Measurement of Nitrate Concentration Distribution in Vegetables by Near-Infrared Hyperspectral Imaging

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The purpose of this research is to develop a laboratory-based near-infrared (NIR) hyperspectral imaging system to measure the two-dimensional distribution of nitrate concentrations in a vegetable leaf as a tool for precisely analysing nitrate metabolism. Komatsuna leaves were analyzed by hyperspectral reflectance in the range from 607 to 967 nm with a resolution of 9 nm. The reflectance was standardized by a reference plate and was converted to relative reflectance. An algorithm to select the effective wavelength to predict the nitrate concentration was developed in conjunction with partial least squares (PLS) regression and principal components regression (PCR). As for preprocessing methods for the spectra, mean-centre and standard normal variate transformations were examined. Estimation accuracy of the developed models was evaluated by the weighted average of standard error (WSE). The estimation accuracy of the wavelength-selected models was improved and the WSE was smaller than that of the full-spectrum model (41 wavelengths). The calibration model that used 21 wavelengths achieved the best WSE of 1446 ppm with a correlation coefficient of 0.870. The nitrate distribution in Komatsuna leaves were visualized in digital images with a spatial resolution of 2.5 × 10⁻⁶ mm/pixel. These images showed that the transporting route of nutrients contains higher nitrate ion concentration than other areas in the Komatsuna leaf.

Keywords: hyperspectral camera, image processing, Komatsuna, near-infrared spectroscopy

INTRODUCTION

Nitrate (NO₃⁻) is found naturally in the environment and is an important plant nutrient. It is present in varying concentrations in all plants and is a part of the nitrogen cycle. Since nitrate is the most stable oxidation state, nitrite (NO₂⁻) is not usually present in significant concentrations except in a reducing environment. In general, the most important source of human exposure to nitrate is through vegetables. In a human body, nitrite can be formed by the microbial reduction of nitrate, which is one of the constituents in vegetables. Nitrites can lead to methemoglobinemia and the formation of N-nitroso compounds, which are carcinogenic (Guidelines for drinking-water quality, 2008).

In order to decrease nitrate concentration in vegetables, it is necessary to understand nitrate metabolism, especially the activation of nitrate reductase (NR). In a plant body, nitrate ion is re-
duced to ammonium ion by NR and nitrite reductase (NiR). Presently, it is known that an increase of nitrate concentration inside a cell induces NR activation, but this is confirmed for the leaf as a whole and not for each point inside a leaf. It is unclear whether NR becomes active at the point where the nitrate concentration is high; it is possible that something other than nitrate ion can activate NR. That is, an unknown signalling transmitter, which communicates the increase of the nitrate concentration inside a leaf, can induce NR activation. For the precise analysis of nitrate metabolism, the spatial distribution of nitrate concentration in the leaf plane is one of the most important pieces of information to acquire.

The purpose of this study is to obtain the distribution image of nitrate concentration in a leaf plane in order to accurately analyze the relationship between nitrate concentration and NR activation. The distribution image will be used to determine the nitrate concentration at each point inside a leaf. If NR is active at the point where the nitrate concentration is low, a signalling transmitter may exist. This imaging technology will help clarify the signalling pathway that activates NR.

A high-spatial-resolution distribution image of the nitrate concentration is required for this research. It is difficult to measure the nitrate concentration at a small point in the leaf by means of constituent analyzers such as liquid chromatography because the mass of the point sample is extremely less. Near infrared spectroscopy (NIRs) was used in this research. NIRs has been an important tool for the qualitative and quantitative analyses of the internal properties of fruits and vegetables, such as kiwifruit (Shaare and Fraser, 2000), tomato (Khuriyati and Matsuoka, 2004, 2005; Suhandy et al., 2006) and oilseed rape leaves (Liu et al., 2009). For the measurement of nitrate concentration, Ito et al. (2003) examined visible-NIR absorption spectra obtained from rhizomes of Japanese radishes; the calibration model that used only four wavelengths was derived by a stepwise regression method. Matsumoto et al. (2009) developed models to measure nitrate concentration in the whole body of a lettuce by using absorption spectra in the range of 700–960 nm; the potential to measure nitrate concentrations in vegetables by NIRs has been reported.

The spectra measurements shown in the above report do not resolve the spatial distributions of constituents within a sample. To solve this problem, hyperspectral imaging is an effective tool to measure a spectrum at each pixel in digital images. Recently, ground-based or laboratory-based hyperspectral imaging systems have been developed. Evans et al. (1998) developed a hyperspectral imaging system with a liquid-crystal tuneable filter, and the system was used to evaluate the vigour of bush bean plants grown under different nitrogen treatment. Martinsen and Shaare (1998) visualized the distribution of soluble solids in kiwifruit. The calibration model used the selected wavelengths that were derived from the iterative partial least squares method (Osborne et al., 1997). Kim et al. (2001) developed the hyperspectral reflectance and fluorescence imaging system, and the versatility of the system was demonstrated on apples with fungal contamination and bruised spots. Cogdill et al. (2004) predicted the moisture and oil contents of single maize kernels from near-infrared hyperspectral image data. A hyperspectral imaging system was used to analyze the internal properties of strawberries (Kobayashi et al., 2006; Nagata et al., 2006a; Tallada et al., 2006) and sweet potatoes (Nagata et al., 2006b). Suzuki et al. (2006, 2008a) used ground-based hyperspectral imaging to measure the chemical composition of grass. Okamoto et al. (2007) and Suzuki et al. (2008b, 2009) measured the hyperspectra and developed algorithms to distinguish plants that were grown in a particular field. Ye et al. (2006) developed a ground-based hyperspectral imaging system and used it to recognize the new or old leaves of a citrus tree. Ariana and Lu (2009) detected hollow or bloater damage inside pickles from images of the second principal component score that was calculated from the reflection and transmittance spectra. Junkwon et al. (2009) analyzed the hyperspectral images of oil palms to estimate ripeness, oil content and free-fatty-acid content.

NIRs and hyperspectral imaging are very effective tools to analyze the two-dimensional distributions of the internal properties of plants. In this research, the distribution of nitrate concentration in a Komatsuna leaf was derived from spectra, which were measured at small points in the leaf by
a near-infrared hyperspectral imaging system. Finally, digital images of the nitrate distribution in Komatsuna leaves were generated.

MATERIALS AND METHODS

Sample preparation for calibration
A Komatsuna (‘Hakatakomatsuna’, Brassica rapa var. peruviridis) leaf was examined. It was cultivated by four fertilization treatments (chemical, organic, a mixture of fertilizers and hydroponics) to make the nitrate concentration range sufficiently broad. The wide variation of the objective variable, which is the nitrate concentration in this research, is necessary in order to develop an effective regression model. Small size is preferable because the spectral image had a very fine spatial resolution. This camera measures spectra of all pixels inside the sample region. The mean spectrum over pixels inside each sample region was used for calibration. If the area of the sample is large, the variation of nitrate concentration inside the sample plane tends to be wide. Therefore, actual characteristics of the sample will not be represented by the mean spectrum. Further, the prepared sample for calibration should be as small as possible. However, the minimum size was limited by the ion chromatograph specifications. The extraction concentration from a sample that is extremely small becomes very low and falls below the specified measurement range. The adequate weight of a leaf piece was about 0.02 g and area was about 1 cm² in this research. The nitrate concentration of the petiole and leaf vein is higher than the leaf blade. Blocks, which did not contain petiole and leaf blade, were cut off from the sample leaves by a scalpel. The prepared leaf pieces were covered with aluminium foil to prevent evapotranspiration until the spectrum measurement and were transported by the carriage of a uniaxial robot (BST-250, ERC YAMAHA, Japan). The leaf pieces were arranged in a staircase pattern on a black rubber plate.

Hyperspectral imaging system
The hyperspectral imaging system consisted of a detector, light source and sample stages (Fig. 1 (a)). The detector was composed of a charge-coupled device (CCD) camera (BCi4 C-Cam, Belgium), a spectrograph (Inspexeter V10, Oulu, Finland) and an f1.4 C-mount lens (16 mm Television lens COSMICAR, Japan). The horizontal and vertical axes on the CCD were spatial and spectral information, respectively, and the dynamic data range of luminous intensity was 8-bit. A spectral image contains 1,280 spatial pixels and 1,024 spectral bands (from 395 to 1,088 nm). Because the resolution of the spectrograph is 9 nm, only 70 spectral bands are applicable. As shown in Fig. 1 (a), the camera was fixed downward. The length from the lens to the object was set at 0.64 m and the spatial resolution of the captured image was $\frac{2.500 \times 10^{-4}}{\text{m/pixel}}$ with this condition. As for the light source, two reflector flood lamps (RF100V300W National, Japan) were adopted to measure the NIR reflectance. Illuminance on the object surface was 37400 lx as measured by an illuminance meter (T10 MINOLTA, Japan). This camera measures the spectra of points on one scanning line; thus, the object has to be moved to create a two-dimensional spatial image.

![Hyperspectral imaging system without (a) and with (b) interception boards.](image)
Objects were moved by the uniaxial robot with a velocity of 1 mm/s in order to make the aspect ratio of the captured image equal to that of the objects. A black plate made from sponge rubber (SP-04 SANYU SANGYO, Japan) was laid on the carriage of the uniaxial robot. Objects were placed on the rubber plate and were captured against the background of the rubber. This rubber absorbs almost all of the visible-NIR light. Reflectance from the rubber was almost zero at every wavelength.

A heat interception board was used because strong infrared rays radiated from the reflector flood lamps and damaged to the rubber plate and sample leaves. The carriage was covered with two acrylic plates (Fig. 1 (b)). Black felt cloth was placed on the acrylic plates to intercept the heat rays. The light from the flood lamps illuminated the sample leaves through a narrow gap between the two plates to reduce heat damage. Sponge rubber was pasted on the under side of the plates to prevent diffused reflection under the plates.

The sampling period and the sampling number of the spectrum image were adjustable by using a software written in the C/C++ language. The sampling period was set to 15 ms. The aperture stop of the camera lens was adjusted manually so that the maximum reflectance of a reference plate does not get saturated. The focus was adjusted to make the spectrum edge of the reference plate sharp (Fig. 2 (b)).

Dynamic range of each wavelength

The spectrometer’s photometric response was not uniform over the wavelength. Figure 2 shows a spectrum measurement of a reference plate on the sponge rubber and an output image from the hyperspectral camera. Because the white reference plate reflects the entire incident light, the reflectance of all wavelengths should be large and uniform. However, the spectrum of the reference plate was not uniform as shown in Fig. 2 (b). This meant that the spectrometer’s sensitivity was not uniform over the wavelength and dynamic range of the measured values. In order to determine the dynamic range of each wavelength, the spectra of the reference (upper limit) and rubber (lower limit) plates were measured.

The hyperspectral camera used was a line sensor. The camera measures object spectra of each pixel in one line. The horizontal and vertical axes of the spectral image are the spatial and wavelength axes, respectively (Fig. 2 (b)). Hundreds of spectral images were captured to measure the spectra of the whole object by moving the carriage of the uniaxial robot. The spectral image, which was obtained from the line on the centre of the reference plate, was extracted to get the dynamic range of each wavelength. First, a two-dimensional spatial image (2DSI) at 538 nm was obtained (Fig. 3 (a)). The vertical and horizontal axes of 2DSI were both spatial axes. The 2DSI is like a normal picture captured by a conventional camera except that a wavelength has to be specified. One row that was located at a vertical position of 538 nm in the spectral image (example, Fig. 2 (b)) was extracted from the entire captured spectral image. The extracted rows were placed in the same order as they were captured, and then the 2DSI was obtained (Fig. 3 (a)). In order to detect the centre of the reference plate, the reference plate and other (sponge rubber) regions must be distinguished first. The labelling process, which was a type of image-processing method, was applied to a binary image that was a result of threshold processing. The region of reference plate was then identified and the coordinate of the centre (d, e) was detected as shown in Fig. 3 (b). The vertical coordinate of the centre coincides with the capturing number of the spectral image. The eth image shown in Fig. 4 was the spectral image of the line on the centre of the reference plate. For the intensity measurement of the reference and rubber plates at wavelength f, a mean intensity over 100 pixels on a row whose centre was the horizontal coordinate d at vertical coordinate f was calculated for the reference plate. A mean intensity over 100 pixels on another row whose centre was 300 pixels apart from the centre of the reference plate at vertical coordinate f was calculated for the rubber plate. This procedure was repeated until the mean intensity of the reference and rubber plates of the entire wavelength were measured. Finally, the dynamic range of each wavelength was
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Fig. 2  Reference and rubber plates on carriage of uniaxial robot (a) and spectral image obtained from a scanning line (b).

Fig. 3  Composed image of reference plate against the background of rubber plate at 538 nm (a) and coordinates of the centre of the reference plate in two-dimensional spectral image (b).

Fig. 4  Measured points at wavelength $\lambda$ in spectral image that resulted from a scanning line on centre of reference plate.

obtained from the detected spectral image.

Flat-field corrections

Flat-field (reference plate) correction was applied to all pixels in the two-dimensional spatial image at all wavelengths in order to make the dynamic range uniform over all wavelengths as equation (1) (Kim et al., 2001; Tallada et al., 2006),

$$ R_{\text{rel}} = \frac{RS_i - RB_i}{RW_i - RB_i} \times 100 $$  \hspace{1cm} (1)

where $R_{\text{rel}}$ is the relative reflectance at wavelength $\lambda$ of pixel $i$, $RS_i$ the intensity at wavelength $\lambda$ of pixel $i$, $RW_i$ and $RB_i$ are the intensities of the reference plate and sponge rubber, respectively; both intensities show the dynamic range of wavelength $\lambda$. 

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Spectral measurement of each leaf piece

On applying the same procedure to the reference plate, hundreds of spectral images of the leaf pieces were captured; 2DSI of the pieces after a flat-field correction was generated to recognize regions of each leaf piece. The 2DSI at 539 nm, which was the wavelength of green colour, was used. The dynamic range of the relative reflectance \( R_{\lambda} \) (0–100) was converted to the dynamic range of digital images (0–255) by a proportional conversion and Fig. 5 (a) was generated. Thresholding was applied to the 2DSI with the threshold value of 10 and labelling was conducted (Fig. 5 (b)). Regions of each leaf piece in the 2DSI were recognized. The labelling algorithm scans pixels from left to right and from top to bottom. This is the reason why leaf pieces were set in a staircase pattern.

Absorption \( A_{\lambda i} \) at wavelength \( \lambda \) of pixel \( i \) was derived from the relative reflectance \( R_{\lambda i} \) as equation (2).

\[
A_{\lambda i} = \log_e \frac{100}{R_{\lambda i}}
\]  

(2)

The mean absorptions over pixels inside each piece region of 2DSI at all wavelengths were calculated. Finally, the absorption spectra of leaf pieces were obtained. The absorption spectrum consisted of 70 wavelength bands in the range 400–1,021 nm with a resolution of 9 nm.

Absorption spectra preprocessing

In this research two types of preprocessing were tested; one was mean-centre and the other was the standard normal variate transformation. The former was adopted to remove a spectral offset as equation (3) and the latter was used for removal of additive and multiplicative spectral effects as shown in equation (4),

\[
MSX_{\lambda i} = A_{\lambda i} - \bar{A}_{\lambda}
\]

\[
SNVX_{\lambda i} = \frac{A_{\lambda i} - \bar{A}_{\lambda}}{\sigma_{\lambda}}
\]

where \( MSX_{\lambda} \) is mean-centre transform of absorption \( A_{\lambda} \), \( \bar{A}_{\lambda} \), and \( \sigma_{\lambda} \) are mean and standard deviations of \( A_{\lambda} \), respectively and \( SNVX_{\lambda} \) is the standard normal variate transform of \( A_{\lambda} \).

Nitrate concentration measurement

After the capture of spectral images, each leaf piece was immediately put into 2 ml tubes with hundredfold distilled water. The tubes were boiled for 15 min. The nitrate concentration of the extraction liquid was measured by using RQflex plus10 (MERCK, Germany). RQflex was usually used as a guide for ion chromatography analysis; however, the measured values of nitrate concentration by RQflex were used in this research because of a mechanical problem with the ion chromatograph.

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Fig. 5 Two-dimensional spatial image of leaf pieces at 539 nm after flat-field correction (a) and generated image after labelling processing (b).
Data modelling

In the above measurement, 232 sets of absorption spectra coupled with the nitrate concentration of leaf pieces were obtained. Some leaf pieces showed very low concentrations that were lower than the measurement range; also, there were some mistakes in the measurement of concentrations. Further, 160 sets of the predictor variable, which was the absorption spectra, coupled with the objective variable, which was the measured nitrate concentration, were prepared. The data sets were divided into two groups, namely, calibration data to develop model equations and validation data to probe the accuracy of the models. The ratio of the number of calibration data to that of validation data was 2 to 1. The data sets were split in order to make the statistical characteristics of the objective variable equal as follows. First, all the data sets were sorted in order of the nitrate concentration. The sorted data sets were segmented into many blocks with a three data interval. In each block, the first and second data were assigned as the calibration data, and the third data was allocated to the validation data. The remainder, after dividing 160 by 3, was assigned to the calibration data. Finally, the number of calibration and validation data became 107 and 53, respectively.

Modelling combined with method of wavelength selection

Models to estimate the nitrate concentration were developed by using principal component regression (PCR) and partial least square (PLS) methods. A number of factors were decided as follows.

Step 1: First, a model was derived from the first factor of the calibration data. The validation data were substituted into the model and the correlation coefficient between the measured and estimated nitrate concentrations of the validation data was calculated.

Step 2: The next factor was added and a new model was obtained from the factors. Estimations of the validation data were computed by the new model and the new correlation coefficient between the measured and estimated nitrate concentrations of the validation data was calculated.

Step 3: If the new correlation coefficient was larger than the old one, step 2 was repeated until the new correlation coefficient becomes lesser. Finally, the number of factors that were used in the calculation of the last old correlation coefficient was adopted.

In this research, a wavelength selection method was developed. Osborne et al. (1997) developed iterative partial least squares (IPLS) method that was based on the PLS method incorporated with wavelength selection like a forward stepwise regression. The accuracy of the developed model by the IPLS was affected by selection of starting wavelengths or step size of the wavelength in the stepwise operation. In this method, a candidate wavelength, which was used in the next step, was selected randomly and the judgment of whether the wavelength was adopted to a model depended on standard error of estimations.

In our research, a criterion to select the candidate wavelength was variance of absorption over the calibration data at each wavelength. Absorption at the effective wavelength to estimate the nitrate concentration must vary as a variation of nitrate concentration and will show large variance. This wavelength selection was one of the backward selection methods. Full wavelengths were examined at first and the number of wavelengths was reduced to two as in the procedure explained below. The effect of resolution of wavelength was also analysed in this selection procedure as follows:

Step 1: The initial resolution of the wavelength was set artificially. The resolutions of 9, 18, 27 and 36 nm were considered. Because the specification as per the wavelength resolution of the spectrograph was 9 nm, absorption data at the unused wavelengths were removed when the resolutions of 18, 27 and 36 nm were selected.

Step 2: Variances were calculated as absorption at each wavelength selected in step 1 over all the calibration samples.
Step 3: A model to estimate the nitrate concentration was developed by the PCR or PLS method with the absorptions at the selected wavelength that was reduced from full wavelengths to 2 in step 4. Three kinds of standard error were calculated, namely, standard error of calibration (SEC), standard error of prediction (SEP) and weighted average of SEC and SEP (WSE) as shown in equations (5)–(7),

\[
SEC = \sqrt{\frac{1}{n_p-1} \sum_{i=1}^{n_p} (y_{ei} - \hat{y}_{ei})^2}
\]  

(5)

where \( n_p \) and \( p \) are the number of calibration data and wavelengths used, \( y_{ei} \) and \( \hat{y}_{ei} \) are the measured and estimated nitrate concentration of the \( i \)th calibration data.

\[
SEP = \sqrt{\frac{1}{n_v-1} \sum_{i=1}^{n_v} (y_{vi} - \hat{y}_{vi})^2}
\]  

(6)

where \( n_v \) is the number of validation data, \( y_{vi} \) and \( \hat{y}_{vi} \) are the measured and estimated nitrate concentration of the \( i \)th validation data.

\[
WSE = \frac{SEC \times n_p + SEP \times n_v}{n_v + n_p}
\]  

(7)

Step 4: A wavelength, which showed the minimum variance of absorption, was removed from the wavelengths used in step 3.

Step 5: Repeat steps 3 and 4 until the number of wavelengths is reduced to 2.

A model that showed the minimum WSE was adopted as the most effective model to estimate the nitrate concentration. The wavelengths that were used in the best model were effective wavelengths for the estimation.

**Nitrate concentration distribution measurement in leaves**

After harvesting, each leaf in the stock was separated from outside to inside and was numbered in the order of the separation. A new leaf sprouts out between older leaves and then the leaf number of an older leaf becomes smaller.

The strong heat rays made leaf samples recurve during the capture of hundreds of spectral images. Reflection from the recurred surface did not reach the lens of the camera sufficiently. Spectra obtained from the uneven surface did not include enough information. Then the model estimation that was obtained from the incomplete spectrum tends to show large error. Therefore, the petiole of the sample leaf was separated from the leaf blade and several points of the leaf periphery were cut. Each separated part of a sample leaf was impaled on the rubber plate to keep the surface flat. After spectral images of the leaf were captured, the leaf was boiled with distilled water and the nitrate concentration of the extraction liquid was measured by using RQflex.

Similarly, applied to the leaf pieces, hundreds of spectral images of a leaf sample were captured, and after the flat-field correction the 2DSI of the leaf was generated to recognize regions of each separated part. The 2DSI at 742 nm was used because a fine image of leaf sample was obtained at that wavelength. Thresholding and labelling were conducted on the 2DSI with the threshold value of 70. The region of each leaf part in the 2DSI was recognised. The absorption spectrum of each pixel inside the leaf regions was measured. The same preprocessing, which was applied to the development of the best model, was applied to the absorption spectra. The preprocessed spectra were substituted into the model and the nitrate concentration of all pixels inside the leaf regions was estimated. Finally, the nitrate concentration distribution was visualized in a digital image by converting the concentration to greyscale. The mean concentration of all the pixels in the leaf regions was calculated and was compared with the measured value.

The analysis explained above was carried out using the software written by the authors in the C/C++ programming language.
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RESULTS AND DISCUSSION

Data used in this analysis

Figure 6(a) shows the raw spectra of 232 leaf pieces. There was much noise in visible light and infrared light over 1,000 nm. Therefore, the absorption spectrum in the range from 607 to 967 nm was used to estimate the leaf’s nitrate concentration. Figure 6(b) shows raw spectra of 160 leaf pieces that were used in this analysis. The 160 data were split into the calibration and validation data. The ratio of the number of calibration data to that of validation data was 2 to 1. Table 1 shows statistical characteristics of both the data. There was no large difference in the variation of both data. The range of nitrate concentration was sufficiently wide to actually use this measurement. Two types of preprocessing methods were applied to the raw spectra. Preprocessed spectra of the calibration data are shown in Fig. 7. Compared to Fig. 6(b), offsets among the spectra were reduced by the mean-centre method (Fig. 7(a)) and absorption of each spectrum was normalized by the standard normal variate transformation as shown in Fig. 7(b).

Results of modelling

Models in four categories were developed according to the preprocessing and modelling methods. The effects of the initial resolution or the number of wavelengths on the weighted average of standard error $WSE$ were examined for each model. Table 2 shows the four models that show minimum $WSE$ in each category. $WSE$ obtained from PCR was smaller than that from PLS. Although mean-centre was more effective than the standard normal variate transformation, the difference of $WSE$ between the preprocessing methods was not large in the case of PCR. The best accuracy was obtained from the model that was derived from the PCR method by using mean-centre spectra. Figure 8 shows the comparison of measured and estimated nitrate concentration. Adequate corre-

![Fig. 6](image.png)

**Fig. 6** Raw absorption spectra of 232 leaf pieces (a) and absorption spectra of 160 pieces used in this analysis (b).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Variation in calibration and validation data sets (units: ppm).</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of spectra</td>
</tr>
<tr>
<td>Calibration</td>
<td>107</td>
</tr>
<tr>
<td>Validation</td>
<td>53</td>
</tr>
</tbody>
</table>

![Fig. 7](image.png)

**Fig. 7** Absorption spectra of calibration data after preprocessing: (a) mean-centre; (b) standard normal variate transformation.
Table 2 Comparison of developed models.

<table>
<thead>
<tr>
<th>PP</th>
<th>MM</th>
<th>IR (nm)</th>
<th>NW*</th>
<th>NF*</th>
<th>SEC (ppm)</th>
<th>R^2</th>
<th>SEP (ppm)</th>
<th>WSE (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNV</td>
<td>PCR</td>
<td>9</td>
<td>21</td>
<td>21</td>
<td>7.917E+01</td>
<td>1.639E+03</td>
<td>8.609E+01</td>
<td>1.125E+03</td>
</tr>
<tr>
<td>PLS</td>
<td>27</td>
<td>12</td>
<td>6</td>
<td>5.216E+01</td>
<td>2.363E+03</td>
<td>7.128E+01</td>
<td>1.581E+03</td>
<td>2.104E+03</td>
</tr>
<tr>
<td>M-C</td>
<td>PCR</td>
<td>18</td>
<td>21</td>
<td>16</td>
<td>8.019E+01</td>
<td>1.598E+03</td>
<td>8.698E+01</td>
<td>1.138E+03</td>
</tr>
<tr>
<td>PLS</td>
<td>18</td>
<td>21</td>
<td>8</td>
<td>7.909E+01</td>
<td>1.642E+03</td>
<td>8.598E+01</td>
<td>1.160E+03</td>
<td>1.482E+03</td>
</tr>
</tbody>
</table>

* Preprocessing, \* Modelling method, \* Initial resolution, \* Number of wavelengths, \* Number of factors, \* Coefficient of determination of calibration data, \* Correlation coefficient of validation data, \* Standard normal variate transformation, \* Mean-centre

Figure 8 Comparison of measured and estimated nitrate concentration of calibration data (a) and validation data (b).

...loration was found in a wide range of nitrate concentrations for both the calibration and validation data.

Figure 9 shows variation of WSE in the wavelength-selection procedure when the PCR method was applied to the mean-centre spectra. Because this selection method is a backward stepwise procedure, the number of wavelengths was decreased step by step until the number was reduced to two. WSE tended to increase as the number of wavelengths decreased. The initial resolution of the wavelength affected the estimation accuracy. Resolutions such as 27 and 36 nm did not generate an accurate model. However, some models developed from an initial resolution of 18 nm gave smaller WSE than models obtained with an initial resolution of 9 nm. It was found that the wavelength-selected models were more effective than a full-spectrum model.

Measurement of nitrate concentration distribution in a leaf

Figure 10(a) shows a 2DSI at 742 nm. The petiole of the sample leaf was separated from the leaf blade and several points of leaf periphery were cut to keep the surface flat. By using the best model, nitrate concentration of each pixel inside leaf regions in 2DSI was calculated. In order to visualize the nitrate distribution, each pixel was separated by a greyscale, according to the estimation of the nitrate concentration. The nitrate distribution of a leaf is shown in Fig. 10 (b). High intensity shows high concentration. Comparing Fig. 10(a) with Fig. 10(b), the petiole and leaf vein showed higher intensity than the leaf blade. This result means that the transporting route of nutrients contains higher quantity of nitrate ions. However, the edge of the blade that is far from the petiole and leaf vein also showed high intensity. It was considered that the reflection from the edge of the leaf was not sufficient to include a large error in the estimation of the pixel.

Figure 11 compares the measured nitrate concentrations and the estimated concentration of the leaves. The estimation is the average value that was calculated from all pixels inside the leaf area. The horizontal axis of the figure shows the leaf number assigned from outside to inside. A new leaf sprouts out between older leaves and then the leaf number of an older leaf becomes smaller. There was a tendency that the more mature the leaf was, the higher the nitrate concentration.
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**Fig. 9** Relation between number of wavelengths and WSE for an initial resolution with 9 nm (a), 18 nm (b), 27 nm (c) and 36 nm (d), when PCR method was applied to mean-centre spectra.

**Fig. 10** Two-dimensional spatial image of Komatsuna leaf at 742 nm (a) and nitrate concentration distribution of the leaf (b).

**Fig. 11** Comparison of measured and estimated nitrate concentrations of leaves in stock.

Although there were large estimation errors in some leaves, the estimation showed a similar tendency to the measured values. As shown in Fig. 8, the high accuracy of used model was proved but there was a large error in the measurement of average concentration in a leaf. The uneven surface would affect the estimation accuracy and the unevenness was emphasized by the strong heat
ray from the light sources. The unevenness was not observed in the leaf piece because the area of the leaf piece was small. Furthermore, it seemed that it was difficult to receive sufficient light from the blade edge that curls from outside to inside, although the sample leaves were stretched and impaled on the rubber plate.

CONCLUSION

The laboratory-based near-infrared hyperspectral imaging system was developed to measure the nitrate distribution in a Komatsuna leaf for precisely analysing nitrate metabolism. By using the hyperspectral imaging system, the spectra of small leaf pieces were measured and the calibration models were developed by the PCR or PLS methods coupled with the wavelength-selection method. The hyperspectral imaging system measured the spectra of each pixel in a leaf and the developed model estimated nitrate concentration of the pixels. Finally, nitrate concentration distribution in a leaf was visualized in greyscale.

The combination of mean-centre preprocessing, which was applied to raw spectra after the flat-field correction, and the PCR method generated the best calibration model. The WSE and correlation coefficient of the model were 1,446 ppm and 0.870, respectively. This model used 21 wavelengths and the WSE was smaller than that of the full-spectrum model.

The greyscale image of the nitrate concentration distribution showed the high intensity at the petiole and leaf vein and then the transporting route of nitrate was identified clearly.

The uneven surface would decrease the estimation accuracy of the model. Because the strong heat ray made the unevenness more pronounced, an alternative light source should be used.

It was found that the hyperspectral imaging system was effective for the nondestructive measurement of nitrate concentration at fine points in a leaf.

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REFERENCES


MEASUREMENT OF NITRATE DISTRIBUTION


