al. (2005) detected that foliar applied potassium silicate effectively controlled infection by *Podosphaera xanthii* in cucumber, but only via physical barrier and osmotic effects of the silicate applied; no Si absorption was observed. According to Rezende et al. (2009), Si sprayed leaves had higher Si deposit on the adaxial (upper) leaf surface, which is more exposed to Si solution spray, than abaxial (lower) leaf surface. On the other hand, reports were made on increased Si content in plants under foliar applied Si in comparison to check (Guével et al., 2007; Crusciol et al., 2013a; Crusciol et al., 2013b).

It has been reported that foliar applied Si provides benefits to plants; however, there is no clear evidence supporting foliar absorption of Si in plants, especially when no transporters have been found (Liang et al., 2005). Thus, this study was conducted to determine if Si in solution form can be absorbed through leaf surface of rice and translocated within the plant.

3.2.Materials and Methods

3.2.1. Bulk Soil Sampling and Analysis

A silt loam to silt clay, poorly drained soil (Crowley-Vidrine complex) classified as a fine, smectitic, thermic, and typic albaqualfs soil was selected for this study (SSURGO-USDA, 2015). Samples were collected in Evangeline Parish (Louisiana) from a producer's rice field. Composite soil samples were taken, oven-dried at 40°C, and analyzed for soil characterization. The soil has low Mehlich-3 P and K content, slight acidity (1:1 pH in water) and contain 18 g kg^{-1} organic matter. Calcium, Mg, sodium (Na), sulfur (S), copper (Cu) and zinc (Zn) contents as well as cation exchange capacity (CEC) and sum of bases are presented in Table 3.1.

Table 3.1. Soil characterization analysis before experiment establishment.

Texture	pH (1:1 water)	Sum of Bases ($cmol_c$ dm^{-3})	CEC ($cmol_c$ kg^{-1})	Extractable Nutrients (mg kg^{-1})								
				Si	P	K	Ca	Mg	Na	S	Cu	Zn
Silt loam	6.14	5	12	37	16	39	756	112	20	14	1	2

3.2.2. Experiment Establishment

Plastic pots (Encore Plastics®) with 13-L capacity were filled with 11 kg of air-dried, sieved soil. The fertilizer recommendation was based on soil analysis for rice production in

Louisiana. Pre-plant fertilization consisted of triple super phosphate (TSP, 46% P), potassium chloride (KCl, 60% K) and zinc sulfate ($ZnSO_4$, 22.7% Zn) applied at rates of 112, 90 and 6 kg ha^{-1}, respectively.

Ten seeds of the rice variety CL151 were sowed per pot and ten days after germination, plants were thinned to six plants per pot. Flood was established two weeks after sowing, maintaining a 2.5 cm water column. Nitrogen (urea, 45% N) was first broadcast applied to soil right after sowing at 115 kg ha^{-1} while the second application was done 20 days after flooding at 68 kg N ha^{-1}. Second application of K was also done 20 days after sowing at 56 kg ha^{-1}.

3.2.3. Silicon Absorption and Uptake in Tillers Treated with Foliar Si

A solution containing 6000 mg of Si L^{-1} (Taminco®) was used as Si source in this study. The treatments consist of four foliar Si rate: (1) 0 (deionized water) (2) 20 mg of Si L^{-1} (3) 40 mg of Si L^{-1}, and (4) 80 mg of Si L^{-1}, diluted to a final volume of application of 600 L ha^{-1}. Foliar Si solution was sprayed either to whole rice plants or to leaves of the primary third tiller of each plant (Figure 3.1), using a pressurized handheld sprayer (Stihl® SG 10). The foliar Si application was done three times: at early tillering, booting, and early flowering stages. Early tillering was designated as the stage in which the second tiller is emerged from the main plant stem, while booting when stem shows a leaf protuberance (initial of panicle development), and early flowering when panicle is completely out of the flag leaf with opened flowers at the tip. During foliar application, the surface of the pots was covered with a plastic sheet to prevent Si solution dripping into the soil. The amount of Si delivered per application was 12, 24, and 48 g Si ha^{-1} for concentrated solution at 20, 40 and 80 mg of Si L^{-1}, respectively. As the application was done three times during the rice cycle, the total Si applied was 36, 72 and 144 g Si ha^{-1}. One week after each application, biomass samples were collected wherein leaves where separated into two groups: one was washed with DI water before oven-drying and the other left unwashed.

At harvest, panicle and tiller number were determined and separated into straw and panicle before drying in an oven at 65°C. Dry weights were recorded and yield determined. The treatments was arranged in a randomized complete block design with four replications.

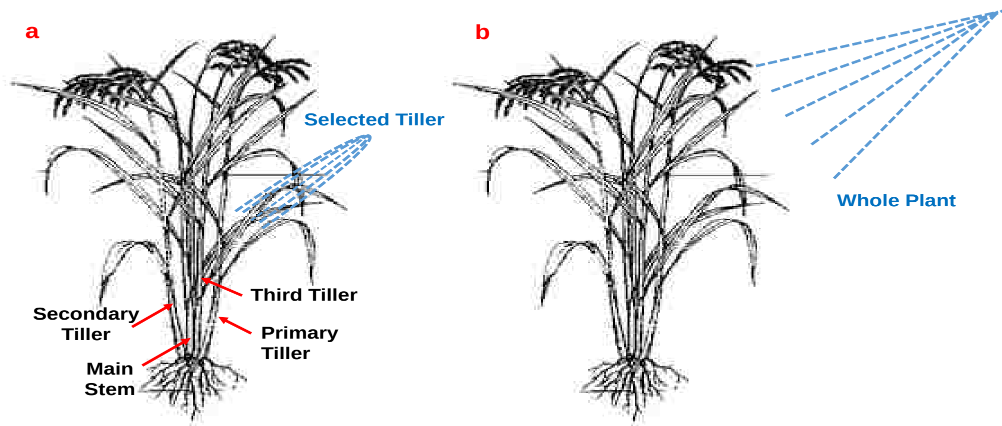

Figure 3.1. Scheme of foliar Si application to leaves of the primary third tiller (a) and to whole rice plant (b).

3.2.4. Silicon Deposition on Adaxial Leaf Surface of Rice Treated with Foliar Si

Three Si sources including a check were tested in this study: (1) foliar-applied Si solution at 80 mg of Si L^{-1} (Taminco®, 6000 mg of Si L^{-1}), (2) soil-applied silicate slag at 4.5 ton ha^{-1} (Plant Tuff®, 14% Si, 23% Ca, and 7% Mg), (3) soil-applied wollastonite at 4.5 ton ha^{-1} (24% Si and 31% Ca), and (4) a check treatment composed by foliar application of DI water.

Silicate slag (Plant Tuff®) and wollastonite (Vansil®) were applied before sowing rice seeds by spreading and incorporating the material into the soil by hand. With silicate slag, the Si applied was equivalent to 690 kg of Si ha^{-1}, whereas with wollastonite was 1190 kg of Si ha^{-1}. Silicon-containing solution was diluted with DI water to come up with Si application rate of 80 mg of Si L^{-1}, which delivered 48 g Si ha^{-1}. Foliar treatment was applied once at early tillering stage. The leaves were hold down and solution was strictly applied to the adaxial leaf surface using a perfume-sprayer (Pete®) (Figure 3.2). Pots were covered during foliar application to avoid runoff of solution into the soil and unexpected Si uptake by roots. One week after application, plants were harvested and separated into two groups: one was washed with DI water and other with 2% nitric acid (HNO_3) before oven-drying. The experiment was arranged in a randomized complete block design with five replications.

Figure 3.2. Foliar Si solution application to adaxial surface of rice leaves.

3.2.5. Washing Procedure

Plants samples were carefully placed into plastic bottles. Washing was done in batches of 12 samples and 100 mL of either DI water or 2% HNO_3 was added to it. Samples were shaken for 2 minutes on reciprocal shaker (Eberbach: E6010.00). Washing solutions for each treatment were collected and analyzed for Si content by Molybdenum Blue Colorimetric (MBC) (Hallmark et al., 1982).

3.2.6. Plant Analysis

Oven dried plants were processed before Si and extractable nutrient analysis. Silicon content was determined by Oven-Induced Digestion procedure (OID) (Kraska and Breitenbeck,

2010) followed by MBC. Ground plant tissue sample (100 mg) was placed into a 50-mL polyethylene centrifuge tubes. Five drops of octyl alcohol and 2 mL of hydrogen peroxide (H_2O_2) were added to the samples. Tubes were placed inside the oven with temperature set to 95°C. After 30 minutes, the tubes were taken out and 4 mL of 50% sodium hydroxide (NaOH) was added to it. Tubes were capped loosely and placed back in the oven for another 4 hours. Within this period, samples were taken out of the oven every 15 minutes for quick mixing using a vortex mixer. After 4 hours, 1 mL of ammonium fluoride (NH_4F) was added and the volume was completed to 50 mL using DI water. References samples (soybean and sugarcane) and blanks were also digested.

An aliquot (2 mL) of plant digest solution was collected and placed into 50-mL centrifuge tubes. Subsequent addition of 10 mL 20% acetic acid and 2 mL 0.26 M ammonium molybdate [$(NH_4)_6Mo_7O_2$, 2 mL] was made to each tube. After 5 minutes, 2 mL of 20% tartaric acid was applied and samples were shaken for 10 seconds by hand. Samples were allowed to stand for 2 minutes and then 2 mL of ANSA (0.5 mg of 1-amino-2-naphthol-4-sulphonic acid, 1.0 g of sodium sulfite and 30.0 g of sodium bisulfite) was added. The final volume was completed to 30 mL with 20% acetic acid. A standard curve was prepared with the same digested background at rates of 0, 0.4, 0.8, 1.6, 3.2, 4.8, and 6.4 ug mL^{-1} of Si. Previous digested references and blanks were also examined. Absorbance readings were measured at 630 nm using a Hach® DR 500 UV-Visible Spectrophotometer.

Plant extractable nutrients were determined by HNO_3-H_2O_2 digestion and inductively coupled plasma (ICP) – Optical Emission Spectroscopy (OEM) analysis. Five hundred milligrams of plant material was weighed into digestion glass tube. Concentrated HNO_3 (5 mL) was applied to samples followed by vortex mixing for 10 seconds. After 50 minutes standing at room temperature (25°C), tubes were placed on heating block at 152°C for five minutes. Samples were allowed to cool before adding 3 mL of 30% H_2O_2. Small glass funnels were inserted into the top of the tubes before placing them back to the digestion block. After 2 hours and 45 minutes of digestion, tubes were taken out of the block and allowed to cool at room temperature before diluting the digested sample to 12.5 mL with DI water. Digest solutions were filtered using Whatman® no. 1 filter paper prior to ICP atomic spectrometry analysis. Reference samples and blanks were also run.

3.2.7. Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray (EDX) Analysis

Scanning electron microscopy coupled to EDX microanalysis mapping was used to determine Si content and deposition on leaf surface. Before drying the leaf samples, small sections were cut and stored in the refrigerator for SEM-EDX analysis. Both the adaxial and abaxial surface of leaf samples were examined. This technology relies on atomic excitation by electron beams, which provides a semi-quantitatively determination of nutrient content by proportionality of scanned area (McMullan, 2006). Three readings per sample were taken to increase data reliability.

3.2.8. Soil Analysis

Soil samples were collected at the end of the experiments. The 0.5 M acetic-acid extraction and MBC procedures were followed for determination of soil Si content (Korndorfer et al., 2001). Soil (2 g) was weighed in a polyethylene centrifuge tube and mixed with 20 mL of 0.5 M acetic-acid. Samples were placed in reciprocal shaker (Eberbach) for 1 hour and filtered using Whatman® no. 1 filter paper. The MBC procedure was done by adding DI water (10 mL), 1:1 HCl:water solution (0.5 mL), and 10% ammonium molybdate (1 mL) to tubes containing 0.5 mL of soil extract. After 5 minutes, 1 mL of 20% tartaric acid was added, samples were swirled for 10 seconds, and then allowed to sit for 2 minutes. The reducing reagent ANSA (1-amino-2-naphthol-6-sulphonic acid) was added at 1 mL and final volume to 25 mL was made with DI water. Absorbance reading was measured after 5 minutes at 630 nm using UV visible spectrophotometer (Hach® DR 500). Standard series with 0, 0.2, 0.4, 0.8, 1.2, 1.6, and 2.0 ug Si mL^{-1}, blanks, and reference samples (sharkey and commerce soils) were also read.

Extractable nutrients were analyzed by Mehlich-3 procedure followed by ICP atomic spectrometry (Mehlich, 1984). For this procedure, 2 g of soil was weighted and mixed with 20 mL of Mehlich-3 extractant (dilute acid-fluoride-EDTA solution corrected to pH 2.5). Samples were shaken using a reciprocal shaker for 5 minutes then filtered using a Whatman® no. 42 filter paper. Clear filtrates were transferred to tubes for ICP analysis. Reference and blanks were included for quality assurance.

3.2.9. Statistical Analysis

Data were evaluated using PROC MIXED in SAS 9.4 software (SAS Institute, 2012). In both experiments, analysis of variance (ANOVA) was used to analyze treatment effect. For experiment two, ANOVA was also conducted to check significant differences between leaves collected from whole-plant and selected-tiller treated-Si rice. For any significant effect detected at $P<0.05$, treatment means were compared using Tukey’s test.

3.3. Results and Discussion

3.3.1. Silicon Absorption and Uptake in Tillers Treated with Foliar Si

Based on ANOVA, all measured variables (e.g. dry mass, Si content) between whole-plant and select-tiller treated Si were not significantly different. The data were pooled then before performing ANOVA among Si sources. Foliar application of Si had no effect on the measured variables, including rice growth and yield (Table 3.2). Similar results were observed by Guével et al. (2007) wherein the growth of plants which received foliar application of two different Si products did not show any improvement in greenhouse condition, except when plants were subjected to disease. Potassium silicate applied as foliar spray was reported to raise photosynthetic rate and growth of chestnut (*Castanea spp.*) when plants encountered heat or water stress (Zhang et al., 2013). Both straw and panicle Si content were not increased by foliar Si application (Figure 3.3). Guével et al. (2007) observed that the Si content of plants applied with foliar Si solution was the same as the check. In contrast, higher Si content was noted by Crusciol et al. (2013a) in rice flag leaves from plants which received foliar Si application in a field condition. In the current study there were no notable stress factors encountered by the rice plants which might partially explain the lack of plant growth response to Si treatments. The increased Si content on plants that were foliarly applied with Si in field conditions might have resulted from Si solution runoff into soil and Si uptake by the roots. The Si content of biomass and panicle from tillers which received Si foliar application was the same as the rest of the plant which was not sprayed with foliar Si (Table 3.3), suggesting that Si absorption did not take place in leaf surface nor the translocation of Si in the plant. Both DI washed and unwashed leaves from the whole and third tiller applied plants had similar Si content (Figure 3.4). This study was conducted using one type of Si solution and it is possible that the properties of carrier and the presence of other nutrients in the solution had an effect on the results. Such effect was reported by Sousa et al. (2010) wherein increased yield of corn foliarly applied with potassium silicate was not only due to Si, but to the joint effect of Si and K in the plant.

Table 3.2. Effect of silicon treatments on rice number of tillers and panicles, biomass, straw, and panicle yield.

Si treatments (mg Si ha^{-1})	Flowering		Harvest			
	Tiller Number	Biomass (g)	Tiller Number	Biomass (g)	Panicle Number	Panicle Yield (g)
Check	15.0 a	44.7 a	19.0 a	35.5 a	19.0 a	86.0 a
20 mg Si L^{-1}	14.6 a	41.2 a	17.8 a	33.0 a	16.6 a	81.2 a
40 mg Si L^{-1}	16.0 a	48.4 a	17.8 a	30.7 a	19.0 a	75.9 a
80 mg Si L^{-1}	14.6 a	42.4 a	19.8 a	33.5 a	16.8 a	81.6 a
P-value	NS	NS	NS	NS	NS	NS

NS = non-significant. Means followed by the same letter within columns are not significantly different at $P<0.05$ according to Tukey's test.

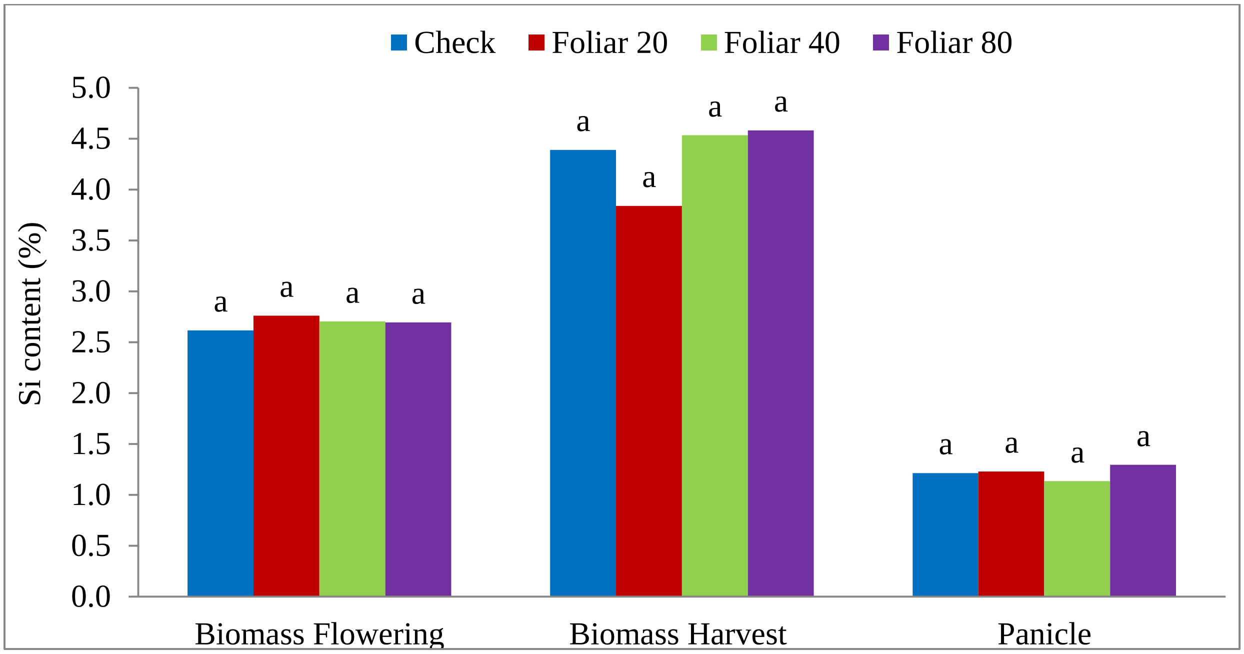

Figure 3.3. Effect of foliar silicon application at different rates on silicon content of biomass at flowering and harvest, and of panicle. Bars labeled with the same letter within sampling type are not significantly different at $P<0.05$ according to Tukey's test.

Table 3.3. Silicon content of biomass and panicle from tillers which received or not foliar application of silicon.

	Biomass Si at flowering (%)	Biomass Si at harvest (%)	Panicle Si (%)
Tiller with foliar Si	2.97 a	4.27 a	1.17 a
Tiller without foliar Si	2.92 a	4.57 a	1.16 a
P-value	NS	NS	NS

NS = non-significant. Means followed by the same letter within columns are not significantly different at $P<0.05$ according to Tukey's test.

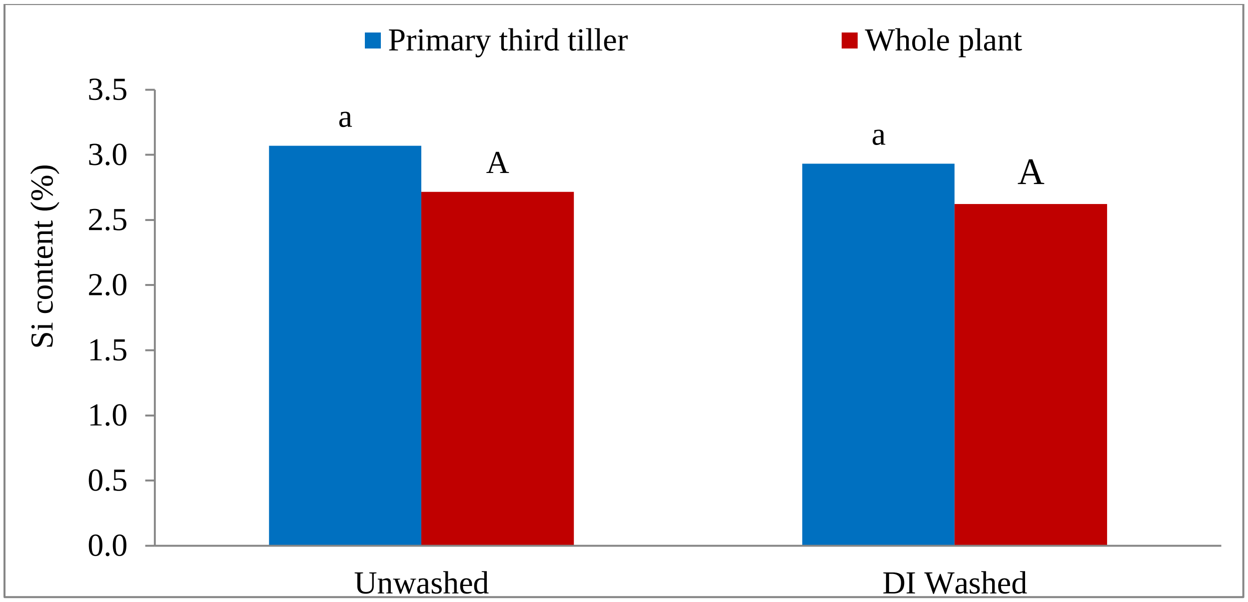

Figure 3.4. Effect of washing the leaves with DI water on straw silicon content across rice growth stage under different silicon treatments. Bars labeled with the same letter within washing treatment are not significantly different at $P<0.05$ according to Tukey's test. Upper case letter for primary third tiller application and lower case for whole plant application.

3.3.2. Silicon Deposition on Adaxial Leaf Surface of Rice Treated with Foliar Si

In general, there was no difference in Si content of leaves washed with DI water and with 2% HNO_3 (Figure 3.5). Among Si treatments, Si content of washing solution was the same (Figure 3.6). However, 2% HNO_3 washing solution showed higher Si content than the DI washing solution for all treatments including the check (Figure 3.7). In addition, higher content of other nutrients, such as Cu, Fe, Mn, P, S, and Zn was detected in 2% HNO_3 washing solution compared to the DI water (Table 3.4). These results suggest that 2% HNO_3 removed Si from leaves, but may not entirely come from washing of dehydrated Si solution in the leaf surface. The enhanced nutrient content of 2% HNO_3 solution could be due to initial leaf digestion as a result of 2% HNO_3 action to plant tissue, especially those with physical damage due to folding. Cell cytoplasm content containing nutrients can easily leak out of cells with disrupted cell wall and membrane.

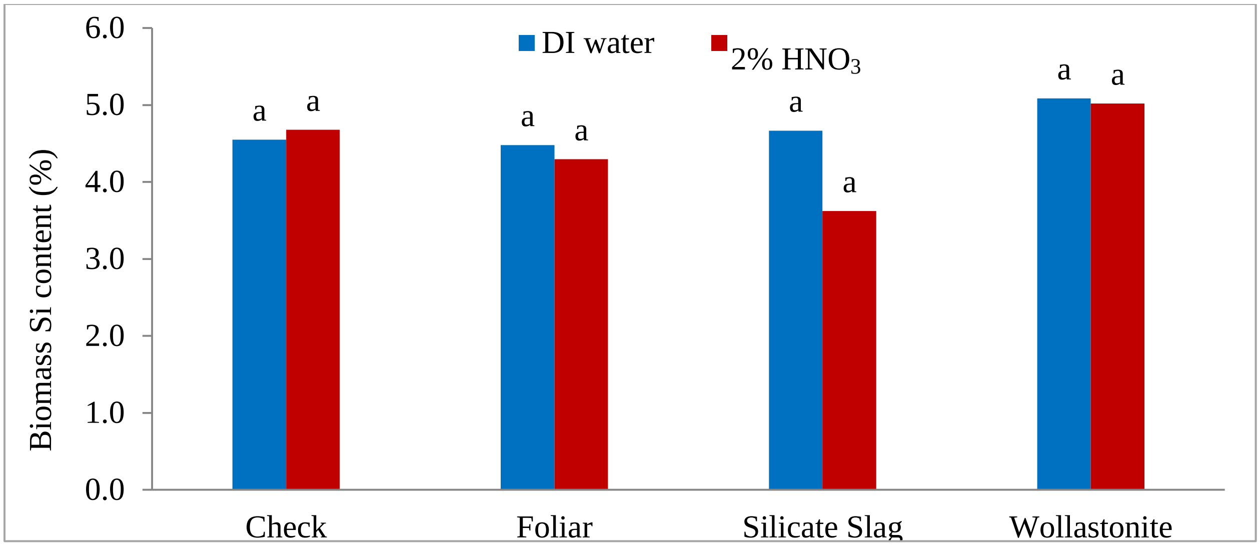

Figure 3.5. Effect of washing the leaves with DI water and 2% HNO_3 on silicon content of rice leaves under different silicon treatments. Bars labeled with the same letter within silicon treatments are not significantly different at $P<0.05$ according to Tukey's test.

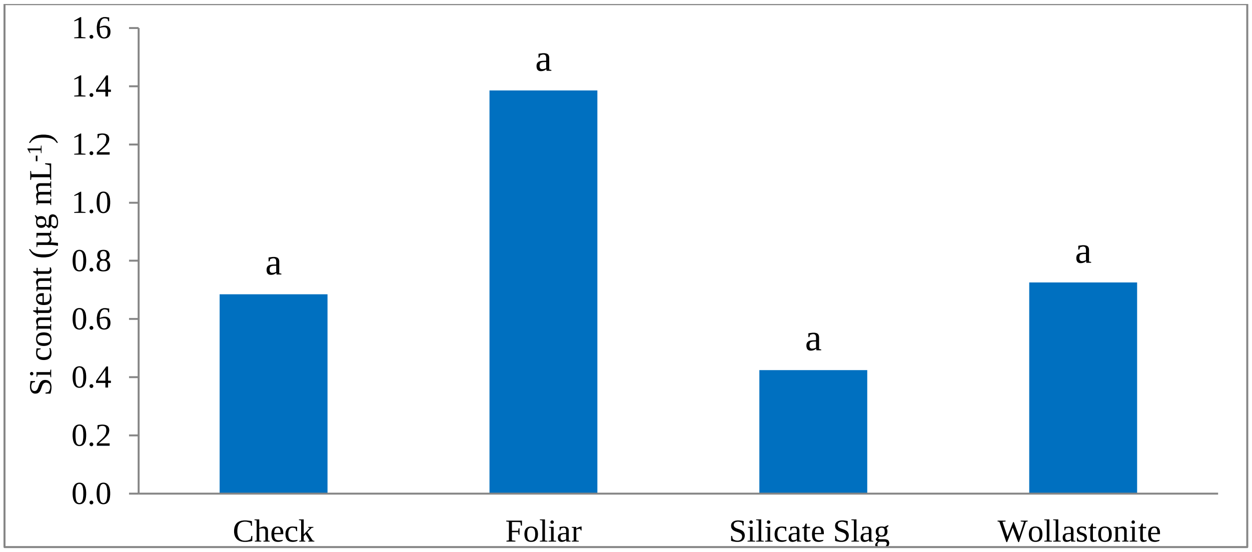

Figure 3.6. Silicon content of washing solution under different silicon treatments. Bars labeled with the same letter are not significantly different at $P<0.05$ according to Tukey's test.

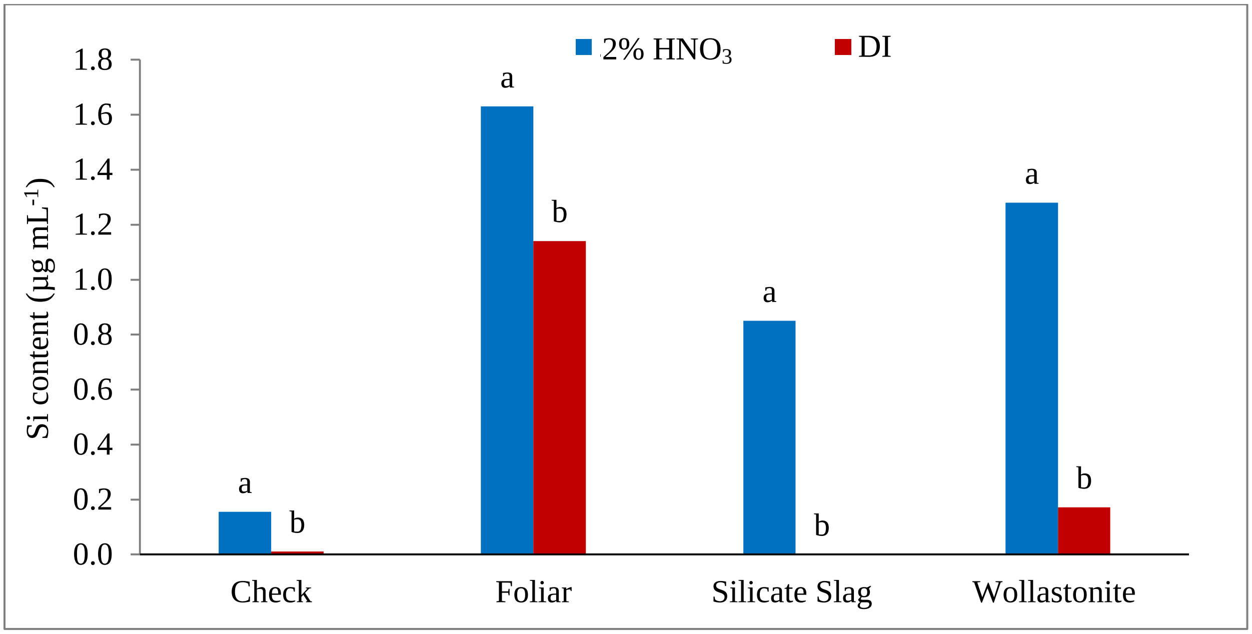

Figure 3.7. Silicon content of DI and 2% HNO_3 washing solution under different silicon treatments. Bars labeled with the same letter within silicon treatments are not significantly different at P<0.05 according to Tukey's test.

Table 3.4. Nutrient content of 2% HNO_3 and DI water washing solution.

	Nutrients (µg mL^{-1})											
	P		S		Mn		Fe		Zn		Cu	
DI water	0.030	b	0.204	b	0.003	b	0.024	b	0.042	b	0.003	b
2% HNO_3	0.498	a	0.382	a	0.351	a	0.367	a	0.101	a	0.013	a
P-value	<0.001		<0.01		<0.001		<0.001		<0.001		<0.001	

Means followed by the same letter within columns are not significantly different at P<0.05 according to Tukey's test.

Foliar application of Si solution did not increase Si content of leaves, whereas wollastonite-treated rice attained the highest Si content (P<0.01) (Figure 3.8). This result agrees with Guével et al. (2007) study which enhanced Si in plants sprayed with Si solutions was not detected. Menzies et al. (1992) also did not notice significant difference in leaf Si content between plants sprayed with potassium silicate and the check, but observed that a coating was formed on the leaf surface of plants applied with Si solution. In contrast, significant increase in biomass Si content was detected when Si solutions was applied to soil or soil-less media (Kanto et al., 2004; Kanto et al., 2006). For example, Kanto et al. (2006) reported that Si content in strawberry was increased by 30% when Si solution was applied to the plots as a soil drench. Similarly, in a hydroponic study the addition of liquid potassium silicate increased the amount of Si uptake by the plant (Kanto et al., 2004). Based on SEM-EDX analysis, Si fertilization did not result in any increase in leaf Si content, except for wollastonite-treated rice compared to foliar application (Figure 3.9). As shown in Figure 3.10, wollastonite treated rice had greater number of silica bodies distributed on leaf surface than foliar-applied leaves. In addition, for all Si treatments including the check, the SEM-EDAX analysis detected greater number of silica bodies on adaxial than abaxial leaf surface (Table 3.5). The microscopic technique used (SEM-EDX) detects Si content on selected areas of leaf surface (Goldstein, 2003). Silicon deposition is not uniform in epidermal cell wall and certain areas on leaf surface might present different Si content; this was confirmed by the variable transpiration intensities at different areas on leaf surface (Kim et al., 2002). Therefore, certain degrees of disagreement in the results between OID-MBC and SEM-EDX analysis are expected.

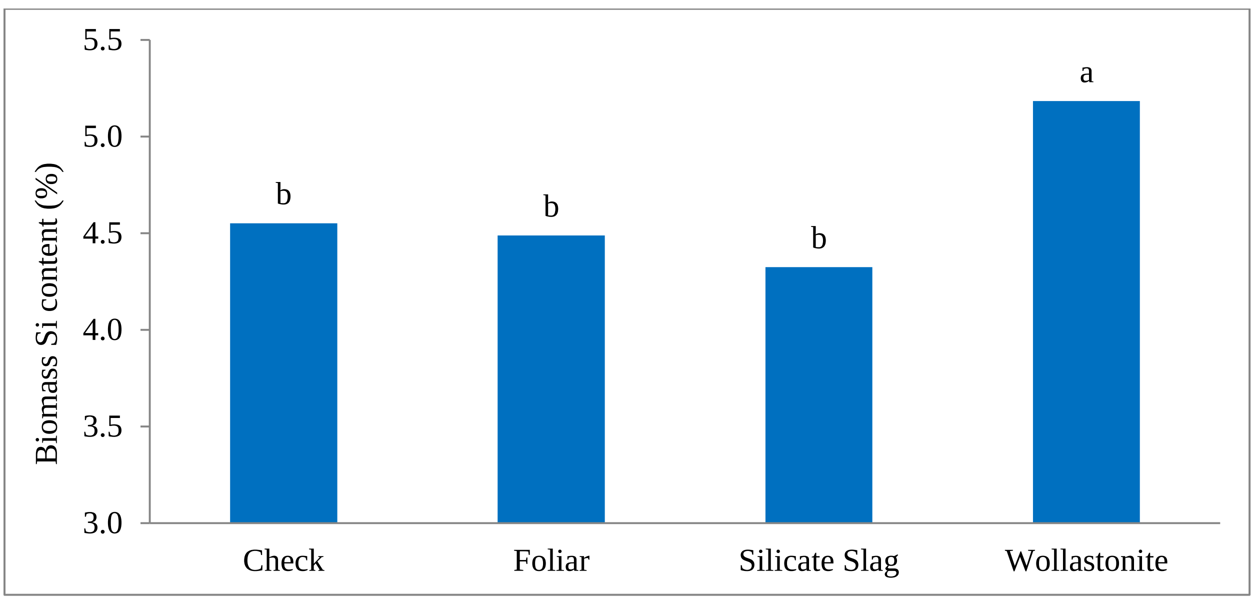

Figure 3.8. Effect of silicon treatments on leaf silicon content by OID-MBC of rice plants. Bars labeled with the same letter are not significantly different at $P<0.05$ according to Tukey's test.

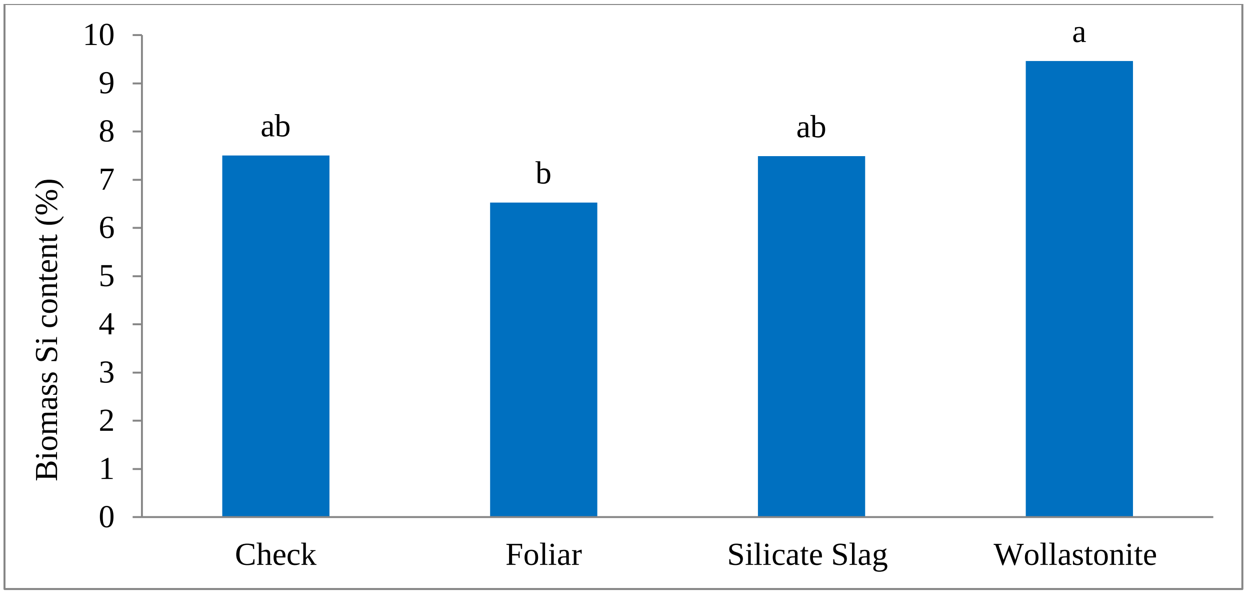

Figure 3.9. Effect of silicon treatments on leaf silicon content by SEM-EDX of rice leaves. Bars labeled with the same letter are not significantly different at $P<0.05$ according to Tukey's test.

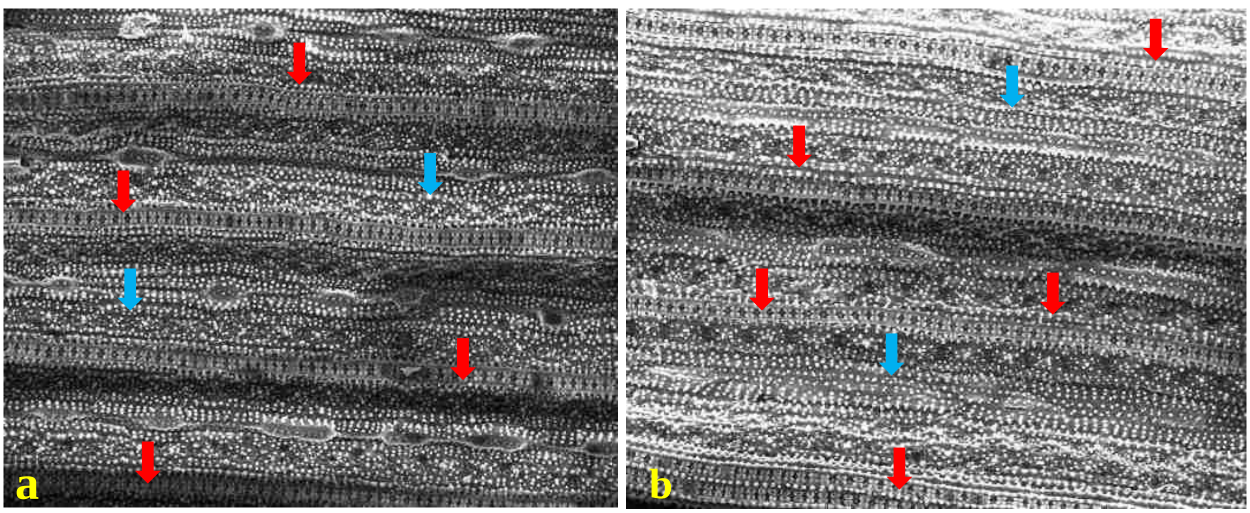

Figure 3.10. Scanning electron microscopy images (400 times magnification) of rice leaves showing silica bodies due to silicon deposition of foliar (a) silicon application and wollastonite (b) application. Red arrows = dumbbell shape silica bodies; blue arrows = globular shape silica bodies.

Table 3.5. Silicon content on rice adaxial and abaxial leaf surface under different silicon treatment.

	Check	Foliar	Silicate Slag	Wollastonite
Si Adaxial (%)	6.70 a	6.53 a	9.00 a	7.97 a
Si Abaxial (%)	6.08 b	6.11 b	6.94 b	6.19 b
P-value	<0.05	<0.05	<0.05	<0.05

Means followed by the same letter within columns are not significantly different at $P<0.05$ according to Tukey's test.

There was no significant increase on total biomass and number of tillers with foliar or soil application of Si (Table 3.6). Prakash et al. (2011) noted no effect of varying silicic acid rates applied foliarly on growth parameters of rice cultivated at hilly zone in India. On the other hand, increased biomass accumulation was recorded by Sousa and Korndorfer (2010) in rice soil-applied Si as wollastonite. It is possible that the lack of response in plant growth of soil applied Si in our study, despite its positive effect on soil Si (Table 3.6), was due to the fact that maximum Si uptake was not attained yet when the plant was harvested (tiller stage) or because plants were not under stress condition (Ma et al., 1989). Both wollastonite and silicate slag application raised soil Si content compared to the check with wollastonite treated soil having higher soil Si than silicate slag treated soil (Table 3.6). This result was mainly due to actual Si content added to soil, which was substantially larger in wollastonite (1190 kg Si ha^{-1}) than silicate slag (690 kg Si ha^{-1}) treatments. Different solubility between wollastonite and silicate slag sources (Haynes et al., 2013) may have also contributed to it. It is notable also that soil pH and EC were significantly increased by the application of wollastonite and silicate slag to soil (Table 3.6). Both sources have high liming potential and contain substantial levels of Ca and/or Mg which can also forms salts with sulfates, carbonates, and chlorides leading to high EC.

Table 3.6. Effect of silicon treatment on tiller number, biomass, soil pH, EC, and soil silicon content.

Si treatments	Tiller Number	Biomass (g)	pH (1:1 Water)	EC (μs cm^{-1})	Si (mg kg^{-1})
Check	11 a	15 a	7.1 b	557 b	29 c
Foliar	14 a	19 a	7.0 b	501 b	24 c
Silicate Slag	12 a	16 a	8.1 a	688 a	125 b
Wollastonite	11 a	15 a	8.2 a	678 a	186 a
P-value	NS	NS	<0.001	<0.001	<0.001

Means followed by the same letter within columns are not significantly different at $P<0.05$ according to Tukey's test.

3.4. Conclusions

There was no clear evidence collected from these series of pot experiments that proves absorption of Si through rice leaf surface. Silicon applied via soil is consistently more effective than foliarly-applied Si in enhancing Si content of rice. However, it depends on source, rate and plant's ability to uptake Si from soil solution. It is also possible that the nature and type of carrier and the presence of other nutrients in Si solution have an effect on its leaf absorption. No effect of Si fertilization was observed on rice growth and yield.

Foliar application of Si can be used in rice production, with the understanding that it may not be absorbed and its reported benefits (e.g. disease suppression, plant growth) may depend on the type of solution and application frequency. For future studies, it is essential to evaluate several types (e.g. different carrier, pH, ionic vs. complexed form) of Si solutions to be able to draw a clear cut conclusion about foliar Si absorption.

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Chapter 4. Conclusions

The use of silicon (Si) in crop production has gained attention from the scientific community especially because of its influence on the dynamics of other elements, and its potential control of plant diseases in a more sustainable and environment-friendly way. However, there is a need for better Si sources, as the common soil-applied sources are amended at high rates, whereas foliar application of Si solution is yet to be proven as effective. In this study, a series of pot experiments were conducted to answer two long-standing questions in Si fertilization: (1) interaction between Si and phosphorus (P), and (2) foliar Si absorption through the leaves.

The well-documented relationship of P and Si was not clearly demonstrated in the present study. Silicon applied to soil (wollastonite and silicate slag) and leaves (Si solution) did not result in significant increase in rice P content and uptake in straw and grain. However, a corresponding increase in soil P content (from 37 to 48 ug g^{-1}) with wollastonite application suggests that these two nutrients (P and Si) have similar soil binding sites. The elevated level of soil Si due to wollastonite application freed some phosphates from the binding sites which eventually caused an increased in soil P as determined by Mehlich-3 procedure. Perhaps, the lack of plant uptake was due to the precipitation of phosphates as a result of pH increasing by Si application. Soil response to this change, i.e. P content, may have also taken place had the native P was at an extremely low level.

There was no clear evidence collected from the series of greenhouse studies conducted that proves absorption of Si through rice leaf surface. Foliar application of Si solution did not increase Si content and uptake by rice and no effect on plant parameters was observed. Silicon applied via soil was consistently more effective than foliarly-applied Si in enhancing Si content of plants, but this result depends on Si source (wolastonite or silicate slag) and rate. There are currently no known transporter genes to move Si through the leaf surface, but the nature and type of carrier and the presence of other nutrients in Si solution might also affect Si absorption through the leaves. Therefore, for future studies it is essential to evaluate several types (e.g. different carrier, pH, ionic vs. complexed form) of Si solutions to be able to draw a clear cut conclusion about foliar Si absorption. Perhaps the unique chemical and physical properties of nanotechnology could help on this absorption issue.

Silicon plays an important role in the mineral nutrition of plants, especially for the high accumulator species, such as rice. Practical means of application, such as lower rates and the use of equipment (e.g. sprayer) commonly used in the field may facilitate adoption of Si fertilization by producers. This research was not able to prove that foliar Si absorption in rice takes place. There were some benefits documented in this research as other did in previous studies; however, they were not directly linked to enhanced Si uptake. Absorption of Si through the roots appear to be the only mechanism thus far by which Si can be taken up by plant. For this reason, in agricultures crops where Si fertilization is required, the application of silicate slag or any Si sources to soil remains a sound approach to sustain crop Si need. With the understanding that Si solution may not be absorbed and further benefits to plants will depend on the type of solution and application frequency, foliar application of Si could be used to sustain plant health. As Si is a mitigator of plant stress, future studies should evaluate foliar application of Si in plants under stress conditions.

Vita

Flávia Bastos Agostinho was born in Uberlândia, Brazil in July of 1990. She attended Federal University of Uberlândia (UFU) and received her Bachelor of Science in Agronomy in December of 2011. After graduating she was accepted into the Minnesota Agricultural Student Trainee (MAST), an exchange program hosted by University of Minnesota (UofM), where she worked in a commercial greenhouse and studied at UofM for 1 year. In August of 2013, she was accepted into the School of Plant, Environmental, and Soil Sciences at Louisiana State University (LSU) through a Brazilian program (Science without Borders). Since then, she has worked in soil fertility under the guidance of Dr Brenda Tubana on silicon fertilization through soil and foliar-applied sources in rice production systems.