Rapid Determination of Silicon Content in Rice

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Abstract: A method for rapid determination of silicon content in rice was introduced. The reliability of this method was verified by using a recombinant inbred line (RIL) population of rice cross Zhenshan 97B / Milyang 46. Two hundred and forty-nine RILs were transplanted in two replications. Simple correlation coefficients on the silicon content in the hull, flag leaf and stem in rice between duplicate samples of 498 rice materials were 0.97954, 0.97026 and 0.98848, respectively. Ten representative samples were selected for measurement using the high-temperature alkaline fusion method. Simple correlation coefficient between the silicon contents determined by the high-temperature alkaline fusion method and by the present method is 0.9993.

Key words: rice; silicon content; determination method

Silicon (Si) is the second most abundant constituent in soil. It constitutes 28% of the total weight in soil, which is only lower than oxygen that is 47% [1]. Rice is a model plant for the genomic research of monocot species and a staple for more than half of the global population. Rice is also a typical silicophilous plant [2-4], owning the ability to absorb and accumulate silicon metabolically while many upland crop plants seem to lack such ability [5]. Numerous experiments have shown that Si deposition in the plant tissues can improve yield, lodging resistance, and biotic and abiotic stress tolerance of rice plants. Silicon has long been recognized as a beneficial element for rice, although it has not been proved as an essential element [6, 7].

Methods for the determination of silicon content have been improving with the advance of Si research in the world. Methods for silicon determination in plant tissue include gravimetry [8], high-temperature alkaline fusion colorimetry [9-12], hydrofluoric acid dissolution spectrophotometry or colorimetry [13, 14] and alkaline digested oxidation spectrophotometry [15]. However, these methods are too complicated and laborious to be applied for large amount of samples, appearing as a limiting factor in the physiological, biochemical and genetical studies of rice silicon. In this paper, a simple method for rapid determination of the silicon content in rice was introduced.

MATERIALS AND METHODS

Materials

In 2003, 249 recombinant inbred lines (RILs) derived from the rice cross Zhenshan 97B/Milyang 46 were transplanted in two replications in the paddy field of China National Rice Research Institute, Hangzhou, China. From each of the 498 lines, flag leaves, stems and grains were collected at maturity, respectively, and used as samples for the determination of silicon content.

Protocol for the measurement of silicon content

Preparation of standard solution

1) Put 1 g ultra pure SiO₂ (99.99%) in a muffle furnace. Heat slowly to 1000°C and keep for 1 h at 1000°C. Cool to room temperature (about 3 h).

2) Weigh 0.1000 g pretreated SiO₂ and put into a nickel crucible. Add 2 g Na₂CO₃. Heat slowly to 1000°C such that a translucent melt is formed. Cool to room temperature.

3) Take the nickel crucible out from the muffle furnace. Add 5 mL boiling ddH₂O into the nickel crucible, and transfer the melt from the nickel crucible completely into a 300 mL plastic cup.

4) Add 150 mL ddH₂O. Stir until the chemical is completely dissolved. Transfer to a 1000 mL volumetric flask. Adjust to room temperature.

5) Transfer the solution to a 1000 mL plastic bottle. Tightly stoppered and stored at room temperature. The solution contains 0.1 mg/mL SiO₂.
Setting up standard curve

1) Transfer 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 mL Si standard solution to a 50 mL volumetric flask, respectively.

2) Add 30 mL acetic acid (20%) and 10 mL ammonium molybdate solution (54 g/L, pH 7.0). Shake up to mix thoroughly. Keep for 5 min and then add 5 mL 20% tartaric acid and 1 mL reducing solution. Adjust to 50 mL with 20% acetic acid.

The reducing solution was made by mixing solution A (2 g of Na₂SO₃ and 0.4 g of 1-amino-2-naphthol-4-sulfonic acid in 25 mL of ddH₂O) and solution B (25 g of NaHSO₃ in 200 mL of ddH₂O), and adjusted to 250 mL with ddH₂O. Stored in a tightly stoppered plastic bottle in the dark.

3) Thirty minutes later, measure the absorbance at 650 nm.

Sample preparation

Flag leaves, stems and hulls collected from each line in either replication were dried in oven at 70°C for 7 d. Each sample was ground and sifted through a 60-mesh sieve. They were then dried at 60°C for 48 h. Two 100-mg samples were weighed from each of 498 hull, flag leaf and stem powders.

Sample pretreatment

Put each 100 mg sample into a 100 mL polyethylene tube. Add 3 mL 50% NaOH and cover it with a loose-fitting plastic cap. Gently vortex, then autoclave at 121°C for 20 min. Transfer to volumetric flask and adjust to 50 mL with ddH₂O.

Sample determination

Transfer 1 mL sample solution to a 50 mL volumetric flask. Add 30 mL 20% acetic acid and 10 mL ammonium molybdate solution (54 g/L, pH 7.0). Shake up to mix thoroughly. Keep for 5 min and then immediately add 5 mL 20% tartaric acid and 1 mL reducing solution. Adjust to 50 mL with 20% acetic acid. Thirty minutes later, measure the absorbance at 650 nm.

RESULTS AND ANALYSIS

Standard curve

Linear regression equation for the determination of the silicon content was acquired as: \( y = 0.0824x + 0.0038 \), \( R^2 = 0.9998 \) (Fig. 1). Where, \( y \) is OD value and \( x \) is silicon content in the solution used for setting up standard curve.

Determination of the silicon contents in Zhenshan 97B/Milyang 46 RIL population

Silicon contents in the hull, flag leaf and stem in the 498 lines showed highly significant positive correlation (\( P < 0.01 \)) between the duplicate samples. Linear correlation coefficients were 0.97954, 0.97026 and 0.98848 for the silicon contents in the hull, flag leaf and stem, respectively (Fig. 2).

In comparison with results obtained by using high-temperature alkaline fusion method

The high-temperature fusion method is commonly applied as a reference method to evaluate the accuracy of other methods measuring silicon content \([9-12]\). Ten samples representing the variation scope of the silicon content in Zhenshan 97B/Milyang 46 RIL population were determined with the high-temperature fusion method. Results from the two
methods were significantly correlated ($P<0.01$, $r = 0.9993$) (Fig. 3). This indicated that the present method is reliable for the determination of silicon content in rice.

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**REFERENCES**