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# Simple and Inexpensive Water Extraction Method for Assaying Potassium Concentration in Tobacco Plant Tissue

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*Potassium (K) in plant tissue is not bound to organic compounds and occurs in soluble forms, thus indicating the ease of its extractability. The conventional methods of plant-sample preparation for K determination are often tedious, time-consuming, and/or require chemicals, making the analysis expensive. In this investigation, we propose a water extraction method for assaying K concentration in tobacco leaf tissue and evaluate it for analytical accuracy and precision in comparison to the established methods, namely, triacid digestion, 1 N ammonium acetate ($NH_4OAc$) extraction, and 0.5 N hydrochloric acid (HCl) extraction. The proposed method entails extracting K from 0.5 g finely ground plant tissue (<0.5-mm sieve) with distilled water at a 1:100 ratio (sample weight to water volume, w/v) by shaking for 20 mins and filtering before K measurement by flame photometry. Results with 25 tobacco leaf samples having a wide range in K concentrations showed very close agreement between the values of K determined by the proposed water extraction method and the established methods. The mean K concentration obtained with water extraction method was within 3 to 6% of those measured by established methods. The correlations between the K values obtained by the established methods and the water extraction method were highly significant ($P = 0.01$), and the relationships are best described by linear regression equations with high values of $R^2$ (>0.99). The standard errors (SEs) and coefficient of variation (CV) for K measurements by different methods followed the order water extraction < HCl extraction < triacid digestion < $NH_4OAc$ extraction. The results suggest that the water extraction method is comparable in accuracy and superior in precision to the established methods for K determination. Being simple, rapid, and inexpensive, the water extraction method could be used as an alternative to the most commonly employed standard, triacid digestion, for routine analysis of K in tobacco plant tissue.*

**Keywords** HCl-extractable K, Potassium concentration, plant tissue, triacid digestion, water-extractable K

## Introduction

Potassium (K) is one of the essential nutrient elements for plant growth and development. It plays a critical role in enhancing plant tolerance to abiotic and biotic stresses and improving the quality of produce in crops such as tobacco (*Nicotiana tobaccum* L.). Generally, K is absorbed by flue-cured tobacco in larger amounts than any other nutrient

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(Krishnamurthy, Ramakrishnayya, and Singh 2003; Su et al. 2006). Concentration of K in leaf tissue is the key factor that enhances tobacco leaf quality in terms of improved leaf size, specific leaf weight, leaf color, pliability, and combustibility (Krishnamurthy, Ramakrishnayya, and Singh 2003). Consequently, plant-tissue analysis is routinely done to monitor K status of crops for developing appropriate fertilization strategy and assessing leaf quality.

Analyses of K in plant tissue involve sample preparation to release K and quantification of the amount of K released by using an analytical technique, which in most cases is flame emission spectrometry. Sample preparation methods typically involve wet digestion in triacid [nitric acid ($HNO_3$) + sulfuric acid ($H_2SO_4$) + perchloric acid ($HClO_4$)], diacid ($HNO_3$ + $HClO_4$), or single acid ($HClO_4$) media (Jackson 1973; Alder and Wilcox 1985). Based on a comparative study of wet-digestion methods, Alder and Wilcox (1985) observed that the amount of K recovered from plant tissue was not different among digestion methods for a particular tissue type. In addition, some nondigestion-extraction methods including 1 N ammonium acetate ($NH_4OAc$) and 0.5 N hydrochloric acid (HCl) extractions are also often suggested for determination of K concentration in plant tissues (Sahrawat 1980). The plant K contents obtained by different extraction methods were reported to be in close agreement with those determined by the standard acid digestion techniques for a range of plant samples (Sahrawat 1984; Rao, Rao, and Takkar 1998). Although these digestion and extraction methods of plant sample preparation for K determination are precise and sufficiently accurate, they are often tedious, time-consuming, and/or require chemicals adding to the cost of analyses. It would be advantageous to estimate K in plant tissue using simple and inexpensive methods that can be adopted for routine plant analyses in large numbers.

Potassium remains in soluble forms in the plant and is not bound to organic compounds in plant cells (Marschner 1986), indicating its easy extractability with water. The amount of K released from plant tissue by water is related to tissue K content (Rosolem, Calonego, and Foloni 2005) and depends on plant-tissue resistance in the form of a physical barrier to K release. Greater K liberation would occur with plant-tissue decomposition or with grinding plant material to a fine powder. The objective of this study was to develop a simple inexpensive extraction method using water for determining K concentration in tobacco plant tissue and to compare its accuracy and precision with some established and standard methods.

## Materials and Methods

### *Plant Samples*

Tobacco (*Nicotiana tobaccum* L.) leaf samples from different geographical locations in India, which were submitted to the Central Tobacco Research Institute, Rajahmundry, for routine assessment of leaf quality, were used in this study. A total of 25 tobacco samples representing different production domains with contrasting soil types and management practices were selected. These samples were expected to have a broad range of K concentrations and could be used to compare the water extraction method with other established methods for the determination of plant-tissue K. Additionally, three more leaf samples with relatively low (<1%), medium (1–2%), and high (>2%) K contents were collected in bulk and used to assess the effect of extraction variables on K extracted by water and the precision of different methods. All plant samples were oven dried at 60 °C for 48 h and finely ground to pass through a 0.5-mm sieve prior to analysis.

### *Evaluation of Extraction Variables*

To evaluate the impact of extraction variables on water-extractable K, finely ground and dried subsamples of tobacco leaf (with medium K content) were extracted with distilled water employing a range of sample-to-water (w/v) ratios and shaking periods. The treatments consisted of combinations of four sample-to-water (w/v) ratios (1:60, 1:80, 1:100, and 1:120) and four shaking periods (10, 20, 30, and 60 min) with three replications. Portions of plant samples weighing 0.5 g were placed into Erlenmeyer flasks. These flasks received 30, 40, 50, or 60 mL of distilled water to create the four different sample-to-water ratios. The flasks were shaken on a reciprocating shaker for 10, 20, 30, or 60 min. The contents were filtered through filter paper (Qualigens 615A equivalent to grade 1; Qualigaens, Mumbai, India). Potassium concentration in extracts was determined by flame emission spectrometry using a flame photometer (model 128, Systronics, Mumbai, India). The K concentration in plant tissue was expressed as percentage of oven-dried plant tissue. Based on the results of this study, the protocol for the water extraction method, with the appropriate sample-to-water ratio and shaking period, was proposed and further compared with the established methods for quantification of plant-tissue K concentration.

### *Comparison of Water Extraction Method with Established Methods for K Determination*

Twenty-five tobacco leaf samples were assayed in triplicate for K concentration using an experimental water extraction method and the established digestion/extraction methods. The results of plant-tissue K concentrations obtained with the proposed water extraction method were compared to those obtained by the following three standard methods (Jackson 1973; Sahrawat 1980):

1. Triacid digestion method: Plant samples weighing 0.5 g were transferred to 250-mL conical digestion flasks. Ten mL of triacid mixture of nitric acid, sulfuric acid, and perchloric acid ($HNO_3 + H_2SO_4 + HClO_4$) in the ratio of 10:1:4 (v/v) was added to the flasks. The samples were allowed to stand in the mixed acid solution for 2 h, followed by digestion for 3–4 h on a hot plate at 250 $^{\circ}$C until the digest was clear or colorless. The flasks were allowed to cool and the contents were diluted with distilled water to 100 mL. The digests were further diluted 10 times by taking 10-mL aliquots and increasing the final volumes to 100 mL using distilled water.
2. Ammonium acetate ($NH_4OAc$) extraction method: Finely ground plant samples weighing 0.5 g were taken in 250-mL Erlenmeyer flasks and shaken with 100 mL of 1 N $NH_4OAc$ solution for 2 h on a reciprocating shaker. The extracts were filtered using qualitative filter paper (Qualigens 615A, equivalent to grade 1). The extracts were further diluted 10 times by taking 10-mL aliquots and increasing the final volume to 100 mL using distilled water.
3. HCl extraction method: Finely ground plant samples weighing 0.5 g were shaken with 40 mL of 0.5 N HCl in 250-mL Erlenmeyer flasks for 5 min. The extracts were filtered using qualitative filter paper (Qualigens 615A, equivalent to grade 1). The extracts were further diluted 20 times by taking 5-mL aliquots and increasing the final volume to 100 mL using distilled water.

Plant sample digestions and extractions were carried out in triplicate. The K concentrations in all the digests and extracts were determined using a flame photometer (model 128, Systronics) and expressed as percentage (%) of oven-dried weight.

### *Precision of Different Methods*

To better characterize the precision associated with standard digestion/extraction techniques and the proposed water extraction method, five independent analyses were performed using tobacco leaf samples with both relatively low- and high-K concentrations.

### *Statistical Analysis*

Analysis of variance (ANOVA) technique was used to test significance (at $P = 0.05$) of the effects of analytical variables (plant sample weight to water volume ratio and shaking period) on plant-tissue K concentration. Separation of means for these variables was done using Duncan's multiple-range test (Gomez and Gomez 1984) at the significance level of $P = 0.05$. Descriptive statistical analysis was performed to estimate variability of plant-tissue K across different extraction ratios and shaking periods. Correlations and simple linear regressions were performed to evaluate the relationship between the K concentrations determined by the proposed water extraction method and standard methods. Coefficient of determination ($R^2$) was calculated for measuring the best fit of the lineal models. Range, mean, standard deviation (SD), and coefficients of variation (CVs) were worked out to assess the precision of K-extraction methods.

## Results and Discussion

### *Effects of Water Extraction Variables on Leaf-Tissue K Concentration*

Analysis of variance for the data on water-extractable K concentration in tobacco leaf tissue showed no significant impact of extraction conditions, namely, plant sample weight to water volume ratio and shaking period (Table 1). The mean values of plant-tissue K concentration (1.51–1.60%) obtained by water extraction method did not differ significantly

**Table 1**
Water-extractable K concentration (%) in tobacco leaf tissue as affected by the extraction variables

| | K concentration (%) | | |
|---|---|---|---|
| Extraction variable | Mean | SE | CV (%) |
| Plant sample to water (w/v) ratio | | | |
| 1:60 | 1.52a | ±0.022 | 2.86 |
| 1:80 | 1.60a | ±0.036 | 4.45 |
| 1:100 | 1.53a | ±0.010 | 1.25 |
| 1:120 | 1.59a | ±0.029 | 3.63 |
| Shaking period | | | |
| 10 min | 1.59a | ±0.044 | 2.76 |
| 20 min | 1.54a | ±0.015 | 0.97 |
| 30 min | 1.59a | ±0.079 | 4.97 |
| 60 min | 1.51a | ±0.043 | 2.86 |

*Notes*. Means with same alphabet are not significantly different according to Duncan's multiple-range test at $P = 0.05$. SE, standard error of mean; CV, coefficient of variation.

(at $P = 0.05$) among the sample-to-water (w/v) ratios ranging from 1:60 to 1.120 and the shaking periods varying from 10 to 60 min. However, the test results were more consistent for the extraction ratio of 1:100 and the shaking period of 20 min, as evidenced from the lower values of standard error (SE) and CV associated with these extraction conditions (Table 1). Based on these results, the following protocol for determination of K in tobacco leaf tissue was proposed and employed in studies aimed at evaluating the proposed method versus the established methods of plant K determination.

### *Protocol for the Proposed Water Extraction Method*

Weigh 0.5 g of finely ground (<0.5 mm) and dried (60 °C) plant sample into a 250-mL Erlenmeyer flask. Add 50 mL of distilled water to the flask to get the extraction ratio of 1:100 (sample to water, w/v). Shake the contents of the flask on a reciprocating shaker for 20 min and filter through filter paper (grade 1). Measure the K concentration in filtrate using the flame photometer and express it as percentage (%) of oven-dried weight of plant tissue.

### *Comparison of Water Extraction Method with Established Methods for K Determination*

The data on K concentration of 25 tobacco leaf samples as measured by experimental (water extraction) and established (triacid digestion, $NH_4OAc$ extraction, and HCl extraction) methods are given in Table 2. The K concentrations (mean of triplicate analyses) among all samples and across different methods of extraction varied from 0.60% to 2.85%. These measured K values are well within the range of tobacco leaf-tissue K contents (0.50–3.88%) reported in the literature (Krishnamurthy and Ramakrishnayya 1993; Krishnamurthy, Ramakrishnayya, and Murthy 1997). The range of K concentrations among 25 tobacco leaf samples was more or less identical for different methods, with the K ranging from 0.60 to 2.65% for triacid digestion, from 0.65 to 2.85% for $NH_4OAc$ extraction, from 0.63 to 2.76% for HCl extraction, and from 0.61 to 2.70% for the water extraction method. Though mean K concentration varied slightly among different methods, it followed the order $NH_4OAc$ extraction (1.71%) > HCl extraction (1.68%) > water extraction (1.61%) > triacid digestion (1.56%). Relatively greater values of plant K obtained with $NH_4OAc$ extraction in the present study corroborate the findings of earlier researchers (Sahrawat 1984; Rao, Rao, and Takkar 1998), who reported that the ammonium acetate–extractable K content of diverse plant materials was greater than the K concentrations obtained with HCl extraction or triacid digestion. The fact that the triacid digestion yielded relatively lower K concentration than the extraction methods might be due to possible loss of K resulting from sample drying and burning toward completion of digestion (Zarcinas, Cartwright, and Spouncer 1987).

In general, K concentrations obtained with the water extraction method tended to be slightly greater than those obtained with triacid digestion but slightly lower than the K values for $NH_4OAc$ or HCl extraction methods (Table 2). The water-extractable K values averaged 103.2, 94.2, and 95.8% of mean K obtained with triacid digestion, $NH_4OAc$ extraction, and HCl extraction, respectively. The proposed water extraction method gave mean K concentration that was within 3 to 6% of that measured by established methods and thus was comparable in accuracy to the established methods.

Relationships of experimental method (water extraction) with established methods (triacid digestion, $NH_4OAc$ extraction, and HCl extraction) for K concentration in tobacco

**Table 2**
Comparison of tobacco leaf tissue K concentration (%) determined by established methods and water extraction method

| | K extraction method | | | |
|---|---|---|---|---|
| Sample | Triacid digestion | $NH_4OAc$ extraction | HCl extraction | Water extraction |
| 1 | 1.41 | 1.52 | 1.48 | 1.42 |
| 2 | 1.07 | 1.20 | 1.15 | 1.08 |
| 3 | 1.81 | 2.03 | 1.92 | 1.82 |
| 4 | 2.05 | 2.17 | 2.17 | 2.15 |
| 5 | 2.46 | 2.69 | 2.73 | 2.46 |
| 6 | 1.42 | 1.64 | 1.56 | 1.42 |
| 7 | 2.29 | 2.45 | 2.38 | 2.33 |
| 8 | 1.48 | 1.54 | 1.56 | 1.47 |
| 9 | 2.52 | 2.70 | 2.69 | 2.57 |
| 10 | 1.31 | 1.50 | 1.36 | 1.40 |
| 11 | 2.65 | 2.85 | 2.76 | 2.70 |
| 12 | 0.60 | 0.65 | 0.63 | 0.62 |
| 13 | 0.61 | 0.65 | 0.65 | 0.61 |
| 14 | 1.14 | 1.22 | 1.24 | 1.17 |
| 15 | 1.17 | 1.26 | 1.24 | 1.16 |
| 16 | 0.68 | 0.72 | 0.71 | 0.63 |
| 17 | 1.79 | 2.00 | 1.99 | 1.95 |
| 18 | 1.04 | 1.17 | 1.19 | 1.10 |
| 19 | 1.30 | 1.43 | 1.45 | 1.35 |
| 20 | 1.26 | 1.37 | 1.36 | 1.24 |
| 21 | 1.22 | 1.32 | 1.37 | 1.30 |
| 22 | 2.25 | 2.48 | 2.57 | 2.38 |
| 23 | 1.63 | 1.83 | 1.80 | 1.74 |
| 24 | 1.68 | 1.99 | 1.90 | 1.93 |
| 25 | 2.12 | 2.26 | 2.22 | 2.21 |
| Range | 0.60–2.65 | 0.65–2.85 | 0.63–2.76 | 0.61–2.70 |
| Mean | 1.56 | 1.71 | 1.68 | 1.61 |
| SE | 0.12 | 0.13 | 0.13 | 0.12 |

*Note.* SE, standard error of mean.



leaf tissue are depicted in Fig. 1. The K concentrations obtained with water extraction method were significantly ($P = 0.01$) correlated with K values measured by triacid digestion ($r = 0.9952^{**}$), $NH_4OAc$ extraction ($0.9966^{**}$), and HCl extraction ($0.9953^{**}$) methods. These correlations indicate close agreement between the K concentrations obtained by the water extraction method and the established methods. Regression of the K concentrations determined by different established methods (y) on K obtained by water extraction method (x) yielded the following linear regression equations:

$$\text{Triacid digestion}: \ y = 0.953x + 0.026 \quad (R^2 = 0.9903) \tag{1}$$

$$NH_4OAc \text{ extraction}: \ y = 1.030x + 0.051 \quad (R^2 = 0.9931) \tag{2}$$

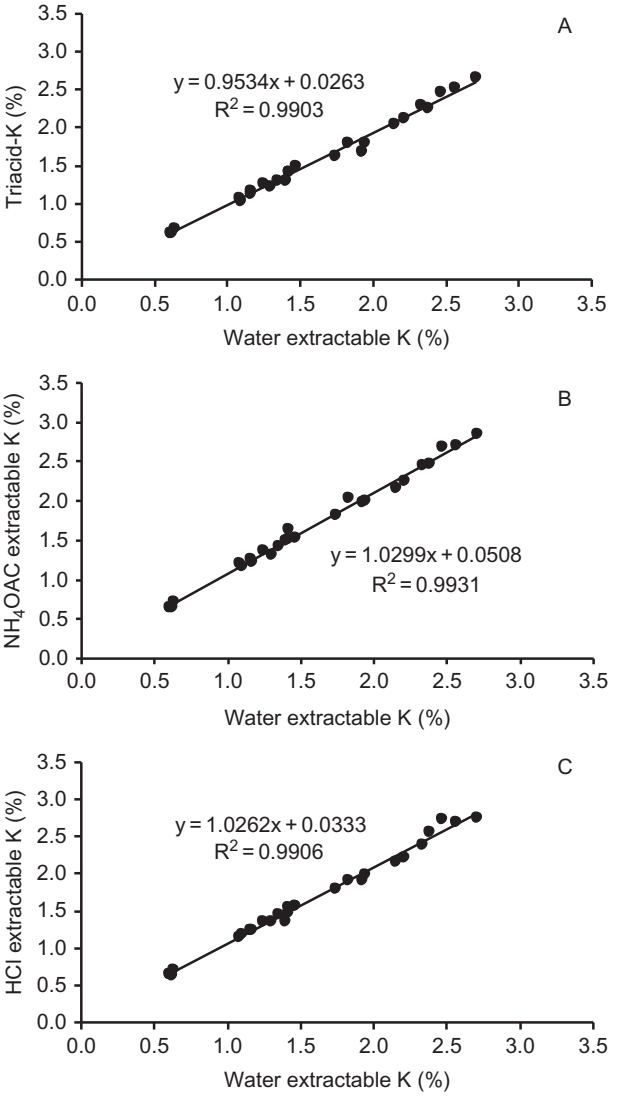


**Figure 1.** Relationship of water-extractable K with (A) triacid K, (B) NH4OAc-extractable K, and (C) HCl-extractable K in tobacco leaf tissues (n = 25).

$$\text{HCl-K extraction} : y = 1.026x + 0.033 \quad (R^2 = 0.9906) \tag{3}$$

Very high $R^2$ values for the linear regression equations (1) through (3) also suggested that the K determined using the water extraction method was predictively related with K quantified by established methods.

### *Precision of K Determination*

To better characterize precision associated with the proposed water extraction method and established methods for K determination, five independent K determinations by different

**Table 3**
Precision of tobacco leaf tissue K concentration assayed by different digestion/extraction methods

| Method | K concentration (%) in tobacco leaf tissue[a] | | | |
|---|---|---|---|---|
| | Range | Mean | SE | CV (%) |
| Low-K sample | | | | |
| Triacid digestion | 0.62–0.66 | 0.648 | 0.008 | 2.76 |
| $NH_4OAc$ extraction | 0.64–0.68 | 0.660 | 0.010 | 3.03 |
| HCl extraction | 0.64–0.67 | 0.653 | 0.006 | 2.05 |
| Water extraction | 0.64–0.66 | 0.656 | 0.004 | 1.36 |
| High-K sample | | | | |
| Triacid digestion | 2.38–2.56 | 2.484 | 0.034 | 3.09 |
| $NH_4OAc$ extraction | 2.52–2.78 | 2.672 | 0.043 | 3.61 |
| HCl extraction | 2.59–2.69 | 2.637 | 0.019 | 1.57 |
| Water extraction | 2.54–2.62 | 2.572 | 0.016 | 1.41 |

[a]Based on five independent analyses.
*Notes.* SE, standard error of mean; CV, coefficient of variation (%).



methods were performed using two tobacco leaf samples (one with low K and another with high K). The results are presented in Table 3. In general, analytical variability of K determination by different methods was low, as illustrated by the small values of standard errors (SEs) of mean K in both low-K and high-K samples, resulting in coefficients of variation (CVs) less than 4%. The CV associated with different methods was similar for low-K and high-K samples, suggesting little or no influence of leaf K concentration on precision of K determination. The K measured using the water extraction method had the lowest CV of 1.36% for the low-K sample and 1.41% for the high-K sample. Among different methods employed for K digestion/extraction, the SE and CV values followed the order water extraction < HCl extraction < triacid digestion < $NH_4OAc$ extraction. It is readily evident from these results that the proposed water extraction method proved more precise than the standard/established methods for determination of K in tobacco leaf tissue.

## Conclusions

The proposed water extraction method for determination K concentration in tobacco leaf tissue involves shaking 0.5-g finely ground sample with distilled water at 1:100 extraction ratio (sample to water, w/v) for 20 min and filtering before K measurement by flame photometry. The accuracy and precision of K concentrations obtained with the proposed water extraction method are comparable or superior to those obtained with standard triacid digestion, $NH_4OAc$ extraction, and HCl extraction methods. Besides providing accurate and precise measures of K, the water extraction method is simple and rapid and virtually precludes the need for costly chemicals and equipment. The proposed water extraction method serves as an inexpensive alternative to the most commonly used standard triacid digestion method for routine analysis of K in tobacco leaf samples. Further investigations are needed to assess the suitability of water extraction method for determining K concentration in diverse plant materials.

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## References

Alder, P. R., and G. E. Wilcox. 1985. Rapid perchloric acid digestion methods for analysis of major elements in plant tissue. *Communications in Soil Science and Plant Analysis* 16:1153–1163.

Gomez, K. A., and A. A. Gomez. 1984. *Statistical procedures for agricultural research*. New York: John Wiley and Sons.

Jackson, M. L. 1973. *Soil chemical analysis*. New Delhi, India: Prentice Hall of India.

Krishnamurthy, V., and B. V. Ramakrishnayya. 1993. Yield and quality of FCV tobacco as affected by potassium nutrition. In *Plant nutrition effects on production and quality of tobacco*, ed. G. Dev, M. S. Chari, and B. V. Ramakrishnayya, 78–102. Gurgaon, India: Potash and Phosphate Institute of Canada, India Programme.

Krishnamurthy, V., B. V. Ramakrishnayya, and K. D. Singh. 2003. *Potassium nutrition of flue-cured tobacco*. Rajahmundry, India: Central Tobacco Research Institute.

Krishnamurthy, V., B. V. Ramakrishnayya, and N. S. Murthy. 1997. Distribution pattern of potassium in different segments of flue-cured tobacco leaf. *Communications in Soil Science and Plant Analysis* 28 (9–10): 665–671.

Marschner, H. 1986. *Mineral nutrition of higher plants*. London: Academic Press.

Rao, C. S., A. S. Rao, and P. N. Takkar. 1998. Evaluation of several methods for determining the potassium content in diverse plant materials. *Communications in Soil Science and Plant Analysis* 29 (17): 2785–2792.

Rosolem, C. A., J. C. Calonego, and J. S. S. Foloni. 2005. Potassium leaching from millet straw as affected by rainfall and potassium rates. *Communications in Soil Science and Plant Analysis* 36 (7): 1063–1074.

Sahrawat, K. L. 1980. A rapid non-digestion method for determination of potassium in plant tissue. *Communications in Soil Science and Plant Analysis* 11 (7): 753–757.

Sahrawat, K. L. 1984. Potassium determination in grain samples using the nondigestion (dilute HCl extraction) method. *Communications in Soil Science and Plant Analysis* 15 (1): 81–86.

Su, F., L. Fu, H. Chen, and L. Hong. 2006. Balancing nutrient use for flue-cured tobacco. *Better Crops* 90 (4): 23–25.

Zarcinas, B. A., B. Cartwright, and L. R. Spouncer. 1987. Nitric acid digestion and multi-element analysis of plant material by inductively coupled plasma spectrometry. *Communications in Soil Science and Plant Analysis* 18 (1): 131–146.