Assessing the silicon status of rice (Oryza sativa)

Joseph Eugene Kraska

Louisiana State University and Agricultural and Mechanical College

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ASSESSING THE SILICON STATUS OF RICE (ORYZA SATIVA)

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
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In
The School of Plant, Environmental and Soil Sciences

By
Joseph Eugene Kraska
B.A., Louisiana State University, 2005
June 2009
This thesis has been made possible by the unwavering support of my parents, brothers, and astounding girlfriend, without your support this would not have been possible.
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Abstract

Low silicon (Si) content in rice, sugarcane and other Si-accumulating crops can adversely affect crop performance due to reduced tolerance to both abiotic and biotic stresses. This research had two specific objectives relative to Si in Louisiana rice: develop a digestion procedure for Si tissue analysis that is robust, accurate, precise, and requires equipment commonly found in most agricultural laboratories; and survey the Si status of rice plants at mid-tiller (Y-leaf) and after harvest (straw) throughout the rice-growing regions of Louisiana. Assessing the Si status of a crop typically depends upon accurately measuring Si accumulation in plant tissue. While wet-digestion methods have several advantages for Si analysis, the accuracy and precision of these methods are doubtful. A systematic study showed that control of excessive foaming during digestion of plant tissue significantly reduced variability and increased recovery. The inclusion of ammonium fluoride to facilitate the release of polysilicic acid prior to colorimetric determination results in reproducible readings that stabilize within 60 min and remain stable for at least 5 h. With this modification, the accuracy and precision of values obtained colorimetrically are comparable or superior to those obtained by ICP-OES analysis. A detailed survey of the Si status of rice grown in the Major Land Resource Areas (MLRA) throughout Louisiana was created to identify areas where possible Si deficiencies occur and where responses to Si fertilization would most likely result in economic responses. Low early season uptake occurred in nearly all rice fields included in this study, though by harvest, only 36% of the fields contained less than 50 g Si kg\(^{-1}\). Parishes showing the lowest seasonal Si uptake were located within the Gulf Coast Prairies MLRA in the southwest corner of Louisiana. Significant correlations (p>0.05) were found between air dry pH and tissue Si at both early and late season, but no relationships were evident between dissolved Si in irrigation flood water or soil pore water and Si uptake by rice.
Chapter 1
Literature Review

Silica is the second most abundant element in soils. It readily combines with oxygen, the most abundant element, to form silicate minerals. These minerals comprise the principal constituents of soils and serve as the primary substrate for plant growth. Though the solubility of silicate minerals vary, the concentrations of dissolved silicates in water of mineral soils commonly range from 0.1 to 0.6 mM (Faure, 1991). These concentrations are similar to typical concentrations of Ca\(^{2+}\) and K\(^+\) and greatly exceed those of PO\(_4^{3-}\).

Silicon is assimilated by plant roots as monosilicic acid (H\(_4\)SiO\(_4\)) where it accumulates in leaves and other plant tissue primarily as amorphous silicates or phytolithic opal (Epstein, 1994). Once deposited in this form, Si is immobile and is not redistributed within the plant (Ma et al., 1989; Epstein, 1994). Hydrated, amorphous silica is deposited in cell lumens, cell walls, and intercellular spaces. It also accumulates in external layers below and above the cuticle of leaves. Silicon is present in roots, leaves, and the inflorescence bracts of cereals, especially in rice, wheat, oats and barley (Epstein, 1999). The uptake of Si by rice and other plants is not well understood, but appears to be influenced by a number of soil and climatic factors. Growth chamber studies comparing the effects of low (4 °C) and high (25 °C) temperatures showed that low temperatures substantially suppressed assimilation of Si by rice and corn (Zea mays) as did chemical inhibitors of metabolism (Liang et al., 2006). Increasing solution concentrations of Si, however, increased Si uptake even at low temperatures, suggesting that uptake is a combination of both metabolic rate and Si availability.
Plant physiologists generally agree that Si is not an essential nutrient because most plants can complete their reproductive cycle when grown in nutrient solutions lacking Si in their formulation. This conclusion may not be valid because of the ubiquity of Si as a contaminant. Nutrient culture studies show that plants accumulate some silica even in carefully controlled studies to exclude its presence in growth solutions (Epstein, 1994). Of all the ‘non-essential’ elements assimilated by plants, Si alone is consistently present at concentrations similar to those of the macro- and secondary nutrients. Si concentrations range from 0.1% (similar that of P and S) to more than 10% of whole plant dry matter (Epstein, 1999).

A growing body of evidence indicates that adequate uptake of silicon (Si) can substantially increase the tolerance of rice (*Oryza sativa* L.), sugarcane (*Saccharum officinarum* L.) and other crops to both abiotic and biotic stresses (Dantoff et. al., 2001; Ma and Takahashi, 2002). While all plants accumulate Si to some degree, the amounts accumulated vary greatly among species. When accumulated Si typically represents more than 1% of dry mass, a species is considered a Si-accumulator (Epstein, 1994). Many species of wetland grasses, notably rice, accumulate 5% Si or more in their leaf tissue.

It is also, generally recognized that most plants assimilate dissolved silicates and that Si is crucial to the healthy growth of many plants (Epstein 1994, 1999). Plants deficient in Si are more susceptible to fungal disease, insect feeding, as well as other biotic and abiotic stresses that adversely affect crop production. Low Si uptake has been shown to increase the susceptibility of rice to blast (*Magnaporthe grisea* (Hebert) Barr), leaf blight (*Xanthomonas oryzae* pv. *oryzae* (Ishiyama) Swings et al.), brown spot (*Cochliobolus miyabeanus* (Ito and Kuribayashi in Ito) Drechs ex Dastur), stem rot (*Magnaporthe salvinii* Catt.), scald (*Monographella albescens*...
The specific mechanisms responsible for Si ability to increase disease tolerance are not fully understood, though thickening of the Si layer in the cuticle and improved stomata control have been suggested as contributing factor (Okuda and Takahashi, 1961; Yoshida, 1965; Ma, 1988). Adequate Si uptake also reduces the susceptibility of plants to chewing insects such as stem borer (*Chilo suppressalis* Walker), possibly by rendering plant tissue less digestible and by causing greater damage to the mandibles of feeding insects (Massey and Hartley, 2006).

Even so, high concentrations of soluble silicate in soil water and large reserves of silicate minerals have led many to dismiss ‘silica deficiency’ in most mineral soils, especially in soils containing appreciable amounts of 2:1 clay minerals such as those that occupy much of Louisiana’s landscape. Sufficient reports of improved crop yields and other benefits to Si applications have been documented in the scientific literature to suggest that Si fertilization merits consideration in all regions used for commercial production of rice, sugarcane, wheat and other crops that accumulate high amounts Si in their tissues and harvested components.

The benefits of silica fertilization on crop yields and quality has been studied extensively in Asia, Africa, South America and most other regions where rice, sugarcane and other Si-accumulating crops are commercially grown (Snyder et al., 1986). The most common form of silicate fertilizer applied to soils is slag, a by-product of steel manufacturing. In addition to calcium silicate, slag
typically contains calcium hydroxide and calcium oxide as well as calcium carbonate and numerous micronutrients. Because slag application causes an increase in soil pH and exchangeable Ca, it may be considered an alternative to aglime (calcium carbonate) application for soils rendered acidic by cropping and fertilization.

Recent investigations into the cause of ‘Localized Decline’ (Breitenbeck et al., 2006), a malady of unknown cause affecting flooded rice in southwestern LA; suggest that Si deficiency may be a contributor, if not a primary cause of this disorder. Tissue analyses have confirmed that toxic levels of Fe and Al in young rice plants are a diagnostic characteristic of this disorder even though soil pH and other soil properties in afflicted fields are not consistent with Fe and Al toxicity. This inconsistency prompted a study to assess the possibility that silica deficiency contributes to the onset of localized decline. Numerous studies have shown that Si uptake mitigates Al and Fe toxicity as well as a range of other abiotic and biotic stresses rice and other crops (Ma and Takahashi, 2002; Epstein, 1999). The specific mechanisms responsible for benefits of silica are not completely understood, but it is clear that Si influences the solubility of Fe and Al in flooded soils, the uptake of these potentially toxic metals by roots, and the ability of plants to tolerate elevated tissue concentrations (Ma and Takahashi, 2002).

The complex relationships among tissue Si levels and the susceptibility of rice to biotic and abiotic stresses complicate the establishment of precise critical levels of Si deficiency and sufficiency. Dobermann and Fairhurst (2000) suggested a critical value of 50 g Si kg\textsuperscript{-1} for Y-leaves during tillering and a similar value for mature rice straw with 80-100 g kg\textsuperscript{-1} as the optimum level. The basis for these widely used recommendations is not discussed. DeDatta (1981) also cites 50 g Si kg\textsuperscript{-1} as the critical concentration of Si for straw collected at maturity
based on the work of Tanaka and Yoshida (1970). After their review of extensive field studies in Asia, Lian (1976) concluded no significant increase in yield occurred when mature straw contained >61 g Si kg\(^{-1}\) when grown in Japan and Korea and >51 g Si kg\(^{-1}\) when grown in Taiwan. India rice varieties growing in tropical regions of Sri Lanka and India appear to respond to Si fertilization at straw concentrations of <37 g Si kg\(^{-1}\). This latter value is similar to value of 34 g Si kg\(^{-1}\) established by Korndorfer et al. (2001) as the economic response to Si fertilization in the Everglades Agricultural Area of Florida. Studies to establish early or late season critical Si values for other rice growing regions of the US have not been reported.

Assessing the amounts of potentially available Si in soils remains problematic. While a number of soil tests to assess Si availability have been proposed, none has found wide acceptance (Haysom and Chapman, 1975; Imaizumi and Yoshida, 1958; Kato and Sumida, 2000; Kitada et al. 1992; Ma and Takahashi, 2002; Matichenkov et al., 2000; Mizuochi et al., 1996; Nonaka and Takashashi, 1990; Savant et al., 1997; Sumida and Ohyama, 1988; Sumida, 1991; Takashashi and Nonaka, 1986; Wang et al., 2004). Quantifying Si accumulated in plant tissue remains the most common approach for routine monitoring of the Si status of crops. A number of methods have been proposed for determination of Si in plant tissue, some requiring either long digestion times or expensive equipment (Fox et al., 1969; Elliot and Snyder, 1991; Haysom and Ostatek-Boczynski, 2006). Perhaps the most widely used method is that proposed by Elliott and Snyder (1991) involving colorimetric determination of Si in tissue digests obtained by autoclaving samples in strong oxidants. This autoclave-induced digestion (AID) method is both relatively rapid and avoids the use of costly, specialized equipment. While some have found it satisfactory (Bell, 1997), others have reported that the results obtained by AID are highly variable and tend to underestimate the amounts of Si present in plant tissue (Taber et. al., 2002; Haysom and Ostatek-
Boczynski, 2006). To assess Si status in rice tissue an inexpensive, rapid, robust, accurate, and precise method is needed that has the ability to be used in agricultural laboratories.

Compared to the impacts of other nutrients on rice production, the economic importance of Si is poorly understood in the South Central US. While a number of soil tests have been proposed to assess Si availability, none of these tests has found widespread acceptance. At present, tissue analysis appears to be the primary means of assessing Si availability, though the reliability of various methods for Si tissue analysis remains in doubt. Even with accurate tissue analyses, supporting data to relate plant uptake to crop performance is unavailable for most of the US. A more complete understanding of the Si status of rice grown in the South Central US is needed to allow comparisons to other rice growing regions, and thereby characterize the potential for Si deficiency and the merits of additional Si research in our region.

References


Chapter 2

A Simple, Robust Method for Quantifying Silicon in Plant Tissue

Introduction

A growing body of evidence indicates that adequate uptake of silicon (Si) can substantially increase the tolerance of rice (*Oryza sativa* L.), sugarcane (*Saccharum officinarum* L.) and other crops to both abiotic and biotic stresses (Dantoff et. al., 2001; Ma and Takahashi, 2002). As a result, many laboratories are receiving increased requests to assess Si in plant tissue. To accommodate this demand, an accurate and precise method is required that is also sufficiently simple, rapid, and robust for adoption by laboratories processing agricultural samples in volume. While all plants accumulate Si to some degree, the amounts accumulated vary greatly among species. Plant species that typically accumulate more than 1% Si in their dry mass are considered Si-accumulators (Epstein, 1994). Many species of wetland grasses, notably rice, accumulate 5% Si or more in their leaf tissue. Plant physiologists generally agree that Si is not an essential nutrient because most plants can complete their reproductive cycle in nutrient solutions lacking Si. Even so, it is now generally recognized that Si is crucial to the healthy growth of many crops, especially Si-accumulators such as rice and sugarcane.

Silicon is assimilated by plant roots as monosilicic acid (H$_4$SiO$_4$) where it accumulates in leaves and other plant tissue primarily as amorphous silicates or phytolithic opal (Epstein, 1994). Once deposited in this form, Si is immobile and is not redistributed within the plant (Ma et al., 1989; Epstein, 1994). Hydrated, amorphous silica is deposited in cell lumens, cell walls, and intercellular spaces. It also accumulates in external layers below and above the cuticle of leaves.
Silicon is present in roots, leaves, and the inflorescence bracts of cereals, especially in rice, wheat, oats and barley (Epstein, 1999).

Silicon availability in soil is not fully understood and currently there is no universally accepted Si soil test. Quantifying Si accumulated in plant tissue remains the most common approach for routine monitoring of the Si status of crops. A number of methods have been proposed for determination of Si in plant tissue, some requiring either long digestion times or expensive equipment (Fox et al., 1969; Elliot and Snyder, 1991; Ostatek-Boczynski and Haysom, 2003). Perhaps the most widely used method is that proposed by Elliott and Snyder (1991) involving colorimetric determination of Si in tissue digests obtained by autoclaving samples in strong oxidants. This autoclave-induced digestion (AID) method is both relatively rapid and avoids the use of costly, specialized equipment. While some have found it satisfactory (Bell, 1997), others have reported that the results obtained by AID are highly variable and tend to underestimate the amounts of Si present in plant tissue (Taber et. al., 2002; Haysom and Ostatek-Boczynski, 2006). The latter observations are consistent with our own when attempting to use the AID method to survey the Si status of flooded rice grown in Louisiana. A systematic study was conducted to compare established and experimental methods of determining Si in homogeneous samples of rice or sugarcane tissue.

Materials and Methods

Samples of rice (straw) and sugarcane (leaves), two globally important silica-accumulating crops, were used in these experiments to evaluate established and experimental methods for
Tissue analyses of Si involve oxidation of plant material to release the various forms of Si and an analytical technique to quantify the amount of Si released. Oxidation techniques typically involve wet digestion in strong oxidizing agents or dry combustion in a muffle furnace. The primary techniques used to quantify Si are Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) analysis and colorimetric determination using the molybdenum blue reaction (Weaver et al., 1968). In these studies, the precision and accuracy of colorimetric (MBC) and ICP-OES analyses of samples processed by autoclave-induced digestion (Elliot and Snyder, 1991), microwave-induced digestion (Ostatek-Boczynski and Haysom, 2003), and alkali fusion (Fox et al., 1969) were compared to those of experimental oxidation techniques. At least three reagent blanks were included in each sample set of tissue oxidations described below.

_Tissue Oxidation Methods_

1. Autoclave-Induced Digestion (AID). Dry, ground tissue samples (100 mg) were digested as described by Elliot and Snyder (1991).
2. Modified Autoclave Digestion (MAD). This method is a modification of the AID method. Elliot and Snyder (1991) noted that vigorous foaming occurs when H$_2$O$_2$ and NaOH combine in the sample tube. This rapid excessive foaming can cause deposition of sample on the upper tube wall that often fails to digest during autoclaving. To eliminate excessive foaming, 5 drops of octyl-alcohol were added prior to adding H$_2$O$_2$ and NaOH. The samples were then autoclaved as in AID.

3. Alkali Fusion (AF): Dry, ground tissue samples (500 mg) digested by dry combustion and fusion with alkali as described by Haysom and Ostatek-Boczynski (2006).

4. Microwave Digestion (MWD): Dried, ground tissue samples (200 mg) were digested using microwave-assisted acid-base hydrolysis as described by Haysom and Ostatek-Boczynski (2006). Digestions were performed using a CEM Mars 5 programmable microwave system (CEM Corp., Matthews, NC).

5. Proposed Wet Digestion Method-Oven-Induced Digestion (OID). Dry, ground tissue samples (100 mg) were weighed into 50-mL polyethylene screw-cap centrifuge tubes that were previously washed with 0.1 M NaOH, rinsed with DI water and dried. To reduce foaming, 5 drops of octyl-alcohol were added prior to adding H$_2$O$_2$ and NaOH. Samples were wetted with 2 mL of 30\% H$_2$O$_2$, washing the sides of the tube free of sample. The tube was tightly capped and placed in a convection oven at 95 °C. After 30 min, the tubes were removed and 4 mL of 50\% NaOH added to the hot samples. The sample tubes were then gently vortexed,
capped tightly, and returned to the oven (95 °C). After 4 h, samples were removed and 1 mL of 5 mM $\text{NH}_4\text{F}$ was added to facilitate the formation of monosilicic acid prior to quantitative transfer to 50-mL Nalgene® volumetric flask and diluted to final volume with DI water. During routine analysis, final dilution can be performed directly in the digestion tubes as most 50-mL polyethylene centrifuge tubes are marked to indicate volume of contents. If this expedient is employed, it should be recognized that printed tube markings are less precise than those of volumetric flasks. Studies in our laboratory showed that dilution directly in digestion tubes added as much as 2% to experimental error during routine analysis.

**Determining Si Concentrations**

Molybdenum Blue Colorimetry (MBC): This spectrophotometric procedure for determination of Si in digests follows that described by Hallmark et al. (1982). A 1-mL aliquot of diluted sample digest was added to a 15 mL polyethylene screw-cap centrifuge tube previously washed with 0.1 M NaOH, rinsed with DI water and dried. A larger aliquot may be needed for tissue containing low amounts of Si (<20 mg g$^{-1}$). Add 5 mL of 20% ammonium acetate to each tube, cap tightly, and shake vigorously for 10 sec. Uncap and add 2 mL of 0.3 M ammonium molybdate. After 5 minutes, add 1 mL of 20% tartaric acid, cap tightly and shake vigorously to thoroughly mix reagents. Uncap and add 1 mL of reducing solution, cap tightly and again shake vigorously. Finally, dilute samples to a final volume of 15 mL with 20% acetic acid, cap and shake. Allow color to develop for 60 minutes unless otherwise specified. Shake tubes vigorously to mix prior to determining absorbance using a spectrophotometer calibrated at 630 nm. A suitable standard curve can be established by adding 0, 1, 2, 3 and 4 mL of 100 mg L$^{-1}$ standard Si solution to 15 mL polyethylene tubes prior to addition of reagents described above for sample Si determination. Calculate tissue Si concentration as follows:
g Si per kg dry tissue = \( (A_{\text{smpl}} - A_{\text{blk}}) \times C_{fs} \times V_t / V_a \times 1 / S_{wt} \times (10^6 \mu g \ g^{-1} / 10^6 \ mg \ kg^{-1}) \)

Where

\( A_{\text{smpl}} \) = absorbance reading of sample

\( A_{\text{blk}} \) = absorbance of reagent blank

\( C_{fs} = \mu g \ Si \ ml^{-1} \) derived from standard curve per unit absorbance, assuming no intercept

\( V_t \) = final volume of digest

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

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

ICP-OES analysis: For automated determination of Si by ICP-OES, a 1-mL aliquot of diluted sample digest is transferred into 13 mm x 100 mm polypropylene tube. A larger aliquot may be needed for tissue containing low amounts of Si (<20 mg g^{-1}). To ensure that the digestion matrix poses minimal hazard to the torch nozzle in the ICP instrument, the sample was further diluted by addition of 6 mL of DI water. Both samples and calibration standards were read at the 251 nm wavelength. Calculate tissue Si concentration as follows:

\( g \ Si \ per \ kg \ dry \ tissue \ = \ (R_{\text{smpl}} - R_{\text{blk}}) \times D \times V_t / V_a \times 1 / S_{wt} \times (10^6 \mu g \ g^{-1} / 10^6 \ mg \ kg^{-1}) \)

Where

\( R_{\text{smpl}} \) = ICP reading of sample (\( \mu g \ Si \ mL^{-1} \))

\( R_{\text{blk}} \) = ICP reading of reagent blank (\( \mu g \ Si \ mL^{-1} \))

\( D \) = final volume of sample in tube submitted for analysis

\( V_t \) = final volume of digest

\( V_a \) = volume of digest used for ICP analysis
\[ S_{\text{wt}} = \text{oven-dry equivalent weight of sample digested (mg)} \]

**Influence of \( \text{NH}_4\text{F} \) on Color Stability During Molybdenum Blue Colorimetry**

The effects of \( \text{NH}_4\text{F} \) on the development and stability of color using the molybdenum blue technique were assessed by comparing absorbance readings obtained at various times with and without the inclusion of \( \text{NH}_4\text{F} \). Treatments consisted of triplicate samples (100 mg) of dry, ground rice straw digested using the OID method with and without 1 mL of 5 mM \( \text{NH}_4\text{F} \). Readings commenced at 15 min after addition of the reduction agent. Absorbance was recorded at 15 min intervals for 120 min and at 60 min intervals for another 180 min.

**Recovery of Si by Various Wet Digestion Methods with and without the Addition of \( \text{NH}_4\text{F} \)**

To assess the measured concentrations and variability in tissue Si determined using various wet digestion methods, 14 replicate subsamples of a homogenized sample of finely ground, dry rice straw were digested by the AID, MAD and OID procedures. After digestion, digests were amended with 1 mL of solutions containing 0, 5, 50, or 500 mM \( \text{NH}_4\text{F} \), and diluted to final volume in 50-mL Nalgene® volumetric flasks. After dilution, digests were analyzed colorimetrically and by ICP-OES. The means, standard error of the means, and 95% confidence intervals were calculated using a statistical software package (Statistica, Statsoft, Tulsa OK).

**Recovery of Known Addition of Si**

To obtain additional validation that the proposed digestion (OID) technique quantitatively recovers Si, five replicate 100 mg samples of dry, ground rice straw was digested with and without a known addition of 10 mg Si. After dilution, the amount of Si recovered was determined colorimetrically and by ICP-OES analysis.
Results and Discussion

The principal advantage of the popular AID method (Elliott and Snyder, 1991) lies in its ability to provide a quick and inexpensive tissue digestion that allows for large sample batches. Bell and Simmons (1997) used the AID method to establish Si concentrations in several NIST plant tissue standards. They did not attempt to validate the assumption that the AID method provides accurate quantification of tissue Si by comparing results of the AID method with other proposed methods of Si determination. Others (Taber et al., 2002; Haysom et al., 2006) found that the AID method underestimated the Si content in some plant tissues such as sugarcane, corn, bluegrass, and peach leaves. Low recovery of tissue Si was attributed to incomplete digestion and possibly the inability of the MBC method to quantitatively measure all forms of Si in digests.

The amounts of Si measured in wet digests of rice straw and sugarcane leaves obtained by the AID method were similar to or less than the amounts in digests obtained when this method was modified (MAD) by addition of five drops of octyl alcohol to control foaming (Table 2.1). A slight increase in measured Si was obtained when a two-phase digestion (OID) method was employed. The principal benefit of the OID method was substantially reduced variability. Standard errors of five replicate samples digested using the OID method were 18% to 80% less than those of corresponding samples digested by the AID method.

Table 2.1 also shows that the Si concentrations measured by ICP-OES analysis were consistently greater than those obtained by colorimetric (MBC) analysis. The instability of color
development during colorimetric analysis may have been the cause of the lower values obtained by this analysis. While the ICP-OES measures all forms of Si in solution, the MBC analysis only measures the concentrations of Si ion. It does not directly measure polysilicic acid, a form of Si present in plant digests and water. While reagents added during MBC strongly favor formation of monosilicic acid, dissolution of polysilicic acid is generally slow (Weitz et. al., 1950). As a consequence, the slow dissolution of polysilicic acid during MBC analysis results in unstable absorbance readings due to prolonged color development after the addition of the reducing agent. To compensate for this instability, it has been suggested that absorbance be measured at an arbitrary time after addition of the MBC reagents (Weaver et. al., 1968). The reliability of this solution, however, depends upon a number of factors including constant temperature and the nature and amounts of silicates initially present in digests.

Table 2.1: Influence of wet digestion method on average Si concentrations, standard error of the mean (SE) and 95% confidence intervals (CI) of 5 replicate samples of rice straw and sugarcane leaves determined by ICP-OES and colorimetrically.

<table>
<thead>
<tr>
<th>Digest‡</th>
<th>Tissue</th>
<th>Colorimetrically</th>
<th>ICP-OES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>g Si/kg</td>
<td>g Si/kg</td>
</tr>
<tr>
<td>AID</td>
<td>Rice Straw</td>
<td>34.5</td>
<td>1.3</td>
</tr>
<tr>
<td>MAD</td>
<td>Rice Straw</td>
<td>34.0</td>
<td>1.5</td>
</tr>
<tr>
<td>OID</td>
<td>Rice Straw</td>
<td>34.9</td>
<td>1.1</td>
</tr>
<tr>
<td>AID</td>
<td>Sugarcane Leaf</td>
<td>17.3</td>
<td>1.2</td>
</tr>
<tr>
<td>MAD</td>
<td>Sugarcane Leaf</td>
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<td>1.1</td>
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<tr>
<td>OID</td>
<td>Sugarcane Leaf</td>
<td>20.6</td>
<td>0.8</td>
</tr>
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</table>

‡AID, Autoclave-Induced Digestion; MAD, Modified Autoclave-Induced Digestion; OID, Oven-Induced Digestion
A more satisfying solution for colorimetric determination of Si may lie in enhancing the complete ionization of polysilicic acid prior to MBC analysis. It is well known that the equilibrium between mono- and polysilicic acids can be accelerated by the presence of fluoride ions in the digest (Iler, 1955). The benefits of adding a small quantity of NH₄F on the intensity and stability of absorbance readings during MBC analysis of wet digests are evident in Fig. 2.1. Addition of 1 mL of 5 mM NH₄F to digests prior to dilution greatly enhanced color development and resulted in absorbance readings that stabilized at 45 min and remained constant when the experiment was terminated after 5 h. In contrast, absorbance never fully stabilized even after 5 h when no NH₄F was added. Moreover, the absorbance values obtained with NH₄F after 45 min were 18% greater than those obtained after 5 h when no NH₄F was added.

![Absorbance vs Time](image)

**Figure 1**: Intensity and stability of absorbance readings during colorimetric analysis of Si in wet digests of rice straw with and without the addition of 1 mL of 5 mM NH₄F prior to dilution for MBC analysis.
To better characterize the accuracy and precision associated with various wet digestion techniques and to identify the optimum concentration of NH$_4$F addition for both colorimetric and ICP-OES analysis of Si, highly replicated studies were performed using rice straw (Table 2.2) and sugarcane leaves (Table 2.3). It is readily evident from these data that digestion method influenced both the amount of Si determined in digest as well as the variability associated with those determinations. For example, the data in Table 2.2 shows that Si released in samples of rice straw digested by the MAD and OID methods were significantly greater (6.9% and 8.5%, respectively) than in samples digested by AID. When sugarcane leaves were used (Table 2.3), Si concentrations in AID and MAD digests were similar. However, they were 8.9% and 10.7% less, respectively, than the values obtained using OID. These findings support the conclusion that the two-phase OID digestion offers a more complete and consistent digestion of plant samples than either the AID or MAD.

Addition of as little as 1 mL of 5 mM NH$_4$F to digests prior to dilution led to increases as great as 51% in Si determined colorimetrically in digests of both rice straw and sugarcane leaves obtained by all three methods (Table 2.2 and Table 2.3). In general, NH$_4$F addition had less effect on Si when measured by ICP-OES, though increases as great as 8.2% occurred. Perhaps more striking than its effect on the amount of Si determined, was the ability of NH$_4$F addition to improve reproducibility especially during colorimetric analysis. Standard errors of MBC analyses were reduced as much as 37% in rice straw and 51% in sugarcane leaves. Standard errors tended to decrease and Si values increased as NH$_4$F concentration of dilute digests was increased for 0.1 to 1 mM NH$_4$F, though these trends were not significant (p<0.05).
Table 2.2: Influence of wet digestion method and ammonium fluoride on the average silica concentration, standard error of the mean (SE) and 95% confidence interval (CI) of 14 replicate samples of rice straw determined by ICP-OES and colorimetrically.

<table>
<thead>
<tr>
<th>Digest‡</th>
<th>NH₄F†</th>
<th>Colorimetrically</th>
<th>ICP-OES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mM</td>
<td>Mean g Si/kg</td>
<td>SE g Si/kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AID</td>
<td>0</td>
<td>30.7</td>
<td>2.2</td>
</tr>
<tr>
<td>AID</td>
<td>0.1</td>
<td>41.0</td>
<td>1.6</td>
</tr>
<tr>
<td>AID</td>
<td>1</td>
<td>42.1</td>
<td>1.5</td>
</tr>
<tr>
<td>AID</td>
<td>10</td>
<td>43.9</td>
<td>1.3</td>
</tr>
<tr>
<td>MAD</td>
<td>0</td>
<td>33.4</td>
<td>0.9</td>
</tr>
<tr>
<td>MAD</td>
<td>0.1</td>
<td>44.2</td>
<td>0.7</td>
</tr>
<tr>
<td>MAD</td>
<td>1</td>
<td>46.7</td>
<td>0.6</td>
</tr>
<tr>
<td>MAD</td>
<td>10</td>
<td>47.2</td>
<td>0.6</td>
</tr>
<tr>
<td>OID</td>
<td>0</td>
<td>31.2</td>
<td>1.3</td>
</tr>
<tr>
<td>OID</td>
<td>0.1</td>
<td>47.2</td>
<td>0.8</td>
</tr>
<tr>
<td>OID</td>
<td>1</td>
<td>47.3</td>
<td>0.6</td>
</tr>
<tr>
<td>OID</td>
<td>10</td>
<td>47.1</td>
<td>0.7</td>
</tr>
</tbody>
</table>

†Concentration of NH₄F (mM) in digests after dilution to 50 mL.
‡AID, Autoclave-Induced Digestion; MAD, Modified Autoclave-Induced Digestion; OID, Oven-Induced Digestion

In general, values obtained by MBC analysis of OID digests tended to be slightly greater than those obtained by ICP-OES analysis, though this difference averaged only about 3% and values obtained by these different analytical techniques were not significantly different (p<0.05) even when analyses were highly replicated. A similar bias was reported by Wang et al. (2004) when comparing MBC analyses of soil Si to those obtained by ICP-OES. A ’known addition’ study (Table 2.4) was performed to determine which of these two analytical procedures provided the most accurate values, and to further assess the ability of wet digestion by OID to quantitatively recover Si.
Table 2.3: Influence of wet digestion methods and ammonium fluoride on the average silica concentration, standard error of the mean (SE) and 95% confidence interval (CI) of 3 replicate samples of sugarcane leaves determined by ICP-OES and colorimetrically.

<table>
<thead>
<tr>
<th>Digest‡</th>
<th>NH₄F†</th>
<th>Colorimetrically</th>
<th>ICP-OES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mM)</td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>AID</td>
<td>0</td>
<td>17.8</td>
<td>1.9</td>
</tr>
<tr>
<td>AID</td>
<td>0.1</td>
<td>20.7</td>
<td>1.2</td>
</tr>
<tr>
<td>AID</td>
<td>1</td>
<td>20.5</td>
<td>0.7</td>
</tr>
<tr>
<td>AID</td>
<td>10</td>
<td>19.9</td>
<td>0.2</td>
</tr>
<tr>
<td>MAD</td>
<td>0</td>
<td>18.9</td>
<td>1.2</td>
</tr>
<tr>
<td>MAD</td>
<td>0.1</td>
<td>19.6</td>
<td>0.3</td>
</tr>
<tr>
<td>MAD</td>
<td>1</td>
<td>19.5</td>
<td>0.2</td>
</tr>
<tr>
<td>MAD</td>
<td>10</td>
<td>18.6</td>
<td>0.1</td>
</tr>
<tr>
<td>OID</td>
<td>0</td>
<td>20.3</td>
<td>1.6</td>
</tr>
<tr>
<td>OID</td>
<td>0.1</td>
<td>21.0</td>
<td>0.7</td>
</tr>
<tr>
<td>OID</td>
<td>1</td>
<td>21.9</td>
<td>0.6</td>
</tr>
<tr>
<td>OID</td>
<td>10</td>
<td>21.9</td>
<td>0.6</td>
</tr>
</tbody>
</table>

†Concentration of NH₄F (mM) in digests after dilution to 50 mL
‡AID, Autoclave-Induced Digestion; MAD, Modified Autoclave-Induced Digestion; OID, Oven-Induced Digestion

In that study, digests were amended to contain 0.1 mM NH₄F after dilution because the data reported in Table 2.2 and Table 2.3 indicates that higher amounts of NH₄F did not noticeably improve Si recovery or variability. Moreover, lower amounts of fluoride are less likely to damage ICP’s quartz nozzle. ICP-OES analysis of digests of 100 mg samples of finely ground rice straw amended with 10 mg Si prior to OID resulted in 94% recovery of added Si, whereas colorimetric analysis resulted in 98%. These recoveries were within the range of experimental error, though a bias toward lower values using ICP-OES analysis remained evident.
This bias was clearly evident in preliminary studies when samples were submitted for ICP-OES analysis on an instrument calibrated using Si standards in water rather than in a matrix of NaOH and peroxides similar to that of digests (data not shown). In that instance, the values obtained by ICP-OES analysis were 41% lower than those obtained colorimetrically. Table 2.5 demonstrates the matrix effect on values obtained for Si standards by ICP-OES analysis when the instrument was calibrated using Si standards prepared in DI water. When known concentrations of Si in water (25-100 mg/L) were measured, recovery ranged from 99.2% to 103.5%. When Si concentrations were prepared using a matrix similar to OID digests, recoveries ranged from 61.9% to 72%, and averaged 35% less than those obtain in a water matrix. This finding underscores the importance of calibrating ICP-OES instruments using the appropriate matrix prior to Si analysis. When such calibration is not possible, further dilution of samples can serve to minimize this matrix effect. Alternatively, a set of Si standards in the appropriate matrix can be submitted with samples and used to adjust Si values. It is noteworthy that the colorimetric (MBC) analysis of Si is not subject to a similar matrix effect (Table 2.4).

Table 2.4: Recovery of a 10 mg Si added to rice straw samples prior to digestion (OID) and subsequently analyzed by both ICP-OES and MBC. Standard errors reported are based on analysis of 5 replicate digests.

<table>
<thead>
<tr>
<th>Determination†</th>
<th>Before addition g/kg</th>
<th>After adding 10mg Si g/kg</th>
<th>Efficiency of recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBC</td>
<td>46.4±0.9</td>
<td>56.2±0.3</td>
<td>98%</td>
</tr>
<tr>
<td>ICP-OES</td>
<td>46.1±0.7</td>
<td>55.6±0.3</td>
<td>94%</td>
</tr>
</tbody>
</table>

†MBC, molybdenum blue colorimetry; ICP-OES, Inductively Coupled Plasma Optical Emission Spectrometry

When it was initially discovered that the high variability associated with AID was unacceptable for a planned survey of crop silica status, two recently published methods for Si tissue analysis
(Haysom and Ostatek-Boczynski, 2006) were assessed: alkali fusion (AF) and microwave-assisted (MWD).

**Table 2.5: OID digestion matrix effect on Si recovery by ICP-OES and MBC using instrument calibrated with Si standards prepared in water.**

<table>
<thead>
<tr>
<th>Analysis†</th>
<th>Si (mg Si/L)</th>
<th>Digestion Matrix‡</th>
<th>Water Matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recovered mg Si/L</td>
<td>% recovered</td>
<td>Recovered mg Si/L</td>
</tr>
<tr>
<td>ICP-OES</td>
<td>25.0</td>
<td>15.5</td>
<td>61.90%</td>
</tr>
<tr>
<td>ICP-OES</td>
<td>50.0</td>
<td>33.1</td>
<td>66.2%</td>
</tr>
<tr>
<td>ICP-OES</td>
<td>75.0</td>
<td>51.1</td>
<td>68.1%</td>
</tr>
<tr>
<td>ICP-OES</td>
<td>100.0</td>
<td>71.9</td>
<td>71.9%</td>
</tr>
<tr>
<td>MBC</td>
<td>100.0</td>
<td>99.7</td>
<td>98.7%</td>
</tr>
<tr>
<td>MBC</td>
<td>200.0</td>
<td>197.9</td>
<td>97.5%</td>
</tr>
<tr>
<td>MBC</td>
<td>300.0</td>
<td>298.9</td>
<td>99.5%</td>
</tr>
<tr>
<td>MBC</td>
<td>400.0</td>
<td>402.7</td>
<td>101.7%</td>
</tr>
</tbody>
</table>

† MBC, molybdenum blue colorimetry; ICP-OES, Inductively Coupled Plasma Optical Emission Spectrometry
‡ Digestion Matrix, consisting of H$_2$O$_2$, NaOH, octyl-alcohol, and NH$_4$F addition

Despite several attempts to replicate these methods as described, we were unable to obtain the outstanding accuracy and precision reported (Ostatek-Boczynski and Haysom, 2003; Haysom and Ostatek-Boczynski, 2006). Table 2.6 compares the results for rice straw obtain by the proposed OID method and those obtained by AF and MWD. The AF method gave values that were significantly (p<0.05) lower and more variable than those obtained by OID. A principal difficulty of this method lies in dissolving the fused alkali and ash after combustion. After allowing the recommended 10 mL of warm DI water to stand in crucibles over night,
considerable chipping and scraping with a spatula was necessary to quantitatively transfer the digest for dilution and analysis. Even so, incomplete transfer may have contributed to lower values and precision. Microwave digestion (MWD) resulted in very low recoveries for most samples with an occasional higher value. The aid of a technician with considerable experience in microwave digestion of plant samples did not improve the performance of this method. Though the accuracy and precision obtained by the AF and MWD methods may have improved with practice, the failure of several initial attempts by competent technicians suggests that these methods may not be sufficiently reliable and robust for routine analysis of plant Si.

Table 2.6: Comparison of digestion method on average Si concentration, standard error of the mean (SE), and 95% confidence intervals (CI) of 9 replicate samples of rice straw analyzed by ICP-OES and colorimetrically.

<table>
<thead>
<tr>
<th>Digestion‡</th>
<th>Colorimetrically</th>
<th>ICP-OES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.E.</td>
</tr>
<tr>
<td>Si/kg</td>
<td>g Si/kg</td>
<td>g Si/kg</td>
</tr>
<tr>
<td>OID</td>
<td>47.3</td>
<td>0.8</td>
</tr>
<tr>
<td>AF</td>
<td>40.2</td>
<td>1.6</td>
</tr>
<tr>
<td>MWD</td>
<td>22.8</td>
<td>3.6</td>
</tr>
</tbody>
</table>

‡AID, Autoclave-Induced Digestion; AF, Alkali Fused Digestion; MWD, Microwave Digestion

Conclusions

A two-phase wet digestion process that includes octyl alcohol to control foaming results in more complete and consistent release of Si in plant tissue samples. The addition of NH₄F prior to
dilution of digests facilitates the release of polysilicic acid resulting in colorimetric absorbance readings that stabilize within 1 h and remain stable for 5 h.

These Si values obtained colorimetrically are comparable, if not superior, in both accuracy and precision to those obtained by ICP-OES analysis. It should be noted that the digestion matrix (NaOH, H₂O₂, octyl alcohol) causes quenching of the Si signal during ICP-OES analysis. Consequently, underestimation of Si will occur unless the ICP instrument is calibrated using standards prepared in a matrix similar to that of the sample digests. When compared to other known methods (AF, MWD, AID), the proposed two-phase digestion method provides substantially more accurate and precise measures of Si. Moreover, it is a simple, robust method using inexpensive equipment common to most laboratories and lends itself to automated sample digestion systems (DEENA) being adopted by many commercial and state laboratories.

References


Chapter 3

Survey of the Silicon Status of Flooded Rice (*Oryza sativa*) in Louisiana

Introduction

A growing body of evidence indicates that adequate uptake of silicon (Si) can substantially increase the tolerance of rice (*Oryza sativa* L.), sugarcane (*Saccharum officinarum* L.) and other crops to both abiotic and biotic stresses (Dantoff et. al., 2001; Ma and Takahashi, 2002). While all plants accumulate Si to some degree, amounts accumulated vary greatly among species. When accumulated Si typically represents more than 1% of dry mass, a species is considered a Si-accumulator (Epstein, 1994). Many species of wetland grasses, notably rice, accumulate 5% Si or more in their leaf tissue. Plant physiologists generally agree that while Si accumulates in many species, it is not an essential nutrient because most plants can complete their reproductive cycle in nutrient solutions lacking Si. Even so, it is now generally recognized that Si is crucial to the healthy growth of many crops, especially Si-accumulators such as rice.

The benefits of Si fertilization on crop yields and quality has been documented extensively in Asia, Africa, South America and most other regions where rice, sugarcane and other Si-accumulating crops are commercially grown (Snyder et al., 1986). While silicon fertilization is routine in many countries, it is not widely practiced in the United States. The Everglades Agricultural Area (EAA) of south Florida is a notable exception. Because the organic mucks and sandy soils offer very low Si availability, many rice and sugarcane fields are treated with silicate slag to increase Si availability (Snyder, 2003). Silicon fertilization has largely been overlooked in Louisiana and other rice growing regions of the US where most soils contain appreciable amounts of 2:1 layered silicate clay minerals, and therefore are presumed to supply
adequate amounts of silicates to crops. There is little evidence, however, to support the assumption these mineral soils supply sufficient Si to fully meet the needs of rice, sugarcane and other Si-accumulating crops.

Recent work to identify the causes of a mysterious early season disorder labeled ‘localized decline’ in rice has led to the suspicion that we may have overlooked the importance of Si nutrition in rice grown in south Louisiana (Breitenbeck et al., 2006). During the past decade, numerous rice fields in southwestern Louisiana have begun to display symptoms similar to the devastating nutritional disorder ‘Akagare' that occasionally occurs in Japanese rice fields. Symptoms of ‘localized decline’ are invariably associated with uptake of excessive levels of iron and aluminum by young rice plants. When soils from fields with a consistent history of this disorder are incubated under flooded conditions, the amounts of plant-available iron and aluminum released are negligible. Affected plants are often low in zinc and potassium, but applications of these nutrients has failed to offset the onset of this disorder. A preliminary survey of Louisiana rice at mid-tiller showed that affected plants also contained low levels of Si (12-36 mg/kg), suggesting that Si deficiency may be a contributing factor to ‘localized decline’. While most Si research in rice has focused on its beneficial effects in controlling fungal diseases and insect damage, several studies in the past few years suggest that increasing Si uptake mitigates Al and Fe toxicity as well as a range of other abiotic stresses in rice and other crops (Ma and Taskahasi, 2002).
The primary goal of this study was to survey the Si status of rice plants at mid-tiller (Y-leaf) and after harvest (straw) throughout the rice-growing regions of Louisiana. A detailed survey of the Si status of rice grown in the Major Land Resource Areas (MLRA) throughout Louisiana can be used to identify areas where possible Si deficiencies occur and where responses to Si fertilization would most likely result in economic responses. Silicon concentrations at mid-tiller and harvest were also compared with flood water Si, pore water Si, field moist and air-dried pH to identify possible relationships.

**Materials and Methods**

**Survey strategy:** With the help of LSU AgCenter Extension Agents, representative fields of “good to better” rice were identified visually in each of the rice growing parishes of Louisiana for inclusion in the survey. Sampling sites included all fields within the state’s Rice Verification Program, a program designed to represent the principal growing environments and practices in Louisiana.

**Sampling:** At mid-tiller, rice fields (100+ sites) were sampled for flood water, soil and the most recent fully expanded leaf (Y-leaf). Flood water samples were collected in clean, screw–cap polypropylene bottles. Bottles were rinsed with the flood water three times before filling and capping. After transport to the laboratory, water samples were stored at 4 °C. Soil samples were collected from the surface 15 cm, placed into re-sealable plastic bags, and drained of free water. Bags were placed into a cooler and transported to laboratory where samples were stored at 4 °C until analysis. The geographical coordinates of each sampling site were recorded using a Garmin Vista GPS. After harvest, sites were revisited and samples of straw were collected by cutting
mature plants 10 cm above the soil surface. To minimize soil contamination, leaves and straw were washed in 0.2% detergent, and thoroughly rinsed with DI water (Steyn, 1961; Wallace et. al., 1980) prior to oven-drying (65 °C; 24 h). Dried tissue samples were ground using an Udy Cyclone Mill (Udy Corp., Ft. Collins, CO) to pass through a 20-mesh screen. Ground samples were re-dried for 48 h (65 °C) and placed in snap-cap vials, then stored in a desiccator until use.

**Si Analysis**

*Plant tissue.* Dry, ground tissue samples (100 mg) were digested and Si determined colorimetrically as described by Kraska and Breitenbeck (2009).

*Soil Pore water.* Samples of pore water were collected by centrifuging samples of field-moist soil. In all cases, sites were flooded at time of sample collection and stored in a state approximating saturation. Pore-water was passed through a 0.45 µ filter to remove particulates and the Si concentration of a 3 mL aliquot was determined by molybdenum blue colorimetry as described by Hallmark et al. (1982).

*Flood water.* Flood water Si was determined colorimetrically using filter water by procedures similar to those used to measure soil pore water.

**Determining Field-Moist and Air-Dried Soil pH**

To assess the pH of field moist soil samples, a previously calibrated Cole-Parmer EW-55500-30 AccuFlow™ electrode with Ag/Cl double-junction reference, was placed into the field moist (saturated) soil sample and allowed to stabilize before recording. Air-dried pH was determined in 1:2 soil:water suspensions using samples that had been air-dried under ambient conditions for 10 d.
**Statistical Methods**

Data was analyzed using basic descriptive statistics, one-way analysis of variance and frequency tables using a statistical software package (Statistica, Statsoft, Tulsa OK). Relationships between measured variables were made by plotting simple linear regression comparisons. Forward stepwise multiple linear regression models were used to identify statistically significant (p<0.05) relationships. GIS mapping software (Map Source, Garmin, Salem, OR) was used to create a map showing spatial distribution of flooded rice fields sampled.

**Results**

*Geographical, MLRA and soil group relationships*

Figure 1 shows the locations of the 97 sites sampled in this survey. The density of sampling sites approximates the acreage of rice production throughout the state. Rice production traverses the state diagonally from southwestern to northeastern Louisiana, and includes four Major Land Resource Areas (MLRA). Rice has been a staple crop in southwestern Louisiana since the mid-1800’s and this region continues to produce more than 85% of the state’s rice. This region is comprised of two MRLA’s: the Gulf Coast Plains (GCP) that include most of the more southerly parishes in extreme southwest Louisiana, and the Subtropic Mississippi Valley Silty Uplands (SbMVU) that lie east of the GCP and west of the Atchafalaya Basin. Limited rice production also occurs in central and northeastern Louisiana where acreage has been expanding in recent years. These regions include two MRLA’s: the Southern Mississippi Valley Alluvium (SMVA) that encompasses parishes adjacent to the Mississippi River, and the Southern Mississippi Valley Silty Uplands (SMVU) that lie west of the SMVA.

Many of the soils used for rice in southwestern Louisiana lie within the GCP MRLA and were derived largely from sediments deposited by ancestral Mississippi and Red Rivers. Crowley and
Vidrine are the dominant soils of the GCP in Louisiana. These soil series typically have a cap of mixed loess and alluvium or loamy and clayey subsoil. They are deep, poorly drained and very slowly permeable, making them ideally suited for flooded rice production. In the most western parishes, Mowata-Vidrine associations with strong acid surface horizons predominate. The yield potential for rice tends to be lower on these soils and rice production has declined in recent years in Calcasieu, Allen, and Layfayette parishes (Table 3.1). East of Crowley, LA, where the GCP transitions into the SbMVU, sugarcane begins to rival rice as the dominant crop. The soils of the SbMVU range from silt loams to heavy clays in the uplands along the western edge of the Atchafalaya Basin. Frost and Sharkey are the dominant agricultural soil series. Similar to the silt loams of the GCP, these soil series have deep profiles, moderately low permeability and are poorly drained.

In central and northeastern Louisiana, the soils used for rice were derived from alluvial deposits associated with the more recent course of the Mississippi River. The SMVA MLRA follows the Mississippi River starting at the mouth near Houma, LA and north to Sikeston, MO. Sharkey, a deep, poorly drained clayey alluvium, is the dominant soil series within this MLRA in Louisiana. These soils are well-suited for flooded rice, and production has expanded significantly in Avoyelles and Concordia parishes in recent years (Table 3.1). West of the SMVA lies the SMVU, silty uplands dominated by Dundee-Dubbs complex (silty alluvium) and Grenada-Calhoun complex (loamy loess).
Figure 3.1: Spatial distribution of 97 sampled flooded rice fields in Louisiana

Table 3.1: Average rice production (Mg rough rice) by decade for parishes sampled in survey

<table>
<thead>
<tr>
<th>Parish</th>
<th>MLRA†</th>
<th>Rice Production (Mg) by decade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acadian</td>
<td>GCP</td>
<td>170,175.00</td>
</tr>
<tr>
<td>Allen</td>
<td>GCP</td>
<td>53,445.00</td>
</tr>
<tr>
<td>Avoyelles</td>
<td>SMVA</td>
<td>19,607.00</td>
</tr>
<tr>
<td>Calcasieu</td>
<td>GCP</td>
<td>55,651.00</td>
</tr>
<tr>
<td>Concordia</td>
<td>SMVA</td>
<td>11,380.00</td>
</tr>
<tr>
<td>East Carroll</td>
<td>SMVA, SMVU</td>
<td>29,965.00</td>
</tr>
<tr>
<td>Evangeline</td>
<td>GCP, SbMVU</td>
<td>94,775.00</td>
</tr>
<tr>
<td>Jeff Davis</td>
<td>GCP</td>
<td>169,500.00</td>
</tr>
<tr>
<td>Lafayette</td>
<td>GCP, SbMVU</td>
<td>12,225.00</td>
</tr>
<tr>
<td>St. Landry</td>
<td>SbMVU</td>
<td>45,385.00</td>
</tr>
<tr>
<td>Vermilion</td>
<td>GCP, SbMVU</td>
<td>170,790.00</td>
</tr>
<tr>
<td>West Carroll</td>
<td>SMVA, SMVU</td>
<td>10,754.00</td>
</tr>
</tbody>
</table>

† GCP: Gulf Coast Prairies, SbMVU: Subtropic Mississippi Valley Silty Uplands, SMVU: Southern Mississippi Valley Silty Uplands, SMVA: Southern Mississippi Valley Alluvium
Both these soil series represent very deep profiles with variable permeability and drainage. Even so, many fields are capable of sufficient water retention to support flooded rice production.

Comparisons of the concentrations of Si in y-leaves at mid-tillering and with those in straw after harvest indicate that early season uptake was lower in all fields selected for the survey (Table 3.2). Even so, rice plants in most fields were able to assimilate substantial amounts of Si by harvest. On average, Y-leaves contained 30.5 g Si kg\(^{-1}\) whereas mature rice straw contained 54.7 g Si g Si kg\(^{-1}\). The Si concentrations in Y-leaf ranged from 10.4 to 52.2 g Si kg\(^{-1}\) and those in straw ranged from 21.8 to 80.9 g kg\(^{-1}\). With the exception of two fields in the GCP, the Si content of Y-leaves of young rice was below the 50 g Si kg\(^{-1}\) level suggested as sufficient by Dobermann and Fairhurst (2000) for rice at tillering. By harvest, the Si content of straw in only 34% of the fields was less than the 50 g Si kg\(^{-1}\), a level suggested by DeDatta (1981) and commonly used to assess Si sufficiency. Only six fields, all within the GCP, contained less than 34 g Si kg\(^{-1}\), the level suggested by Korndofer et al (2001) to indicate an economic response to Si fertilization. Ranking the Si contents of straw from various sites according to their respective MLRA’s: SMVA>SMVU>GCP>SbMVA. Parishes showing the lowest seasonal Si uptake were Allen, Calcasieu and Jefferson Davis, all located within the GCP in the southwest corner of Louisiana. In these parishes, 17 of 28 rice fields contained straw with less than 50 g Si kg\(^{-1}\). Four fields containing less than 34 g Si kg\(^{-1}\) were located in western Jefferson Davis Parish, and two in Calcasieu Parish, areas where rice production is declining.

Early season uptake of Si was not a reliable indicator of the amount of Si assimilated by harvest (Fig. 3.2). In no instance did the Si content decrease with maturity, despite the fact that above ground biomass increased approximately 4 times. In a few cases, rice displaying low amounts of Si early in the season remained low until harvest, but most rice fields showing low early season uptake found an adequate supply of Si later in the growing season. The largest increases
in Si status occurred in rice growing in the northeastern region (MRLA’s SMVA and SMVU) where the Si content increased an average of 248% between mid-tillering and maturity. The Si concentration of Y-leaves and straw were used to categorize the Si status of field sites (Fig. 3.2). At mid-tillering, 19% of the sites were deemed ‘very low’ (< 24 g Si kg\(^{-1}\)) and an additional 56% ‘low’ (24-34 g Si kg\(^{-1}\)). Si concentrations suggest that only 3% of the sites contained ‘sufficient’ (45-55 g Si kg\(^{-1}\)) or ‘high’ levels (>55 g Si kg\(^{-1}\)) of Si. By harvest, however, 74% of the rice appeared to contain ‘sufficient’ or ‘high’ levels of Si. Less than 6% of the rice fields could be considered ‘low’ or ‘very low’ in Si uptake.

**Si in flood and soil pore water**

The concentrations of Si dissolved in soil pore water collected during mid-tillering averaged 9.9 mg Si L\(^{-1}\) and ranged from 0.7 to 22.7 mg Si L\(^{-1}\) (Table 3.3). Si concentrations in irrigation flood water collected at the same time averaged 9.6 mg Si L\(^{-1}\) and ranged from 2.0 to 24.7 mg Si L\(^{-1}\). Despite the similarities in mean Si concentrations and their ranges, no significant relationship was evident between Si concentrations of soil pore water and those of the overlying flood water (Fig. 3.3). Even more surprising, no significant relationships were evident between Si concentrations in either soil pore or flood water and the concentrations of Si in plant tissue. Correlation coefficient \((r)\) between y-leaf Si and dissolved Si in pore was -0.1658 and between y-leaf Si and dissolved Si in flood water was only 0.0943. Similarly, the correlation coefficient between Si content of straw and dissolved Si in pore and flood water were only -0.0959 and -0.1339, respectively.
Table 3.2: Average y-leaf and straw Si concentrations and percentages of samples below critical levels of 34.0 or 50 g/kg in the rice producing MRLA’s and parishes

<table>
<thead>
<tr>
<th>MLRA‡</th>
<th>Parish</th>
<th>N</th>
<th>Y-Leaf Mean g Si/kg</th>
<th>% below 50 g Si/kg†</th>
<th>Straw Mean g Si/kg</th>
<th>% below 34 g Si/kg‡</th>
<th>% below 50 g Si/kg¶</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GCP</td>
<td></td>
<td>58</td>
<td>32.1</td>
<td>96.6%</td>
<td>50.9</td>
<td>8.6%</td>
<td>39.7%</td>
</tr>
<tr>
<td></td>
<td>Acadian</td>
<td>16</td>
<td>31.3</td>
<td>93.8%</td>
<td>59.6</td>
<td>0.0%</td>
<td>18.8%</td>
</tr>
<tr>
<td></td>
<td>Allen</td>
<td>3</td>
<td>28.3</td>
<td>100.0%</td>
<td>42.5</td>
<td>0.0%</td>
<td>66.7%</td>
</tr>
<tr>
<td></td>
<td>Calcasieu</td>
<td>13</td>
<td>26.8</td>
<td>100.0%</td>
<td>41.2</td>
<td>15.4%</td>
<td>53.8%</td>
</tr>
<tr>
<td></td>
<td>Evangeline</td>
<td>7</td>
<td>33.6</td>
<td>100.0%</td>
<td>63.3</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td></td>
<td>Lafayette</td>
<td>1</td>
<td>38.4</td>
<td>100.0%</td>
<td>49.1</td>
<td>0.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td></td>
<td>Jeff Davis</td>
<td>12</td>
<td>28.0</td>
<td>100.0%</td>
<td>44.6</td>
<td>25.0%</td>
<td>66.7%</td>
</tr>
<tr>
<td></td>
<td>Vermilion</td>
<td>6</td>
<td>38.0</td>
<td>83.3%</td>
<td>55.9</td>
<td>0.0%</td>
<td>33.3%</td>
</tr>
<tr>
<td>SbMVU</td>
<td></td>
<td>22</td>
<td>24.2</td>
<td>100.0%</td>
<td>45.2</td>
<td>0.0%</td>
<td>40.9%</td>
</tr>
<tr>
<td></td>
<td>Evangeline</td>
<td>8</td>
<td>29.2</td>
<td>100.0%</td>
<td>52.5</td>
<td>0.0%</td>
<td>25.0%</td>
</tr>
<tr>
<td></td>
<td>Lafayette</td>
<td>1</td>
<td>27.6</td>
<td>100.0%</td>
<td>42.4</td>
<td>0.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td></td>
<td>St. Landry</td>
<td>3</td>
<td>29.1</td>
<td>100.0%</td>
<td>46.5</td>
<td>0.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td></td>
<td>Vermilion</td>
<td>10</td>
<td>35.4</td>
<td>100.0%</td>
<td>56.5</td>
<td>0.0%</td>
<td>30.0%</td>
</tr>
<tr>
<td>SMVU</td>
<td></td>
<td>4</td>
<td>23.4</td>
<td>100.0%</td>
<td>56.2</td>
<td>0.0%</td>
<td>25.0%</td>
</tr>
<tr>
<td></td>
<td>West Caroll</td>
<td>4</td>
<td>24.7</td>
<td>100.0%</td>
<td>62.5</td>
<td>0.0%</td>
<td>25.0%</td>
</tr>
<tr>
<td>SMVA</td>
<td></td>
<td>13</td>
<td>27.9</td>
<td>100.0%</td>
<td>66.2</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td></td>
<td>Avoypelles</td>
<td>1</td>
<td>33.8</td>
<td>100.0%</td>
<td>64.1</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td></td>
<td>Concordia</td>
<td>2</td>
<td>21.9</td>
<td>100.0%</td>
<td>69.4</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td></td>
<td>East Caroll</td>
<td>5</td>
<td>27.8</td>
<td>100.0%</td>
<td>67.7</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td></td>
<td>West Caroll</td>
<td>5</td>
<td>27.9</td>
<td>100.0%</td>
<td>63.6</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>All</td>
<td></td>
<td>97</td>
<td>30.5</td>
<td>97.9%</td>
<td>54.7</td>
<td>5.2%</td>
<td>34.0%</td>
</tr>
</tbody>
</table>

†Doberman and Fairhurst (2001)
‡Kornborfer et al. (2001)
¶DeDatta (1981)
¥ GCP: Gulf Coast Praries, SbMVU: Subtropic Mississippi Valley Silty Uplands, SMVU: Southern Mississippi Valley Silty Uplands, SMVA: Southern Mississippi Valley Alluvium
Figure 3.2: Comparisons of Si tissue concentrations at mid-tillering (Y-leaf) and at maturity (Straw)

When stepwise linear regression was used to identify measured variables associated with Si in plant tissue, statistically significant relationships (p<0.05) were found between soil pH and Si concentrations in both Y-leaf and straw (Fig. 3.4). The correlation coefficients between tissue concentrations and pH were greater when pH was determined by 1:2 suspensions of air-dried soils then when pH was determined in field-moist soil samples (saturated).

Table 3.3: Si content of soil pore water and flood water collected at mid-tillering.

<table>
<thead>
<tr>
<th>Water Type</th>
<th>Mean mg Si/L</th>
<th>Median mg Si/L</th>
<th>Min mg Si/L</th>
<th>Max mg Si/L</th>
<th>Range mg Si/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pore Water</td>
<td>9.9</td>
<td>9.2</td>
<td>0.7</td>
<td>22.7</td>
<td>22.0</td>
</tr>
<tr>
<td>Flood Water</td>
<td>9.6</td>
<td>8.9</td>
<td>2.0</td>
<td>26.7</td>
<td>24.7</td>
</tr>
</tbody>
</table>
Figure 3.3: Comparisons of dissolved Si in soil pore water to that in irrigation flood water in 97 fields sampled during mid-tillering of rice.

The relationship between soil pH (1:2) and Si in straw was more evident \((r=0.4198)\) than between pH (1:2) and Si in Y-leaves \((r=0.2309)\), though in both instances pH accounted for only a small portion of the variability in tissue Si concentrations. Some of the fields sampled in this survey had become saline due to the use of saline irrigation water and to coastal flooding caused by hurricanes. The electrical conductivity (EC) of flood water ranged from 51 to 1245 µS cm\(^{-1}\), and no relationships were evident among EC and the uptake of Si by rice or the amount of Si dissolved in soil pore water or irrigation flood water.
Figure 3.4: Linear relationship between air dry 1:2 soil pH and Si concentrations at mid-tiller (Y-leaf) and harvest (straw)

Discussion

Low early season uptake occurred in nearly all rice fields included in this study, though by harvest, only 36% of the fields contained less than 50 g Si kg⁻¹, a level commonly accepted as indicating sufficient Si for optimum production. These observations suggest that if low Si uptake is adversely affecting rice production in Louisiana, it is most likely due to increased susceptibility to early season diseases, insects and abiotic factors commonly associated with restricted Si uptake.
Low Si uptake has been shown to increase the susceptibility of rice to blast (*Magnaporthe grisea* (Hebert) Barr), leaf blight (*Xanthomonas oryzae pv. oryzae* (Ishiyama) Swings et al.), brown spot (*Cochliobolus miyabeanus* (Ito and Kuribayashi in Ito) Drechs ex Dastur), stem rot (*Magnaporthe salvinii* Catt.), scald (*Monographella albescens* Theum), and grain discoloration (Datanoff et al., 1997; Epstein, 1999; Kobayashi et al., 2001; Massey and Hartley, 2006; Mathai et al., 1977; Rafi et al. 1997; Rodrigues et al., 2001; Savant et al. 1997; Volk et al., 1958; Webster and Gunnell, 1992; Winslow, 1992). The specific mechanisms responsible for Si ability to increase disease tolerance are not fully understood, though thickening of the Si layer in the cuticle and improved stomata control have been suggested as contributing factor (Okuda and Takahashi, 1961; Yoshida, 1965). Adequate Si uptake also reduces the susceptibility of plants to chewing insects such as stem borer (*Chilo suppressalis* Walker), possibly by rendering plant tissue less digestible and by causing greater damage to the mandibles of feeding insects (Massey and Hartley, 2006). Enhanced Si uptake can also increase the tolerance of rice to excessive soil iron, manganese, and aluminum (Ma and Takahashi, 2002), suggesting that inadequate Si uptake may contribute to the ‘localized decline’, a devastating early season disorder that occurs in SW Louisiana where plants assimilate excessive amounts of iron and aluminum.

Ma and Takahashi (2002) convincingly argue that adequate Si uptake can directly increase rice yields through more efficient transpiration and by causing more erect, stronger plants that capture light more efficiently and that resist lodging. The complex relationships among tissue Si levels and the susceptibility of rice to biotic and abiotic stresses, however, complicate the establishment of precise critical levels of Si deficiency and sufficiency. Dobermann and Fairhurst (2000) suggested a critical value of 50 g Si kg⁻¹ for Y-leaves during tillering and a
similar value for mature rice straw with 80-100 g kg^{-1} as the optimum level. The basis for these widely used recommendations is not discussed. DeDatta (1981) also cites 50 g Si kg^{-1} as the critical concentration of Si for straw collected at maturity based on the work of Tanaka and Yoshida (1970). After their review of extensive field studies in Asia, Lian (1976) concluded no significant increase in yield occurred when mature straw contained >61 g Si kg^{-1} when grown in Japan and Korea and >51 g Si kg^{-1} when grown in Taiwan. India rice varieties growing in tropical regions of Sri Lanka and India appear to respond to Si fertilization at straw concentrations of <37 g Si kg^{-1}. This latter value is similar to value of 34 g Si kg^{-1} established by Korndorfer et al. (2001) as the economic response to Si fertilization in the Everglades Agricultural Area of Florida for crops grown on sandy or muck soils. Studies to establish early or late season critical Si values for other rice growing regions of the US have not been reported, though the results of this survey suggest that research to assess the influence on Si uptake of rice, especially early season uptake, are merited even in areas where rice is grown on deep, mineral soils containing appreciable amounts of 2:1 clay minerals.

Assuming a value of 50 g Si kg^{-1} in mature rice straw reflects Si sufficiency in Louisiana, Si uptake was adequate by harvest in nearly all parishes with the exception of those in the extreme southwestern portion of the state where more than 60% of the fields surveyed failed to meet this criterion. It may be more than coincidental that declining yields and rising production costs have resulted in decreasing acreage in this area. Certainly, select fields in southwestern Louisiana are suitable candidates for assessing the potential benefits of calcium silicate fertilization on rice productivity.
Between mid-tillering and harvest, tissue concentrations increased an average of 79% while standing biomass increased approximately four times, indicating a increase of more than six times in the amount of Si assimilated into standing biomass. The low early season uptake observed may have been due to reduced Si availability or reduced capacity of young plants to assimilate Si. Most likely, low early season uptake is the result of a combination of both.

Growth chamber studies comparing the effects of low (4 °C) and high (25 °C) temperatures showed that low temperatures substantially suppressed assimilation of Si by rice and corn (Zea mays) as did chemical inhibitors of metabolism (Liang et al., 2006). Increasing solution concentrations of Si, however, increased Si uptake even at low temperatures, suggesting that uptake is a combination of both metabolic rate and Si availability. Temperatures were not monitored in this study, but the influence of variable early season temperatures may account for the lack of a discernable relationship between Si concentrations at mid-tiller and harvest, or between tissue Si concentrations and those in flood water or soil pore water. It is also possible that marked increase in Si uptake between tillering and harvest observed in the more northern rice growing regions of Louisiana was due to lower soil, irrigation water and ambient temperatures.

Si is the second most abundant element in mineral soils, though the solubility of Si in clays and in the various other forms present is not well understood (Faure, 1991). In Japan and other Asian countries where rice has been produced on the same land for hundreds, and possibly thousands of years, it is generally assumed that crop removal has depleted soil reserves of readily soluble Si and routine fertilization with slag or other silicate is practiced (Ohkawa, 1936-1942; Ishibashi, 1936-1939; Hashimoto et al., 1948; Mitsui et al., 1948; Ma and Takahashi, 2002). Rice has been
produced in some fields in SW Louisiana for more than 150 years, and it is possible that reserves of more labile silicates have been depleted as well. Harvesting 5500 kg ha⁻¹ of rough rice containing 50 g kg⁻¹ Si results in the removal of 275 kg of Si. Additional Si losses occur due to the discharge of flood water. In previous years, when water-leveling was widely practiced, the discharge of sediment-laden flood water undoubtedly led to substantial losses of fine clays and dissolved Si.

Assessing the amounts of potentially available Si in soils remains problematic. While a number of soil tests to assess Si availability have been proposed, none has found wide acceptance (Haysom and Chapman, 1975; Imaizumi and Yoshida, 1958; Kato and Sumida, 2000; Kitada et al. 1992; Ma and Takahashi, 2002; Matichenkov et al., 2000; Mizuochi et al., 1996; Nonaka and Takashashi, 1990; Savant et al., 1997; Sumida and Ohyama, 1988; Sumida, 1991; Takashashi and Nonaka, 1986; Wang et al., 2004). Given the interdependence of the many factors influencing Si uptake, this is not surprising. The poor correlations shown here (Fig. 3.2 and 3.3) between dissolved Si in flood or soil pore water and tissue concentrations suggest that development of a reliable soil test will be challenging, though continued efforts are merited. At present, tissue analyses appear to be the most dependable means of assessing Si status. Even so, without supporting research correlating Si status with yields in specific rice growing areas, it is difficult to estimate the economic impact of Si or predict the benefits of Si fertilization.

Conclusions

Comparison of Si tissue concentrations measured in this survey with critical values established for other rice-growing areas indicate that Si uptake is adequate by harvest in most rice-growing areas of Louisiana. On the basis of these data, it is unlikely that Si fertilization would benefit
rice production with the possible exception of extreme SW Louisiana where tissue Si in harvested straw was less than 50 mg kg\(^{-1}\) in more than 60% of the fields surveyed. Tissue concentrations at mid-tillering were consistently less than 50 mg kg\(^{-1}\) level used to indicate adequacy in some rice-producing regions. The influence of low early season Si uptake on the severity or occurrence of common early season disorders in rice merits further investigation.

References


Vita

Joseph Eugene Kraska was born in March 1983, in Bay City, Michigan. He attended Louisiana State University at Baton Rouge, Louisiana, where he received his Bachelor of Science degree in agronomy in 2005 with a minor in agricultural business. While an undergraduate, Joseph worked for the Dr. Gary Breitenbeck as a student worker on a wide range of projects. After graduation, Joseph worked one year as a research associate under Dr. Breitenbeck working on nutritional disorders in rice as well as salt impacts from hurricane storm surges. In January of 2007 Joseph began working on his Masters of Science under Dr. Breitenbeck with a focus on developing a method for silicon analysis in plant tissue and surveying the silicon status of rice grown in Louisiana.