Simple, Rapid and Non-Destructive Determination of Nitrate Nitrogen Content Using Mid-Infrared Spectroscopic Method

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Abstract

This study aims to develop a simple, rapid and non-destructive determination method of nitrate nitrogen in leaves, which is a place for photosynthesis and could be an index of the plant vigor, using Fourier transform infrared (FT-IR) spectroscopy with an attenuated total reflection (ATR) method. The spectral characteristics of two types of the leaf model solutions, which were the aqueous solution consisting of chemical reagents according to the concentrations in the typical plant leaf with varying concentration of nitrate nitrogen and leaf juice added the specified concentration of nitrate nitrogen after filtration, were studied. At around 1350 and 1392 cm⁻¹ in which the peaks characterizing nitrate nitrogen were observed, the relationship between the absorbance spectrum of the model solution and the nitrate nitrogen content showed the excellent linearity. Additionally, for a leaf model that was prepared by impregnating the model solution into a dried filter, the nitrate nitrogen content could be determined by the developed FT-IR/ATR method.

Keywords: Mid-infrared spectroscopy, plant leaf, nitrate nitrogen

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 by a simple, non-destructive, simultaneous, and rapid method. In addition, the measured data need to be retrieved for future simulation using various modeling software.

Applications of X-ray fluorescent (XRF) and mid-infrared (MIR) spectroscopy, which could respectively grasp the elemental and organic information, to the simple, non-destructive, simultaneous, and rapid monitoring of the nutrient information of fresh plants are desirable as the high potential implements. We reported the great potentiality of the both spectroscopic methods to get the quantitative nutrient information of tomato (Hashimoto et al., 2004; Hashimoto et al., 2005; Hayashi et al., 2007). It was found that the developed XRF spectroscopic method was useful as the quantitative analytical method for the elemental contents of the fresh leaf by comparing with an Inductively Coupled Plasma method which is one of the standard methods (Hashimoto et al., 2006).

We also developed a MIR spectroscopic method to accumulate the quantitative spectral information relating to the organic components, such as the saccharides and organic acids, of
fresh leaf (Hashimoto et al., 2005; Hashimoto et al., 2006). The content balances of the different modes of nitrogen could be important and effective index for the cultivation control. Then this study aims to develop a simple, rapid and non-destructive determination method of nitrate nitrogen in the leaf models, which is a place for photosynthesis and could be an index of the plant vigor, using Fourier transform infrared (FT-IR) spectroscopy with an attenuated total reflection (ATR) method (Harrick, 1967). This is the first approach to the simultaneous determination of the nitrogen contents in different modes. The spectral characteristics of two types of the leaf model solutions, which were the aqueous solution consisting of chemical reagents according to the concentrations in the typical plant leaf with varying concentration of nitrate nitrogen (Leaf Model Solution 1) and leaf juice added the specified concentration of nitrate nitrogen after filtration (Leaf Model Solution 2), were studied. In addition, for a Leaf Model that was prepared by impregnating the Leaf Model Solution 2 into a dried filter, the spectral behavior with the change of the nitrate nitrogen content was also quantitatively studied.

**Experiments**

**Materials**

Two types of leaf model solutions were prepared for understanding the spectral features of nitrate nitrogen. Leaf Model Solution 1 is the chemical reagent mixture composing of the plural modes of nitrogen. Casein, which 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 1). The standard concentrations were fixed according to the concentrations in the typical plant leaf. In addition, the tomato leaf juice added the specified concentration of 0.1 N sodium hydroxide solution after filtration (Leaf Model Solution 2) was also used for the spectral measurements. The tomatoes were cultivated in a green house at the Experimental Farm of Mie University. Then Leaf Model Solution 2 includes not only the components in Leaf Model Solution 1 but also the minors.

Furthermore, a filter was impregnated with Leaf Model Solution 2. So the filter has the polysaccharide and the fresh leaf juice components with the complicated geometrical structure. The filter was dried in a desicator at the room temperature (298 K) until the weight change was negligible, and the Leaf Model for the spectral measurements was then prepared.

<table>
<thead>
<tr>
<th>Table 1. Concentrations of nitrogen in Leaf Model Solution 1.</th>
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<tbody>
<tr>
<td>solutions</td>
</tr>
<tr>
<td>no nitric acid</td>
</tr>
<tr>
<td>1/2-fold dilution</td>
</tr>
<tr>
<td>2/3-fold dilution</td>
</tr>
<tr>
<td>standard solution</td>
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<tr>
<td>2-fold dilution</td>
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A Fourier transform infrared spectrometer (FT-IR; TravelIR, SensIR TECHNOLOGIES) equipped with an ATR sampling accessory with a diamond internal reflection element (IRE) was used to collect the MIR spectral information of the samples. The liquid samples were put on the IRE crystal and the spectra were collected. The Leaf Model was pressed with a metal pole on the IRE crystal in order to stabilize the sampling point (Hashimoto et al., 2006). Then the MIR spectra were obtained from 4000 to 800 cm\(^{-1}\) at 4 cm\(^{-1}\) resolution.

As the measurements of the reference values, the analysis of nitrate nitrogen was made by a Tosoh (Tokyo, Japan) ion chromatograph (IC) system, comprised of a model DP-8020 pump and a model CM-8020 electro conductivity detector. A TSK-gel IC-ANION-PW (Tosoh, Tokyo, Japan) IC analytical column (4.6×50 mm) was used. The mobile phase was the boric acid buffer after filtration through a 0.2-\(\mu\)m membrane filter. The Leaf Solution Models 1 and 2 and the extracted solution of the Leaf Model to hot water were prepared for the filtration. The flow rate was 1.0 ml/min, and the column temperature was maintained at 313 K with a column oven (CO-8020, Tosoh, Tokyo, Japan). The electro conductivity of the elute was monitored.

### Results and Discussion

**Spectral characteristics of nitrate nitrogen in aqueous solutions**

Figure 1 shows the MIR spectra of the Leaf Model Solution 1 for the various concentrations of nitrate nitrogen with the spectrum of the correlation coefficient between the absorbance and the concentration over the wavenumber range. The peaks relating to nitrate nitrogen at 1350 and 1392 cm\(^{-1}\), which are could be attributed to the NO stretching and bending modes (Chemical Society of Japan, 1993), respectively, were observed. Additionally, the \(r^2\) value shows higher than 0.998 in the wavenumber range of 1300cm\(^{-1}\) to 1400 cm\(^{-1}\). On the other hand, 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 in Figure 1. Then the spectral information in the wavenumber range from 1400 to 1300 cm\(^{-1}\) could be effective for the nitrate nitrogen content determination for the aqueous solution and the wet materials.
In order to understand the spectral features of the leaf juice as the natural product containing the minor components except nitrate and proteinic nitrogen, the spectral characteristics of the Leaf Model Solution 2 were analyzed by focusing our attention on the wavenumber range from 1400 to 1300 cm\(^{-1}\). Figure 2 displays the MIR spectra of the Leaf Model Solution 2 for the various concentration of nitrate nitrogen. The correlation coefficient for the Leaf Model Solution 2 including the minor components indicated the similar excellent value to that for the Leaf Model Solution 1 composed from the pure chemical reagents in the above wavenumber region. These results experimentally suggest that the MIR spectroscopic method could be acceptable to the nitrate nitrogen content determination in the chemically complicated liquid solution such as the leaf juice.

**Spectroscopic determination of nitrate nitrogen content in Leaf Model**

The spectral features of the Leaf Model, which has the complicated geometrical structure with the leaf components of the Leaf Model Solution 2 and of the filter such as cellulose, were analyzed by focusing our attention on the absorption bands of nitrate nitrogen in order to develop the quantification method. Figure 3(a) indicates the influence of the nitrate nitrogen
content on the MIR spectra of the Leaf Model and the spectrum of the correlation coefficient between the absorbance and the concentration over the wavenumber range. The absorption peaks due to nitrate nitrogen are not obvious in the wavenumber range from 1400 to 1300 cm$^{-1}$, since the absorption peaks for the filter components were much stronger than those for nitrate nitrogen. However, as shown in the magnified graph (Figure 3(b)), the absorption at 1350 and 1392 cm$^{-1}$ increased in the increase of the nitrate nitrogen content, and the good correlation was obtained.

We performed simple and multiple linear regression analysis about the relationship between the absorbance at the wavenumber mentioned above and the nitrate nitrogen content in the Leaf Model and the comparison of the calculated values using the spectral information and those obtained by the HPLC method is displayed in Figure 4. The determination coefficients, $r^2$, for the simple linear regression analysis were 0.72 and 0.68 using the spectral information at 1350 and 1392 cm$^{-1}$, respectively. The coefficient for the multiple linear regression analysis using the both spectral information was 0.73 and very slightly higher than those for the simple linear regression analysis. The results differences between the Leaf Model Solutions and the Leaf Model could reflect the chemical and geometrical complications. Hence the developed MIR spectroscopic method could be effective for the content determination of nitrate nitrogen in the Leaf Model, which has the complicated geometrical structure with the components of the fresh leaf juice and the polysaccharides such as cellulose.
Concluding Remarks

A simple, rapid and non-destructive determination method of nitrate nitrogen in the Leaf Model, which has the fresh leaf components with the complicated geometrical structure, was developed by the FT-IR/ATR method. Therefore, the method and results in this study might contribute to simultaneously determine the plural modes of nitrogen in fresh leaf, which is a place for photosynthesis and could be an index of the plant vigor.

References