X-Ray Fluorescent and Mid-Infrared Spectroscopic Measurement of Leaf Model

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Abstract

This study aims to develop a simple, rapid, simultaneous and quantitative method for evaluating nutrimental elements of the plant vigor using X-ray fluorescent (XRF) and mid-infrared (MIR) spectroscopy. We focused on tomato leaves for tomato is one of the typical agricultural products cultivated by hydroponics and the leaves would be an index of the nutrimental conditions. We developed a quantitative method to analyze the XRF spectroscopic information, and studied the differences of the element content balances between branches of a trunk in order to grasp the element flows from the bottom to the top. We also studied the simultaneous determination of the nitrogen contents in different modes using the MIR spectroscopic method. The experimental results presented a great potentiality to obtain the nutrient information on the chemical elements such as P, K and Ca and the nitrogen in the plural modes using the both spectroscopic methods. This study presents an important step in the development of XRF and MIR spectroscopic analysis of the plant vigor in the agricultural fields.

Key words: X-ray fluorescent spectroscopy, mid-infrared spectroscopy, tomato leaf, nutrimental elements.

1 Introduction

The nutrimental components in plants are important information for the diagnosis and the cultivation control of the agricultural products. For such measurement, it is very important that not only the nutriment contents but also their balance in fresh plants are monitored in a simple, non-destructive, simultaneous, and rapid method. In addition, the measured data need to be retrieved for future simulation using various modelling software.

Application of X-ray fluorescent (XRF) and mid-infrared (MIR) spectroscopy to the above measurement is desirable as a high potential implement. Incidentally, in developments of the portable spectrometers, both spectroscopic methods provide substantial potential as the quantitative tools in the fields for the chemical components relating to the plant vigor.

This study aims to develop a simple, rapid, simultaneous and quantitative method for evaluating the nutrimental elements of the plant vigor using the XRF and MIR spectroscopy. The XRF and MIR information of the inorganic nutrients such as K, Ca, P and S and of the nitrogen in different modes in tomato leaf was respectively obtained, because tomato is one of the typical agricultural products cultivated by hydroponics and the leaves would be an index of the nutrimental conditions. The fresh leaves and the leaf models, which were prepared by impregnating the nutrient mixture solutions with the dry filters or leaves, were prepared as samples. After developing the quantitative method to analyze the XRF spectroscopic information, we studied the differences of the element content balances between branches of a trunk in order to grasp the element flow from the bottom to the top. We also studied the simultaneous
determination of the nitrogen contents in different modes using the MIR spectroscopic method.

2 Experiments

2.1 Model leaves

A filter was impregnated with the nutrient element (kalium: K, calcium: Ca, and phosphorous: P) mixture solution, which was prepared roughly according to the composing balances of the chemical element contents as listed in Table 1. The original solution with the highest concentrations was successively diluted with pure water, and the concentrations of the 16-fold dilution are at the actual level for the common tomato trees. The filter was dried in a desicator at the room temperature (298 K) until the weight change was negligible. The filters including the K, Ca and P elements with different concentrations were used as leaf models for the X-ray fluorescent spectral measurement of the plural nutrient elements.

<table>
<thead>
<tr>
<th>solutions</th>
<th>concentration [mmol/l]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K</td>
</tr>
<tr>
<td>original solution</td>
<td>399</td>
</tr>
<tr>
<td>2-fold dilution</td>
<td>200</td>
</tr>
<tr>
<td>4-fold dilution</td>
<td>100</td>
</tr>
<tr>
<td>8-fold dilution</td>
<td>50</td>
</tr>
<tr>
<td>16-fold dilution</td>
<td>24</td>
</tr>
<tr>
<td>32-fold dilution</td>
<td>13</td>
</tr>
</tbody>
</table>

In addition, we prepared the model leaves for the MIR measurement of the nitrogen in the plural modes. Casein, contains about 15% nitrogen, was dissolved into 0.1 N sodium hydroxide solution to obtain a solution containing about 0.6% proteinic nitrogen. By adding nitric acid or sodium nitrate into the casein solution, the nitrogen mixture solutions with the different ratio of the nitrate nitrogen content to the proteinic one were prepared (Table 2). The solutions and water were impregnated into the dried tomato leaves and these leaves acted as model for the MIR measurement.

<table>
<thead>
<tr>
<th>solutions</th>
<th>concentration [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>casein</td>
</tr>
<tr>
<td>2-fold dilution</td>
<td>3.932</td>
</tr>
<tr>
<td>standard solution</td>
<td>3.647</td>
</tr>
<tr>
<td>2/3-fold dilution</td>
<td>3.956</td>
</tr>
<tr>
<td>1/2-fold dilution</td>
<td>3.953</td>
</tr>
<tr>
<td>no nitric acid</td>
<td>3.963</td>
</tr>
<tr>
<td>water</td>
<td>0.000</td>
</tr>
</tbody>
</table>

2.2 Tomato leaves

The tomato plants under experiment were hydro-cultivated in a greenhouse (Figure 1), located in the Research Institute of Kagome Co., Ltd., Nasushiobara, Tochigi, Japan were prepared for the XRF spectral measurements. The leaves were sampled triplicate (on 29th August, 26th September and 21st November 2002). We studied the differences of the element content balances among branches, which were numbered as displayed in Figure 2(a), for the understandings of the element flow from the bottom to the top. The branch positions for such XRF spectral measurements were determined according to the growth stages. The second apiculus leaves as described in Figure 2(b) were sampled for the XRF spectral measurements.
2.3 X-ray fluorescent spectral measurement

An energy dispersive XRF spectrometry (Rayny EDX-700, Shimadzu, Kyoto, Japan) was used to measure the chemical element components such as K, Ca, P and S of the leaf models and the tomato leaves. The XRF spectra of the center part of the sample with the press using a metal ring were measured. During the spectral measurements, the sample compartment was purged with helium gas for getting the sensitive XRF information successively.

2.4 Mid-infrared spectral measurement

Fourier transform infrared spectrometer (FT-IR; Magna-IR 750, Nicolet) equipped with an ATR (attenuated total reflection; Harrick, 1967; Mirabella and Harrick, 1985) sampling accessory (Graseby Specac) with a diamond internal reflection element (IRE) was used to collect the MIR spectral information of the some modes of nitrogen of the model leaves. The model leaf was pressed with a metal pole on the IRE crystal in order to stabilize the sampling point (Figure 3). And the MIR spectra were obtained from 4000 to 800 cm⁻¹.
3 Experimental results and discussion

3.1 Quantitiability of X-ray fluorescent spectral measurement

In order to quantitatively analyze the XRF spectra of the leaf models pressed with the ring, we standardized them using the peak intensity of Rh \( \text{K}\alpha \) that was the target for X-ray generation, because the intensity value of Rh \( \text{K}\alpha \) could be theoretically constant for all samples without the Rh element.

Figure 4 shows the XRF spectra standardizing the measurement results for the pressed samples with the different concentrations of the nutrient elements. As shown in Figure 4, the differences of the spectral features between the peaks of Rh \( \text{K}\alpha \) and Rh \( \text{K}\beta \) for all samples were negligible both qualitatively and quantitatively. Additionally, the peaks relating to P \( \text{K}\alpha \), K \( \text{K}\alpha \) and Ca \( \text{K}\alpha \) were observed around 2.01, 3.32 and 3.69 keV, respectively. The peak intensities became higher with increase in the chemical element contents impregnated into the leaf models. Generally the XRF spectrum was significantly affected by the geometrical structure near the irradiated points of the sample. Therefore, pressing the leaf model with the ring would moderate the problems of the deflection near the irradiated point. This experiment suggests that it could be possible to quantitatively evaluate the standardized XRF spectral features of the sample with the complicated geometrical structure such as leaves.

![Figure 4](image1.png)

**Fig.4** Standardized XRF spectroscopic characteristics of leaf models with different concentrations of nutrient elements.

Figure 5 represents the calibration curves between the peak areas for the standardized XRF spectra of the leaf models for P \( \text{K}\alpha \), K \( \text{K}\alpha \) and Ca \( \text{K}\alpha \) shown in Figure 4 and the concentrations of the nutrient element mixtures impregnated into them. For each chemical element, the excellent linearity was observed and all coefficients of determination \((R^2)\) were higher than 0.998. The results indicated in Figure 5 means that the nutrient element contents of the leaf model could be quantified using the standardized XRF spectra using the peak intensity of Rh \( \text{K}\alpha \) as the target for the X-ray generation.

![Figure 5](image2.png)

**Fig.5** Calibration curves between peak areas of standardized XRF spectra of leaf models for P \( \text{K}\alpha \), K \( \text{K}\alpha \) and Ca \( \text{K}\alpha \) and concentrations of nutrient element mixtures impregnated into them.
3.2 Inorganic nutrient balances in tomato leaf

Based on the above discussion, we measured the XRF spectra of the fresh tomato leaves by holding the irradiated point and standardized them using the peak intensity of Rh Kα. The difference of the element content balances between branches of the trunk was then studied.

The standardized XRF spectra of the tomato leaves from A2 to A8 (Figure 2) measured on 29th August are displayed in Figure 6 and qualitatively and quantitatively agreed with each other around the peaks of Rh Kα and Rh Kβ as well as the leaf models shown in Figure 4. The method developed in this study could also be applicable for the quantitative analysis of the actual fresh leaves.

![Figure 6](image1)

As shown in Figure 6, the observed peaks were related not only with P Kα (2.01 keV), K Kα (3.32 keV) and Ca Kα (3.69 keV) but also with Si Kα (1.73 keV) and S Kα (2.31 keV). The peak for Ca Kα was very stable and was significantly influenced by the branch positions. The peak intensity of the tomato leaf of the lowest branch (A2) was the highest among the four branches and that on the highest branch (A8) was the lowest. For K Kα, influences of the branch position on the standardized XRF peaks were very small, but consistent. The influences on the P Kα and S Kα peaks were also very negligible. The area of the Si Kα peak was negligible relatively to the others. Furthermore, the experimental results similar to Figure 6 were obtained on 26th September and 21st November. Thus the difference of the element content balances among branches on a trunk reflected on the standardized XRF spectra.

Figure 7 indicates the relationship between the areas of the P Kα, S Kα, K Kα and Ca Kα peaks as shown in Figure 6 and the branch positions. The peak area of Ca Kα definitely decreased with the increase in the branch number and that of P Kα slightly increased with the increase in the branch number. The significant variations were not observed for the S Kα and K Kα peaks. Thus the results revealed that the leaf on the branch located at the lower position contained much Ca component.

![Figure 7](image2)
3.3 MIR spectral characteristics of leaf model

Figure 8(a) shows the MIR spectra of the fresh tomato leaf and the leaf models. The spectra of the leaf models represented the very similar spectral features of the fresh tomato leaf over the wavenumber range measured in this study. Figure 8(a) suggested the spectroscopic suitability of the leaf models both qualitatively and quantitatively. However, the very high peaks at 3360 and 1650 cm\(^{-1}\) where the OH stretching and bending modes mainly due to water are respectively observed characterizing the rough spectral features. We then magnified Figure 8(b), and the magnification is show in Figure 4(b) where the absorption bands relating to the nitrate and proteinic nitrogen components exist. At 1350 cm\(^{-1}\), the very weak peaks are observed, and could be attributed to the NO stretching mode of the nitric functional groups. The stable absorption peaks of the amino functional groups at 1650 and 1550 cm\(^{-1}\), where the very high peak due to the OH bending of water are observed, could not be easily recognized from Figure 4(b).

![Fig.8 MIR spectra of fresh tomato leaf and the leaf models.](image)

3.4 MIR spectral information of plural nitrogen in leaf model

Figure 9 shows the MIR spectra of the leaf models after the spectral subtraction of the leaf model impregnated with water and could provide the MIR spectroscopic information of the leaf model components. The slight spectral pattern differences were observed among the leaf models, but significant peaks characterizes the nitric acid and amino functional groups. At 1350 cm\(^{-1}\), where the absorption peak of the NO stretching mode relating to nitric acid would be observed, the order of the absorbance accorded with that of the nitric acid concentration of the leaf model. Moreover, the relationship between the proteinic nitrogen concentration in the leaf model and the absorbance at 1650 and 1550 cm\(^{-1}\), where the absorption peak of the functional modes due to the amino I and II would exist, could not be extracted from Figure 9. The results shown in Figure 9 could reflect the chemical and geometrical complications of the leaves as the living body.

![Fig.9 MIR spectra of leaf models after spectral subtraction of leaf model impregnated with water.](image)
We focused our attention on the absorption peak related to the NO stretching mode because the absorbance order at 1350 cm\(^{-1}\) was according to that of the nitric acid concentration in Figure 9. Figure 10 indicates the relationship between the absorbance at 1350 cm\(^{-1}\) in Figure 9 and the nitric acid concentration. The excellent linearity was obtained and the correlation coefficient was 0.997. Therefore, Figure 10 explains that the nitrate nitrogen content in the leaf model could be accurately determined.

![Graph](image1)

**Fig. 10** Relationship between absorbance at 1350 cm\(^{-1}\) of MIR spectra of leaf model components and nitric acid concentration of nitrogen mixture solution impregnated into leaf model.

Furthermore, we examined the simultaneous quantitative analysis of the nitrate and proteinic nitrogen components. In consideration of the results shown in both Figures 9 and 10, we discussed the ratio of the nitrate nitrogen content to the proteinic one. Figure 11 represents the relationship between the content ratio and the absorbance one. The denominator of the vertical axis of Figure 11 is the summation of the absorbances at 1650 and 1550 cm\(^{-1}\) respectively relating to the amino I and II components, and the numerator is the absorbance at 1350 cm\(^{-1}\) due to the NO stretching of the nitric acid. Then the value of \(\frac{A_{1350}}{A_{1350} + A_{1550}}\) in the vertical axis would be concerned with the content ratio of the nitrate nitrogen content.

![Graph](image2)

**Fig. 11** Relationship between \(\frac{A_{1350}}{A_{1550} + A_{1650}}\) shown in Figure 10 and concentration ratio of nitrate nitrogen to proteinic ones in nitrogen mixture solution impregnated into leaf model.

Figure 11 displays the relationship and the value of \(\frac{A_{1350}}{A_{1550} + A_{1650}}\) approximately increased with the increase of the content ratio of the nitrate nitrogen to the proteinic one. The correlation coefficient was higher than 0.8 and the good result was obtained. As Figure 10 indicates the possibility of the MIR spectroscopic determination of the nitrate nitrogen content in the leaf model, the simultaneous determination of the nitrate and proteinic nitrogen is possible by combining the analysis of Figure 11 with that of Figure 10.
4 Concluding remarks

The XRF spectra of the leaf models indicated the peaks relating to PKα, KKα and CaKα at about 2.01, 3.32 and 3.69 keV, respectively, by holding the irradiated point and standardized the XRF spectrum on the peak intensity of RhKα that was the target for X-ray generation. This developed method could be used for stable and quantitative XRF measurement of the actual fresh leaves. On the other hand, the MIR spectra of the model leaves represented peaks relating to nitric acid and amides I and II at about 1350, 1650 and 1550 cm⁻¹, respectively, and displayed almost same patterns as those of the fresh leaves. These results show the great potentiality to obtain the nutrient information on the chemical elements (such as P, K and Ca) and the nitrogen in the plural modes using the both spectroscopic methods. This study presents an important step in the development of XRF and MIR spectroscopic analysis of the plant vigor in the agricultural fields. Additionally, the XRF spectral information could desirably be associated with the other information relating to the plant vigor (Oka et al., 2004), especially with the image information obtained by a digital camera (Hashimoto et al., 2002) in a easy and cheep way.

5 References


