Synchrotron -Based X-Ray Fluorescence (XRF) Technique to Localize K and Fe in Cassava Leaves

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Abstract

Nutrient deficiency in cassava can be diagnosed by plant tissue analysis with a chemical method. However, the chemical method is time-consuming and required a large number of plant samples. Synchrotron-based XRF (SR-XRF) technique has been exploited to study the content and localization of mineral nutrients in plant tissues. The mappings of mineral nutrient distributions in plant leaves may relate to leaf positions or leaf veins, especially in the nutrient-deficient leaves. The objectives of this research are to study K and Fe status and to determine K and Fe relationship with leaf positions or leaf veins in cassava using the XRF technique. The field experiment on cassava was conducted using a randomized complete block design (RCBD) with four replications. Treatment included three methods of fertilizer applications: 1) Control (no fertilizer), 2) N+P + K application, and 3) N+P+K+ micro-nutrient application. Leaf K and Fe were analyzed by chemical technique and SR-XRF technique. The result showed that, with the chemical analysis, leaf K and Fe contents in control were in the deficient range. While those in treatments adding nutrients, K and Fe were in the sufficient range. The SR-XRF technique analysis results showed a similar trend as chemical analysis. The mapping of K and Fe distribution on leaves showed that variation of count K and Fe in control was less than those in the fertilizer added treatments. The distribution of K and Fe in leaf samples of control and fertilizer added treatments had no relationship with leaf positions and leaf veins. However, when a further investigation was done in K and Fe severely deficient leaves, the Fe and K distribution tended to relate with the leaf veins.

Keywords: *K, Fe, cassava, SR-XRF, Synchrotron, mapping*

1. Introduction

Cassava is one of the economic crops of Thailand, which is inferior only to rice, rubber, and sugar cane. In 2014, Thailand produced 31.2 million tons of cassava (Bank of Thailand, 2015). The most cultivated area is in the northeastern part of Thailand. It was reported in 2013-2014 that the harvested area of cassava was about 4.2 million rai, that is, 50% of the total harvest area (North Eastern Tapioca Trade Association, 2013). The average yield of cassava is very low at 3 tons per rai (Office Agriculture Economics, 2014), which is caused by many factors such as drought, cassava diseases, and insect pests. Other limiting factors are soil malnutrition, sandy soil, and soil acidity, which negatively affect plant growth and yield. Moreover, continuously cultivating cassava for a long period will result in nonequilibrium of mineral nutrients since these nutrients are absorbed from soil to leaves, stems, and roots differently according to plant demand. K is the highest amount of macro-nutrient, while Fe is the highest of micro-nutrient required by cassava. The high amount of absorbed K and Fe are removed from cassava fields by storage root harvesting and stem cutting for new cultivation, which results in the reduction of these elements in the soil. For sandy soil, these elements were also leached easily because the ability of sandy soil to absorb those elements is low. Most cassava farmers usually apply only N, P, and K fertilizers and not based on the initial availability of these elements in the soil that reduces the effectiveness of fertilizer application and leads to nutrient imbalance. Even though Fe initially exists in soil sources, the acidity of the sandy soil in this area causes Fe to precipitate and not to be in the ready-to-use form for plants. From the above reasons, the deficiency of K and Fe often occurs in the cassava farmers' fields in this area.

K and Fe are critical for cassava productivity and quality because K plays an important role in moving nutrient from leaves to storage root and changing into the form of starch. K is a co-factor of many enzymes involving several plant metabolisms, the main enzymes are pyruvate kinase and 6-phosphofructokinases enzymes, both enzymes involved in the glycolytic pathway (Ambasht, & Kayastha, 2002). Hence, K is very important in starch formation in cassava. Lacking K causes a decrease of starch in the storage roots, thus reducing the quality of cassava yield. K deficiency in cassava can be observed from leaf symptoms. Typical symptoms include brown scorching and curling of leaf tips as well as chlorosis (yellowing) of the old leaves. Fe is one of the elemental compositions of chlorophyll and involves the functioning of several enzymes. Lacking Fe in cassava can also be observed from its leaves. The primary symptom of Fe deficiency is interveinal chlorosis of young leaves (Howler, 2002) but in severe cases, the entire leaf turns yellow or white. Since K and Fe deficiency in cassava always causes unique symptoms on the leaves, the analysis of total nutrients in the leaves is used for the evaluation of cassava K and Fe status. Furthermore, the analysis of nutrient distribution on the leaf may be more accurate to early determine the K and Fe status in cassava.

The chemical analysis by the AAS technique has been a standard method for nutrient analysis in plant tissues. However, this method is time-consuming and required a large number of plant samples, which cannot be used to analyze the distribution of nutrients on plant leaves. In this study, the Micro X-ray fluorescence (Micro-XRF) technique is exploited to study the nutrient content and distributions in cassava leaves. Previously, the application of XRF was used to study elements uptake and accumulation in plants down to sub-cellular level (Zhao, Moore, Lombi, & Zhu, 2014). XRF technique could be used to analyze elements (such as nutrients or toxicants) with a low amount of fresh and hydrated tissues (Donner, De Jonge, Kopittke, & Lombi, (2013). Xin et al. (2009) studied the distribution of nutrients in any part of spinach by XRF technique and found that nutrient inconstantly distributed, young leaves had more Mn than old leaves, K, Ca, Ni, and Cu had more distribution in the middle area of leave. Blonski, Appoloni, Parreira, Aragao, & Nascimento (2007) studied Fe status in leaves by XRF technique and found that the concentration of Fe varied from 44-192 $\mu g.g^{-1}$ in healthy leaves and 363-704 $\mu g.g^{-1}$ in infected leaves. From the previous researches, it was found that the XRF technique could be used to analyze several plant nutrients effectively. Moreover, it can be applied to study nutrient distribution in leaves or plant tissue, which might be more advantageous than chemical analysis.

2. Objectives

The objectives of this research were to study K and Fe status, K and Fe relationship with leaf positions and leaf veins, and to study the possibility of using the XRF for K and Fe analysis in cassava leaves.

3. Materials and Methods

3.1 Field Experiment: Cassava CMR89 (Department of Agriculture, Thailand) were cultivated in sandy soil at the Suranaree University of Technology experimental farm, Thailand in May 2014. The experimental design was a randomized complete block design with four replications. Treatments included three methods of fertilizer application: T1) Control (no fertilizer), T2) N+P+K application, and T3) N+P+K + micronutrient application. The number of nutrients applied was based on soil analysis. All fertilizers were applied one month after planting.

3.2 Synchrotron XRF analysis: At 4 months after planting the cassava leaves at the 7th position below the fully expanded leaves (Uthaiwan, 2013) were collected, cleaned, and oven-dried at 50°C for 48 hours. Then, they were cut in square shape of the studied areas including the rim, leaf blade, and leaf vein (Figure 1a). The elemental distributions of leaves were investigated by the Micro X-ray fluorescence (μ-XRF) technique. The analysis was conducted at the beamline BL6b, Synchrotron Light Research Institute (SLRI) Thailand. At BL6b, the synchrotron radiation source is obtained from the bending magnet producing a high photon flux of the white beam. Without using the monochromator, the polychromatic X-ray beam, whose energy is ranging from 2 to 15 keV, was focused on by the polycarpellary half-lens, thus obtaining 30 × 30 μm^2 of the micro X-ray beam at the sample position. The single element Vortex EM-650 silicon drift detector was used to count the X-ray fluorescence emitted from the exposure of the X-ray beam onto the specimens. The detector was placed 45 degrees to the incident X-ray beam and perpendicular to the CCD camera. The selected area of cassava leave was positioned vertically in the sample holder. The elemental distribution maps were collected using a 500 µm (h) x 500 µm (v) pixel size, with 50 seconds of X-ray exposure time per pixel. PyMca software (Sole, Papillon, Cotte, Walter, & Susini, 2007) was used to analyze the X-ray fluorescence data.

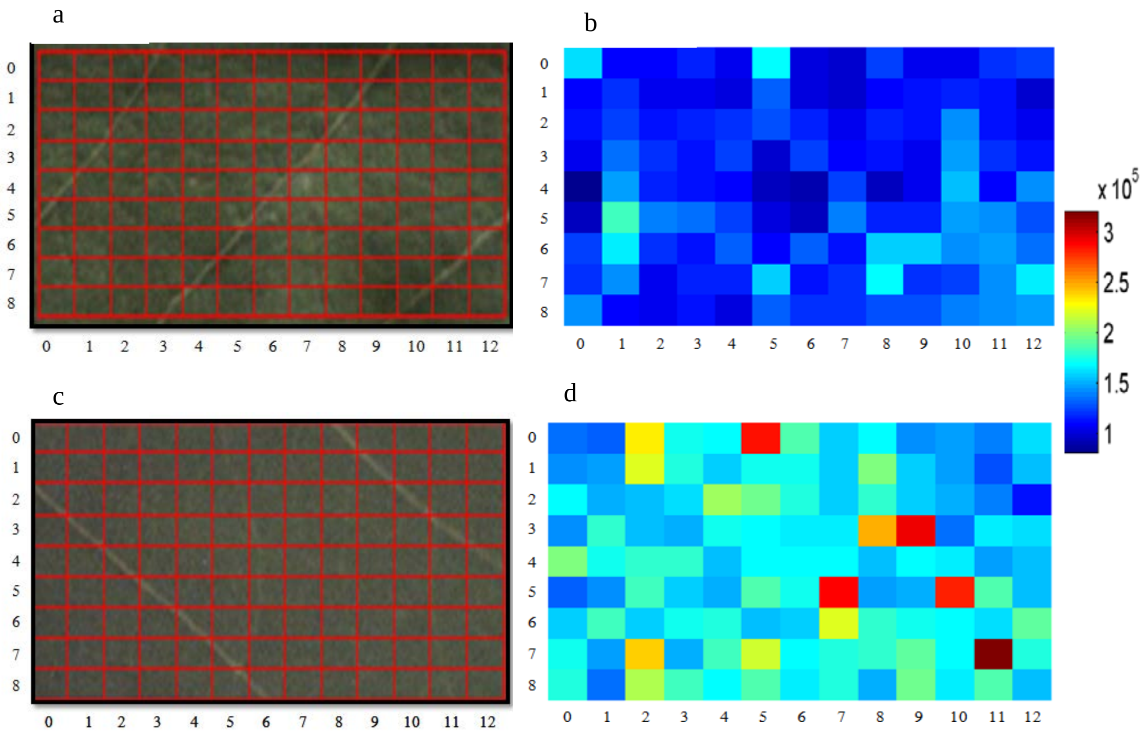

Figure 1 K distribution on cassava leaves determined by XRF technique. The picture of the studied area of control (a) and K added treatment (c), the picture of K distribution in control (b), and in K added treatment (d)

3.3 Chemical analysis: At the same time as Synchrotron XRF analysis, the leaf samples were also analyzed for K and Fe by the chemical method. Leaf samples were oven-dried at 70 ^{0}C and digested using HNO_3 and $HClO_4$. Then, K and Fe in the digested samples were determined by the atomic absorption spectroscopy (AAS) technique (Jones, 2001).

4. Results and Discussion

Leaf K and Fe content

From the chemical analysis result (Table 1), it was found that the K content of cassava leaves in the control treatment (T1) was 1.2 %, which is in the range of deficiency, while K content in N+P+K treatment (T2) was 1.5%, which is in the sufficiency range (Table 2). The result suggested that the application of macronutrients according to soil analysis could elevate K uptake by cassava up to the optimal level. The result was in agreement with the previous research (Ayoola, & Makind; 2007). Similar to chemical analysis, the XRF spectrum of K in cassava leaves of T1 was lower than that of T2 (Table 1). The consistent result of both methods may imply that the XRF analysis could be used to determine the K status in cassava leaf. The result was in agreement with other previous researches (Xin et al., 2009 and Patsara, Sodchol, Somchai, & Waraporn, 2016).

From the chemical analysis of Fe (Table 1), it was found that the Fe content of cassava leaves in N+P+K application treatment (T2) was 108 mg/kg, which is in the range of deficiency, while Fe content in N+P+K + micro-nutrient application treatment (T3) was 200 mg/kg, which is in the high range (Table 2). The application of micronutrients including Fe also promoted the Fe uptake, which is in the agreement with the previous research work (Howler, 2002). A similar result was found with the XRF analysis, ie. the spectrum of Fe in cassava leaves of T2 was lower than that of T3 (Table 1). Zhao et al. (2014) and Kyriacou et al. (2014) also found a good relationship between chemical and XRF technique. The overall result confirmed that the XRF technique could be used to determine the Fe and K status in cassava.

Table 1 The chemical analysis result and total count by XRF technique of K and Fe in cassava leaves

Nutrient	Treatment	Chemical analysis (%)	Nutrient states	Total Count
K	T1) no fertilizer	1.2	Deficient	15,357,120
	T2) N+P+K application	1.5	Sufficient	22,549,160
Fe	T2) N+P+K application	108 mg/kg	Deficient	718,067
	T3) N+P+K + micro-nutrient application	200 mg/kg	High	765,399

Table 2 Concentration of K and Fe in cassava leaves at 3-4 months after planting (Howler, 2002)

Nutrient	Nutrient status					
	Very deficient	Deficient	Low	Sufficient	High	Toxic
K (%)	<0.85	0.85-1.26	1.26-1.42	1.42-1.88	1.88-2.40	>2.40
Fe (mg/kg)	<100	100-110	110-120	120-140	140-200	>200

K and Fe distribution on leaves

Besides the total nutrient content analysis, the XRF technique can be used to evaluate nutrient distribution at various positions of the leaf. Figure 1 showed K distribution on cassava leaf determined by the XRF technique. It was found that in K added treatment (Fig 1d), the total counts of K were consistently higher than those in the control treatment. Moreover, it can be seen that the variation of K in the K added treatment ($1\text{-}3*10^5$) was greater than those in the control treatment ($1\text{-}1.7*10^5$). However, in both treatments, the variation of K content did not relate to leaf positions and leaf veins (compared 1b and 1a, 1d and 1c). Figure 2 showed Fe distribution on cassava leaf determined by the XRF technique. Similar to K, the total count and variation of Fe in Fe added treatment were greater than those in the control treatment and the variation of Fe content did not relate to leaf positions and leaf veins. The result was in contrast with Xin et al. (2009) who studied the distribution of nutrients in a spinach leaf by the XRF technique and found that K had more distribution in the middle area of leave than in the other parts. No relationship between Fe and K content and leaf positions in this study may be since all leaf samples were pre-mature leaves (the 7th position below the fully expanded leaves), the deficiency of K and Fe was not severe even in the control treatment. There were no deficiency symptoms on the leaf samples. Usually, the deficiency symptom of K is found in old leaves while Fe is in the young leaves. Therefore, further investigation was performed on the leaves with severe deficiency symptoms of K (old leaves) and Fe (young leaves) from the control treatment. Figure 3 showed K and Fe distribution determined by the XRF technique on cassava leaves with severe deficiency symptoms. It can be seen that the nutrient distribution tended to relate with leaf veins ie. both nutrients were more concentrated around the leaf veins. The result suggested that the distribution of nutrients in cassava leaves with the severe nutrient deficit are different from normal leaves and leaves with low nutrient status without deficiency symptoms.

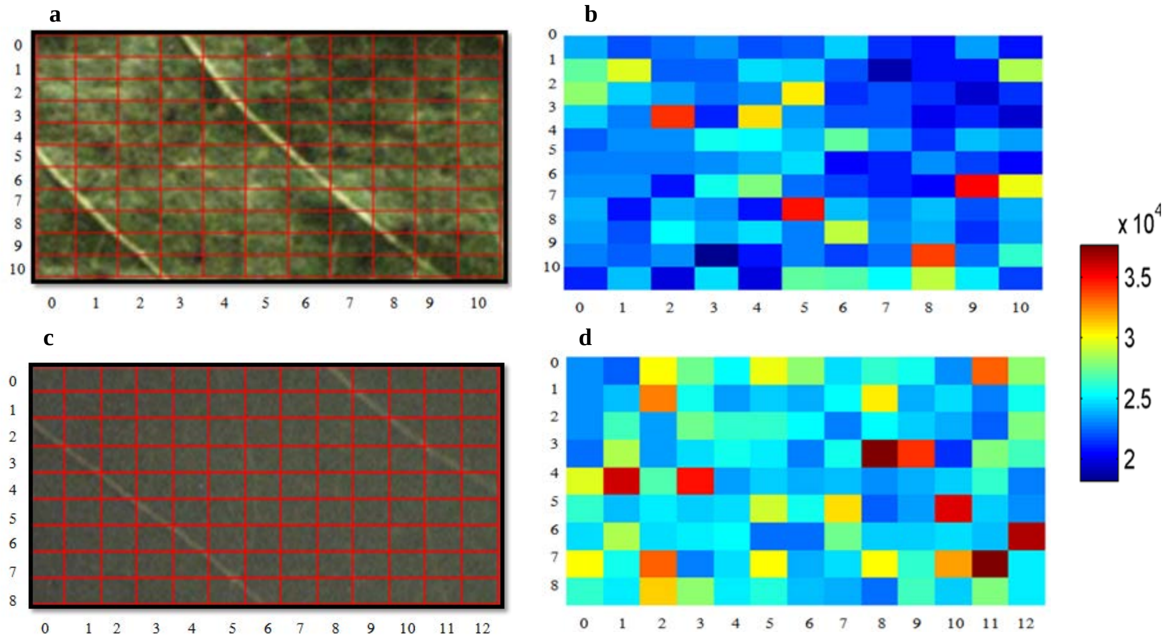

Figure 2 Fe distribution on cassava leaves determined by XRF technique. The picture of the studied area of control (a) and K added treatment (c), the picture of K distribution in control (b), and in K added treatment (d)

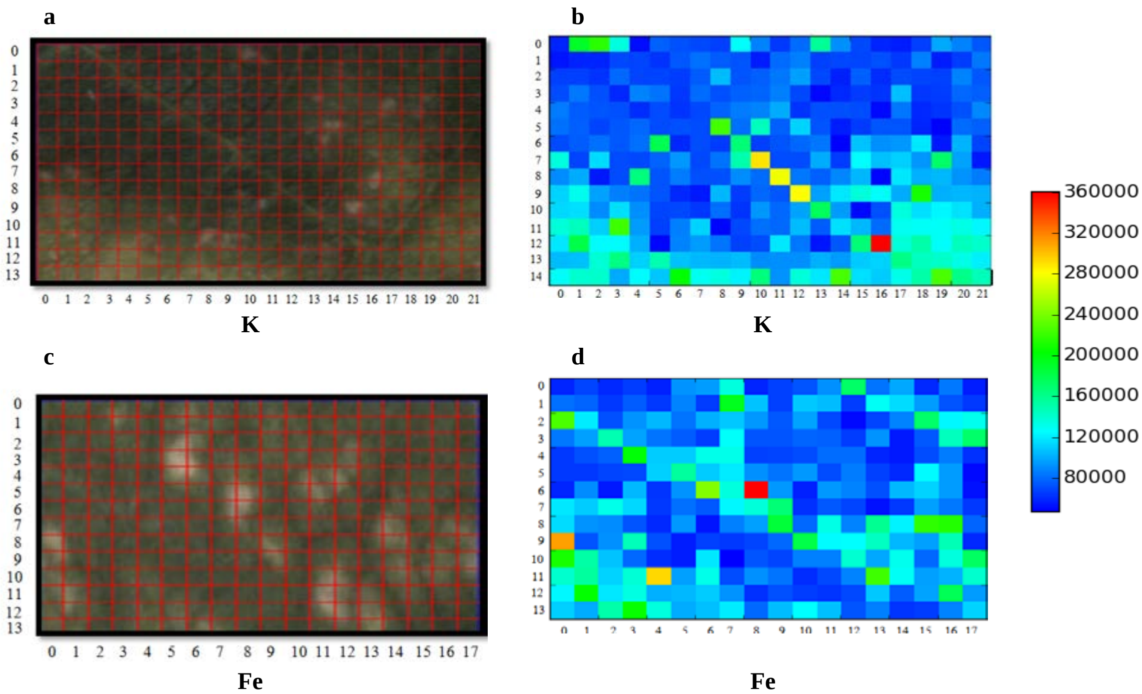

Figure 3 K and Fe distribution on cassava leaves with severe deficiency symptoms determined by XRF technique. The picture of the studied area of control (a and c), the picture of K distribution (b), and Fe distribution (d)

5. Conclusion

The current research investigated K and Fe status in cassava between control and nutrient added treatments by leaf nutrient analysis using chemical and XRF technique and determined the distribution of K and Fe in relation to the leaf positions. The results showed a similar trend in both nutrients and both analysis methods. By chemical and XRF method, leaf K and Fe contents in control were lower than in nutrient added treatments. The distribution of K and Fe in premature leaves had no relationship with leaf position and leaf veins. However, when the investigation was done in severe K and Fe deficiency leaves (old and young leaves), the Fe and K distribution tended to relate with leaf veins. The result confirmed that the XRF technique could be used to determine the nutrients status and nutrient distribution in cassava leaves.

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