Autoclave-Induced Digestion for the Colorimetric Determination of Silicon in Rice Straw†

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Low Si content of rice (*Oryza sativa* L.) straw is known to be associated with poor rice grain yields, and the need for Si fertilization can be assessed on the basis of the Si content of rice straw. Current methodologies for determining Si in plant tissue are tedious and slow and/or involve cumbersome safety precautions. A new method [autoclave-induced digestion (AID)] has been developed. Plant tissue is digested with H_2O_2 and NaOH in an autoclave, and Si is determined by standard colorimetric techniques that can be automated. For rice straw ranging in Si content from <1 to >5%, the AID method was linearly correlated (r = 0.999) with Si determination by NaOH fusion, with an intercept near zero and a slope near 1.

INTRODUCTION

Most plants accumulate Si during their growth, and some, such as rice (*Oryza sativa* L.), accumulate considerable quantities of this element. While Si generally is not considered an element "essential" for plant growth, i.e., required for normal growth and reproduction, many workers have shown that Si improves rice growth. For example, both Yoshida et al. (1959) and Mitsui and Takatoh (1963) have shown that rice plants low in Si developed necrosis in their leaves and were retarded in growth. In pot studies, grain yield has been shown to be lower in the absence of Si (Okuda et al., 1965). More recently, Snyder et al. (1986) have shown that rice grown in organic soils produces higher grain yields when fertilized with calcium silicate slag, which increases the Si content of the plant. Yields of certain other Gramineae species have been shown to be substantially decreased in the absence of Si (Vlamis and Williams, 1967). Thus, it has been concluded that although Si is not indispensable for vegetative growth of many plants, it appears necessary for the healthy development of plants such as rice and certain other grasses (Lewin and Reimann, 1969).

Plant tissue Si concentration has been used to indicate the presence and extent of Si deficiency and to develop methods for fertilizing with Si (Snyder et al., 1986; Elawad and Green, 1979). Current analytical methodologies for the determination of plant tissue Si include gravimetry (Yoshida et al., 1976), high-temperature fusion followed by generation of a silicomolybdous chromophore and subsequent colorimetry (Kilmer, 1965), and hydrofluoric acid solubilization of plant material or ash followed by atomic absorption spectrophotometry or colorimetry (Novozamsky and Houba, 1984; Vorm, 1987).

Gravimetry, in which Si is freed from the tissue matrix and weighed as SiO_2, requires considerable time for the oxidation of organic matter using potentially hazardous oxidizing acids or dry ashing. Gravimetry requires time-consuming and tedious weighings and filtration. The methods involving fusion are both time and labor intensive, require potentially hazardous manipulation of molten flux, and are subject to losses by spattering during the melting process. Hydrofluoric acid solubilization requires the use of hazardous reagents and special modifications of the atomic absorption spectrophotometer.

The autoclave-induced digestion (AID) method described herein is more rapid than previous methods. It is adaptable to relatively high volume routine analysis of straw samples and involves fairly low cost equipment and a minimum of hazardous chemicals.

MATERIALS AND METHODS

Six rice straw samples representing a range of Si concentration were ground in a Wiley mill to pass a 20-mesh screen, dried at 60 °C for 48 h, and analyzed in triplicate by the two methods described below.

1. **Na Fusion.** Nickel crucibles containing 100 mg of ground plant tissue were covered and placed in a cold muffle furnace. The temperature was increased to 300 °C and maintained for 30 min and then increased to 550 °C and maintained for an additional 30 min. After cooling, 4.5 g of 50% NaOH solution was added to each of the crucibles, after which they were placed partially covered on a hot plate and evaporated to dryness. The samples were slowly heated over a Meeker burner to cherry red and maintained for 10 min. During evaporation and fusion, care was exercised to avoid losses due to spattering. Approximately 35 mL of demineralized water (DM) was added to the cooled melt, which was allowed to stand overnight. Samples then were brought to 50 mL and transferred to 0.1 M NaOH rinsed polyethylene bottles. Dilutions of reagent grade SiO_2, fused as described above, served as calibrants for tissue samples digested by NaOH fusion and by the AID method.

2. **AID.** Samples of plant tissue weighing 100 mg were wetted with 2 mL of 50% H_2O_2 in 100-mL polyethylene tubes previously rinsed with 0.1 M NaOH and DM. To each tube was added 4.5 g of 50% NaOH at ambient temperature, and each tube was gently vortexed. The tubes were individually covered with loose-fitting plastic caps. The rack of tubes was placed in an autoclave at 138 kPa for 1 h. After atmospheric pressure was reached, the tubes were removed and the contents brought to 50 mL with DM.

Silicon in dilutions of samples processed by the two methods was determined colorimetrically by the following procedure, which is a modification of the colorimetric procedure described by Horwitz (1970): To each sample were added with mixing 35 mL of 20% acetic acid, 10 mL of ammonium molybdate solution (54 g/L, pH 7.0), 5 mL of 20% tartaric acid, 1 mL of reducing solution (described below), and 20% acetic acid sufficient to bring the volume to 50 mL. Five minutes elapsed between the addition of the ammonium molybdate and the tartaric acid. The reducing solution was made by combining solution A (2 g of Na_2SO_3 in 25

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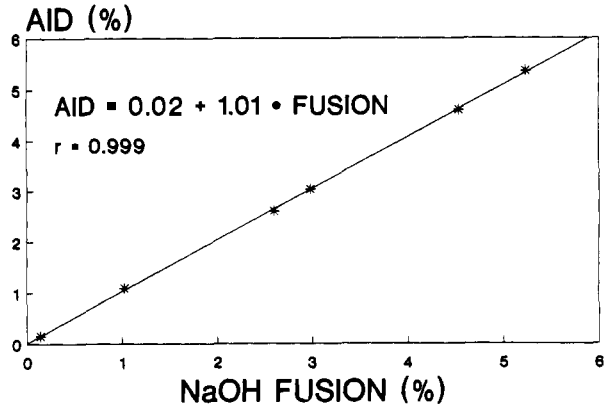

Figure 1. Relationship between Si determination in rice straw by the autoclave induced digestion (AID) method and the NaOH fusion method.

mL of DM plus 0.4 g of 1-amino-2-naphthol-4-sulfonic acid) and solution B (25 g of $NaHSO_3$ dissolved in about 200 mL of DM) and diluting to 250 mL. Following the addition of all the reagents, the samples were mixed well and allowed to stand for 30 min. Plasticware rinsed with 0.1 M NaOH was used throughout. The color was read at 650 nm in a Bausch and Lomb Spec21 with a 1 cm path length. Results were statistically analyzed by SAS (1985) ANOVA, CORR, and GLM procedures.

RESULTS AND DISCUSSION

Si determined by the AID method and Si determined by NaOH fusion were found to be linearly correlated ($p < 0.0001$), with an intercept near zero and a slope of 1.01 (Figure 1). The correlation coefficient (r) was 0.999. The coefficients of variation (CV) for the NaOH fusion and AID methods were 3.6 and 2.3%, respectively. A CV of approximately 3% has been reported elsewhere (Kilmer, 1965) for the NaOH fusion method. The lower CV for the AID method may be attributable to reduced sample handling and processing by this method, as compared to the NaOH fusion technique.

The rationale of the AID method is that at elevated temperatures (126 °C) NaOH hydrolyzes and dissolves the plant tissue organic matrix which subsequently undergoes oxidation by the H_2O_2. The autoclave pressure causes the oxidant to remain within the digestion matrix, augmenting oxidation of the organic matter. This results in (i) partial clarification of the digestate and (ii) the release of Si from the matrix which allows dissolution by NaOH to proceed to completion.

The NaOH fusion method requires approximately 24 h between sample weighing and colorimetric determination, and close attention to individual samples is required during the NaOH drying and fusion process. In our laboratory, only 20 samples can be processed comfortably in a single batch by one technician. A standard gravimetric procedure for determining Si in rice straw and a more rapid gravimetric method have been found to require approximately 17 and 7 h, respectively, to process 30 samples (Elliott et al., 1988). The AID method, by comparison, requires less than 2 h between weighing and colorimetry, and a technician can process 40 samples in a batch. Little attention to individual samples is involved. Furthermore, sample handling by the AID method is conducted at or near room temperature, whereas manipulation of molten NaOH is required by the NaOH fusion method, making the latter inherently more dangerous.

In the AID method, foaming was observed when NaOH was added to the H_2O_2-wetted tissue, which indicates consumption of the oxidant. However, it was determined that delaying placement of the digestion mix into the autoclave for up to 30 min did not affect the Si analysis (data not presented). The digestates generally contained some precipitates, probably insoluble metal hydroxides and some organic matter, so the method is not proposed for elements other than Si. In our laboratory, sample processing by the AID method is sufficiently rapid to warrant the use of automated colorimetric analysis (e.g., Technicon AutoAnalyzer II) for Si determination of the digestates.

The AID method appears to have usefulness for rapid determination of Si in rice straw and therefore has potential value for augmenting Si nutrition research involving rice and possibly other grasses.

LITERATURE CITED

Elawad, S. H.; Green, V. E., Jr. Silicon and the rice plant environment: a review of recent research. *Riso* **1979**, *28*, 235–253.

Elliott, C. L.; Snyder, G. H.; Jones, D. B. Rapid gravimetric determination of Si in rice straw. *Commun. Soil Sci. Plant Anal.* **1988**, *19*, 1543–1550.

Horwitz, W. Silicon. In *Official Methods of Analysis*; Association of Official Analytical Chemists: Washington, DC, 1970; p 7.

Kilmer, V. J. Silicon. In *Methods Of Soil Analysis*; American Society of Agronomy: Madison, WI, 1965; Part 2, pp 959–962.

Lewin, J.; Reimann, B. E. F. Silicon and plant growth. *Annu. Rev. Plant Physiol.* **1969**, *20*, 289–304.

Mitsui, S.; Takatoh, H. Nutritional study of silicon in graminaceous crops. *Soil Sci. Plant Nutr. (Tokyo)* **1963**, *9*, 49–58.

Novozamsky, R. van Eck; Houba, V. J. G. A rapid determination of silicon in plant material. *Commun. Soil Sci. Plant Anal.* **1984**, *15*, 205–211.

Okuda, A.; Takahashi, E. The role of silicon. In *The Mineral Nutrition of the Rice Plant*; Johns Hopkins Press: Baltimore, MD, 1965; pp 123–146.

SAS Institute. *SAS/STAT Guide for Personal Computers*; SAS Institute: Cary, NC, 1985; Version 6.

Snyder, G. H.; Jones, D. B.; Gascho, G. J. Silicon fertilization of rice on Everglades Histosols. *Soil Sci. Soc. Am. J.* **1986**, *50*, 1259–1263.

Vlamis, J.; Williams, D. E. Manganese and silicon interaction in Gramineae. *Plant Soil* **1967**, *27*, 131–139.

Vorm, P. D. J. van der. Dry ashing of plant material and dissolution of the ash in HF for the colorimetric determination of silicon. *Commun. Soil Sci. Plant Anal.* **1987**, *18*, 1181–1189.

Yoshida, S.; Onishi, Y.; Kitagishi, K. The chemical nature of silicon in rice plant. *Soil Plant Food* **1959**, *5*, 23–27.

Yoshida, S.; Forno, D. A.; Cock, J. H.; Gomez, K. A. In *Laboratory Manual for Physiological Studies of Rice*, 3rd ed.; International Rice Research Institute: Los Banos, Laguna, The Philippines, 1976; pp 17–22.

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