

## Measuring K<sup>+</sup> in Rice Basal Stem Sap with a Cardy Meter

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### Abstract

A rice (*Oryza sativa*) experiment was conducted to determine the accuracy of a fast tissue testing method using a Horiba Cardy K<sup>+</sup> meter. Rice was field-grown in three soil potassium regimes. At three early reproductive growth stages (R0, R1, and R2), stem samples from the basal six inches of rice plants were collected and analyzed for K content. Basal stem samples were tested with extracted sap analyzed by a Cardy K<sup>+</sup> meter and digested stem tissue analyzed with an Atomic Absorption Flame Spectrophotometer (AAS). Regression correlations between basal stem sap K and total K ranged from  $r^2 = 0.57$  to 0.89 for the three growth stages. Both methods of measuring lower stem K content at the R1 growth stage were equivalent in their ability to predict grain yield response to potassium fertilization.

### Introduction



Fig. 1. Potassium deficiency in rice at mid-season.

Potassium deficiency in rice can reduce grain yields and increase lodging. Visual symptoms of K deficiency in rice first appear in older leaves (Fig. 1). These symptoms include a yellowing of leaf tips, decreased disease resistance, and reduced yields (3,19). Kono and Takahashi (11) report that K deficiency decreases the accumulation of starch in the rice culm. This suggests a close correlation between K content of the lower culm and the breaking strength of the stalk.

Increased stalk strength and decreased lodging are associated with proper K nutrition (5,8).

Tissue K levels in rice are affected by the stage of growth. The uptake of K by rice plants closely parallels the accumulation of dry matter from emergence until anthesis. Estimates of critical levels in rice leaf tissue are a function of plant growth and developmental stage (16). Adequate K in rice tissue during the late vegetative and early reproductive stages is important for producing optimum yields. During these growth stages, rice plants rapidly accumulate K in leaf and stem tissue (9,16). After seed development begins, K uptake slows dramatically. Transfer of K from lower leaves largely account for K accumulation in the hull and seed.

Rice farmers normally apply K fertilizer before planting or before establishing a permanent flood at first tiller growth stage. However, visual K deficiency symptoms in rice plants or tissue testing sometimes indicate that K fertilizer is needed at mid-season. In a 3-way split fertilizer N program, aerial K applications can be broadcast into floodwater in conjunction with scheduled mid-season urea-N applications. On a K-deficient soil, Stevens et al. (18) found that rice grain yields for 'Bengal' cultivar rice were significantly increased by mid-season K applications. Plant tissue analysis from upper mature leaves indicated that K levels were below the 1% K sufficiency level (13). Visual K deficiency symptoms were not observed. These results indicate that rice grain yields can be increased in K-deficient rice if the problem is diagnosed in a timely manner.

Crop management specialists need a fast, accurate method of measuring in-season rice K tissue levels. Plant tissue analysis for K is currently available with traditional digestion procedures from many university and independent laboratories. A common problem of traditional digestion laboratory analysis is the time lag between sample collection and the potential use of the results by crop management specialists. This time lag may be a few days to a few weeks. Sampling and conducting the tissue analysis the same day can eliminate this time lag. One method of same-day analyses is measuring  $K^+$  in plant sap with a Cardy portable electrode-based ion meter (Horiba, Ltd., Kyoto, Japan). The Cardy  $NO_3^-$ ,  $Na^+$ , and  $K^+$  ion meters offer crop management specialists the ability to quickly evaluate tissue nutrient levels in the field. Cardy meters have been widely used in vegetable production, with  $K^+$  thresholds established for several crops (12). Despite the acceptance and utilization of Cardy meters in vegetable production, very little research has been conducted to determine the utility of these meters in row crop production. During the past decade several researchers (2,7,10,17) have investigated the utility of Cardy meters as diagnostic tools in cotton but their utility for rice is not known.

A review of the available literature indicates that Cardy meters have not been previously evaluated for use in rice production. The objective of this study was to evaluate the accuracy of measuring the K status of rice plants using a Cardy meter at three early reproductive growth stages as compared to a traditional laboratory method.

### Experimental Design

A rice study was conducted on a field at the Missouri Rice Research Farm (36°N, 90°W) in Dunklin County, Missouri in 2003. Rice was planted on a Crowley silt loam soil (fine, montmorillonitic, thermic Typic Albaqualf). The soil has a silt loam eluvial horizon that overlies a thick silty clay loam argillic horizon. This is a typical soil for producing drill-seeded rice in southeast Missouri (6). Rice was cultivated in 2002 and the field was conventionally tilled prior to seeding rice in 2003. Soil samples collected before planting in 2003 showed the soil pH of the surface horizon was 6.2. There was no record of potassium fertilizer ever being applied on the site. The soil ammonium acetate extractable K level in the test area was 55 lb/acre. The University of Missouri soil test recommendation for K fertilizer using an 8-year buildup program was  $K_2O$  at 50 lb/acre (1). No P or Zn fertilizer applications were recommended. A randomized complete block design with four replications was used. Before planting, KCl fertilizer treatments were applied by hand at rates of  $K_2O$  of 0, 50, and 200 lb/acre. Fertilizer was incorporated with tillage to a 3-inch soil depth. Rice, cultivar 'Cocodrie,' was drill-seeded using standard cultural practices including N fertilization. Grain yields were collected from the center of each plot and adjusted to 13% seed moisture content.

### Rice Tissue Collection

Whole plants were removed from approximately one foot of a single drill row in each plot at R0 (stem development), R1 (inter-node development), and R2 (beginning elongation) growth stages (4). The above-ground portion of this sample was separated from the roots using a garden pruning shear. The remaining portion of the lower stem was washed of soil and algae using tap

water. The basal six inches of the plants were separated from the leaves and retained for analysis. The upper mature leaves were also retained for analysis. The stems sections were blotted dry with paper towels. To avoid sample bias, half the stems were then placed oriented up and the other half oriented down. The basal stems were then cut into two 3-inch pieces for tissue analysis.

### Rice Tissue Analysis

Upper mature (flag) leaves and one-half of the collected 3-inch basal stem samples were tested with an established laboratory tissue testing procedure using an AAS (13). These samples were dried at 212°F, ground, digested using  $H_2SO_4$  and  $H_2O_2$ , and analyzed for K content using a PerkinElmer AAS (Shelton, CT). We also tested sap from basal stems by a procedure modified from instructions supplied by the Cardy meter manufacturer. The remaining 3-inch basal stem samples were cut again into smaller 0.5-inch pieces (Fig. 2). These pieces were frozen overnight and sap was extracted using a sap press (Fig. 3). The sap was collected in 10-ml glass beakers. Two to three drops of the extracted sap was then poured on the electrode surface of the Cardy  $K^+$  meter to analyze for  $K^+$  content (Fig. 4). The electrode surface was washed between determinations with deionized water. Three analyses were made from each beaker of sap and the results were averaged. Results of these two basal analyses and flag leaf analyses were then compared.



Fig. 2. Basal rice stems are cut into one half inch pieces and placed on a sap press to extract sap.



Fig. 3. Rice sap is extracted from basal stems using a sap press.



Fig. 4. Rice sap is poured onto a Cardy meter sensor for  $K^+$  determination.

Statistical analyses of the data were preformed with SAS (14) using General Linear Modeling procedures. Fisher's Protected Least Significant Difference (LSD) was calculated at the 0.05 probability level for making treatment mean comparisons. Regression and correlation analyses were performed in accordance with procedures outlined by the SAS Institute (15). Statistical analysis for potassium fertilizer effects on yield, basal stem tissue K, upper leaf tissue K, and basal stem sap K were performed using SAS mixed model procedures. The Mixed Model procedure provides Type III  $F$  values, but it does not provide mean square values for each element within the analysis or the error terms. Mean separation was evaluated through a series of protected pair-wise

contrasts among all treatments. Regression analysis was performed to study the relationship between rice yields and measured K with different methods.

### Extracting Rice Sap

The physiology of rice plants presents a unique challenge for extracting leaf and stem sap. Hodges and Baker (7) found that a Cardy K<sup>+</sup> meter could be successfully used to monitor K levels in cotton plants. Routine lab analysis (2% acetic acid extraction with Inductive Coupled Plasma) of cotton petioles and the Cardy K<sup>+</sup> meter measurements from cotton petiole sap were strongly correlated ( $r^2 = 0.88$ ). Intuitively, obtaining sap samples from an aquatic plant like rice would appear to be easy. We found that extracting sap from the rice leaves was not possible. Our attempts to obtain sap from the basal stem in the late vegetative stages also were not successful. Before inter-node elongation, there was simply not enough stem tissue available to extract the sap. After reproduction began, extraction of a small amount of sap from each rice basal stem was possible. Burmester and Mullins (2) encountered a similar problem with late season cotton petiole samples. The correlations they obtained were lower than Hodges and Baker (7), diminished as the season progressed, and were attributed to the difficulty in obtaining sap extract as the plant matured. Burmester and Mullins (2) concluded that the best use for the Cardy meters would be early season before the petioles hardened.

To increase the amount of sap extracted from each basal stem at early reproductive growth stages, the stems were frozen overnight to rupture the cell walls. Kenty et al. (10) suggested that sap release from cotton petioles was enhanced by briefly freezing the petioles. With rice stems it was necessary to freeze the stems overnight to rupture the cell walls within the stems and allow more sap to be extracted. However, it was necessary to extract from a larger stem sample for rice as compared to cotton petioles to obtain sufficient sap for analysis.

### Cardy Meter versus Established Procedure

Algae residue was present on many of the rice basal stem samples. To reduce the possibility of algal contamination, stems were washed with tap water and blotted dry before sap was extracted. Cardy meter determinations were variable when algae was not removed or the wash water was not dried before sap extraction (*data not shown*). However, cleaning stems resulted in strong linear relationships between K<sup>+</sup> values from the Cardy meter and the AAS procedure (Figs. 5, 6, and 7). The highest regression coefficient between rice K tissue testing methods was found from measurements at the R1 rice growth stage (Fig. 6).

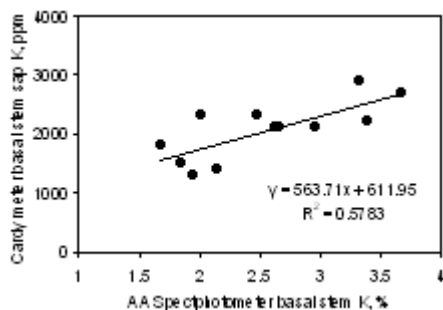


Fig. 5. Relationship between K<sup>+</sup> values from the Candy meter sap readings and total K from the Atomic Absorption Spectrophotometer total K in basal stems at the R0 growth stage in 2003.

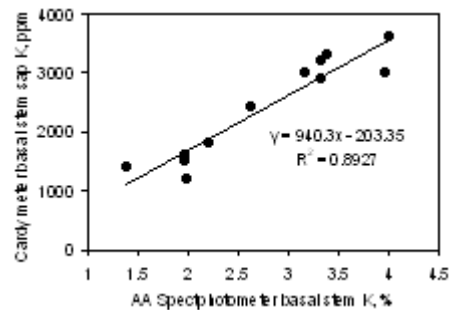


Fig. 6. Relationship between K<sup>+</sup> values from the Candy meter sap readings and total K from the Atomic Absorption Spectrophotometer total K in basal stems at the R1 growth stage in 2003.

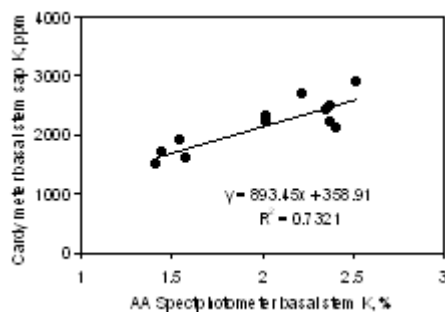


Fig. 7. Relationship between K<sup>+</sup> values from the Candy meter sap readings and total K from the Atomic Absorption Spectrophotometer total K in basal stems at the R2 growth stage in 2003.

Potassium fertilizer applications significantly increased rice yields in this experiment (Table 1). At R1 growth stage, both basal stem tissue test methods (Candy and AAS) detected significant differences in K levels between the 0 and 200 lb K<sub>2</sub>O/acre treatments. Numerical, but not statistically significant, differences were found in the upper leaf AAS analyses. Both basal stem methods showed that the 50 lb K<sub>2</sub>O/acre treatment had K levels numerically greater than the untreated check. But the differences were not statistically significant. Correlations between rice yields were compared between the three methods at R0, R1, and R3 (Table 2). We found that tissue testing basal stems with the Candy and AAS at R1 gave similar results ( $R^2 = 0.30$  and  $0.31$ ). The basal stem methods were more highly correlated with yield than the upper leaf method. After R1, the correlations between yield were better with the upper leaf sampling.

Table 1. Effect of pre-plant K fertilizer applications on tissue K measurements from basal rice stems obtained with a Candy K<sup>+</sup> meter, and a traditional laboratory method using Atomic Absorption Spectrophotometer at R1 growth stage and rice grain yields.

Preplant K fertilizer (lb of K <sub>2</sub> O per acre)	Candy K <sup>+</sup> meter (ppm K)	AA Spectrophotometer * (% K)		Rice yield * (bu/acre)
	basal stem sap *	basal stem	upper leaf	
0	1950 b	2.19 b	1.49 a	95 c
50	2000 b	2.48 b	1.80 a	112 b
200	3275 a	3.67 a	1.94 a	120 a

\* Within each column, values followed by the same letter were not significantly different at an  $\alpha$ -level of 0.05.

Table 2. Correlations between rice yields and Cardy K<sup>+</sup> basal stem sap readings and Atomic Absorption Spectrophotometer total K from basal stems and upper leaves at three early reproductive rice growth stages.

Instrument	Plant part	Rice growth stage (R <sup>2</sup> )		
		R0	R1	R2
Cardy K <sup>+</sup> meter	basal stem sap	0.13	0.30	0.12
AA Spectrophotometer	basal stem	0.42	0.31	0.06
AA Spectrophotometer	upper leaf	0.23	0.24	0.29

### Rice K Management with a Cardy Meter

We found that a Cardy K<sup>+</sup> meter may be useful for detecting K deficiency in the early reproductive growth stages of rice. Extracting sap from rice leaves during vegetative growth stages was not possible. The best rice plant part for testing K<sup>+</sup> with the Cardy meter was the basal stem. When basal stems were cleaned of algae residue and frozen overnight, sufficient sap was obtained for testing with a Cardy meter. There was a strong linear relationship between K<sup>+</sup> in basal stem sap with a Cardy meter and digested basal tissue K with an AAS at R1 growth stage.

Hodges and Baker (7) found that Cardy meters were highly sensitive to changes in temperature and sunlight. This problem combined with the necessity to freeze stem samples will limit the usefulness of Cardy meters for making in-field K<sup>+</sup> rice measurements. In our procedure, we froze the stems overnight. It may be possible to freeze them for a shorter period of time to get "same day" results. This was the first reported study using a Cardy K<sup>+</sup> meter to detect K deficiency in rice. Further work is needed to develop critical K levels for specific rice cultivars at reproductive growth stages.

### Acknowledgment

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