Determination of Leaf Water Potential in Tomato Plants Using NIR Spectroscopy for Water Stress Management

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Quality of tomato fruits can be improved by applying water stress. To avoid the excessive yield reduction and high blossom-end rot incidence, it is highly important to apply water stress at an optimum level. In this research, a calibration model for nondestructive determination of leaf water potential (LWP) was developed using near infrared spectroscopy. A tomato leaf was cut and its spectra in transmittance mode at six different positions were immediately measured. The LWP was determined simultaneously using the pressure chamber method just after spectral acquisition. The result showed that the best calibration model was identified for spectra in wavelength range of 700-990 nm with $R^2 = 0.86$ and standard error of calibration $= 0.076$. The validation result showed that its calibration model had low bias and low SEP. By a 95% confidence pair $t$-test, there were no significant differences between the LWP measured using the pressure chamber method and that predicted by near infrared spectroscopy. This result showed that determination and monitoring of the LWP values using near infrared spectroscopy are possible and can be used for water stress management with high accuracy.

Keywords: leaf water potential, near infrared spectroscopy, tomato plants, transmittance mode, water stress

INTRODUCTION

In Japan, most of tomato plants are grown under controlled environment in plastic greenhouses or glasshouses. To improve the quality of tomato fruits and especially to obtain those with high soluble solid content, a water stress is induced by applying intermittent irrigation or increasing salinity of nutrient solution. Unfortunately, this is always followed by yield reduction and high blossom-end rot incidence (Cuartero and Fernandez-Munoz, 1999; Nuruddin et al., 2003). Therefore, it is very important to apply water stress at an optimum level. Leaf water potential (LWP), one of the physiological status of plants, has been widely used to quantify the water deficits in leaf tissues. As showed by many researchers, under water stress condition, the LWP is highly correlated with physiological parameters of a plant such as stomatal conductance and photosynthetic rate. When the water stress is induced, the LWP, the stomatal conductance and the photosynthetic rate decrease. The LWP is also correlated to leaf rolling, a simple appearance of water stress (O’toole and Cruz, 1980).

In order to realize the optimization of water stress, it is effective to monitor the LWP values of the tomato plant. Then we can use this bioinformation for routine control of water stress level. The process of measurement and identification of plant responses and the use of the plant model

in making decision is known as the "speaking plant approach (SPA)," where the environmental factors are considered to be the input and the plant responses the output (Morimoto and Hashimoto, 2000). However, to apply the water stress management based on the concept of SPA, it needs to determine the LWP values in a rapid and nondestructive method.

Near infrared (NIR) spectroscopy, one of the nondestructive methods has been widely used to detect the water stress condition in several plants both in short and long wavelength. Application of near infrared spectroscopy from a close distance at a single leaf point of view has been conducted to detect water stress in tomato plants (Okamura et al., 2001). However, this study resulted low accuracy of predicting water stress because of very low number of samples used. Hence, the present study is an attempt to realize the potentiality of near infrared spectroscopy to determine the LWP values in tomato plants. In this study, a correlation between the tomato leaf spectra and the LWP was investigated. Then, a calibration model for nondestructive determination of LWP was developed.

MATERIALS AND METHODS

Plant material

Thirty five tomato plants (*Lycopersicon esculentum* cv. "Momotaro Fight", Takii Company, Ltd., Japan) were grown in a glasshouse in Wagner’s pots under same EC level (0.8 dSm⁻¹). Seventy four days after seedling, sampling was conducted for a leaf adjacent to the first truss fruit. Sampling was done on sunny days (May 10, 11, 13, and 14, 2005). Then 103 tomato leaves were used as samples, out of which 55 leaves were used for developing a calibration model and 48 leaves were used for performing a validation test. To gain the broad range of LWP values, a water stress was induced by draining nutrient solution from the roots.

Method of leaf spectra acquisition

A leaf was cut using a sharp razor blade with 2 cm of petiolule and its spectra was immediately measured in transmittance mode using a sample holder for leaf. A specially designed sample holder for leaf is shown in Fig. 1. The sample holder consisted of two main parts known as the upper and the lower parts. Light source and the detector were placed in the upper and lower parts, respectively. The distance between the light source and the detector was 4 mm. Spectral acquisitions for each sample were taken at six different positions using the NIR spectrometer (MMS1; Zeiss, Germany). The instrumentation system for spectral acquisitions is shown in Fig. 2. The spectra were recorded by placing the leaf between the light source and the detector. The adaxial leaf surface was facing with the light source. The spectra were stored in the computer for further analysis through the fiber optics. Since temperature affects leaf spectrum, the leaf temperature was conditioned to 25-30°C. The measuring condition for spectral acquisitions was 30 ms for scanning time and 50 scans for averaging. A ceramic plate with 1.0 mm thickness was used as a reference.

![Fig. 1 A specially design sample holder for tomato leaf in transmittance mode.](image-url)
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![Instrumentation for tomato leaf spectral acquisitions.](image)

The intensity of light transmitted through the ceramic plate was measured, and then NIR measurement was performed by using a leaf in place of the ceramic plate. Spectral acquisition of the ceramic plate was made every time prior to the spectral acquisition of the leaf.

The absorbance spectra in the range of 305 nm to 1100 nm with 3.3 nm intervals for each leaf were measured. Spectra in the near infrared region (700 nm to 1100 nm) were used for spectral analysis. First, the transmittance ($T$) for each leaf was calculated. This value was obtained by using the following formula and comparing the transmitted spectra from the leaf with that from the ceramic plate:

$$T(\lambda) = \frac{I_s(\lambda)}{I_r(\lambda)}$$

where, $I_s(\lambda)$ = Intensity of light transmitted through leaf at $\lambda$ nm

$I_r(\lambda)$ = Intensity of light transmitted through ceramic plate at $\lambda$ nm

Then, the relative absorbance of leaf ($A_s$) was calculated by using the following formula:

$$A_s(\lambda) = \log\left[\frac{1}{T(\lambda)}\right]$$

where, $A_s(\lambda)$ = Relative absorbance of leaf at $\lambda$ nm

$T(\lambda)$ = Transmittance at $\lambda$ nm

**Method of LWP measurement**

Based on Boyer (1967), the LWP can be determined by using the following formula:

$$\Psi_L = \Psi_s - P$$

where, $\Psi_L$ = Leaf water potential (MPa)

$\Psi_s$ = Osmotic potential of xylem sap (MPa)

$P$ = Xylem hydrostatic pressure (MPa)

For accurate determination of the LWP values, those two components must be considered. However, several works have showed that the osmotic potential of xylem component was relatively very small and can be neglected comparing to the xylem hydrostatic pressure (Scholander et al., 1964). In case of tomato plants, Duniway (1971) showed a good correlation between the LWP measured accurately using the thermocouple psychrometer method and the xylem hydrostatic pressure measured by the pressure chamber method. Hence, in this study, the xylem hydrostatic pressure measured by the pressure chamber method (PMS instrument model 600, Oregon, USA) will be considered as the LWP values. Characteristics of sample used for developing calibration and validation is shown in Table 1.

**Data analysis**

The average spectra from six positions were processed using Savitzky-Golay second
Table 1 Characteristics of sample used for developing calibration and validation model for leaf water potential determination.

<table>
<thead>
<tr>
<th>Item</th>
<th>Calibration set</th>
<th>Validation set</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of samples</td>
<td>55</td>
<td>48</td>
</tr>
<tr>
<td>Range</td>
<td>((-1.21)) - (-0.45)</td>
<td>((-1.21)) - (-0.42)</td>
</tr>
<tr>
<td>Mean</td>
<td>-0.84</td>
<td>-0.82</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.20</td>
<td>0.20</td>
</tr>
</tbody>
</table>

\(^1\) Leaf water potential is expressed in MPa unit.

derivative (left and right averaging: 33 nm, polynomial order: 2). Partial Least Squares (PLS) regression was used to develop a calibration model. All of these analyses were performed using The Unscrambler\(^8\) version 7.01 (CAMO, Oslo, Norway), statistical software for multivariate calibration. A student’s t-test was performed using Statistical Package for the Social Science (SPSS) version 11.0 for Windows in order to evaluate the significance level of the model.

RESULTS AND DISCUSSION

Analysis of tomato leaf spectra

The tomato leaf spectra were measured in transmittance mode at six different positions. Then the original spectra for each leaf were transformed to its second derivative spectra. Figure 3 depicts the second derivative of tomato leaf spectra in NIR region with high (-0.32 MPa) and low (-1.21 MPa) LWP values. As shown in Fig. 3, different spectra were identified due to different LWP values. As a result, it is possible to develop a calibration model based on these spectra.

Developing a calibration model

Using the PLS regression method the calibration and validation was performed for original and second derivative spectra (Table 2). Calibration model using the PLS method should have enough number of factors to optimize the prediction model and to avoid over-fitting. Furthermore, the best calibration model can be characterized as follows. These are low factor (F), high coefficient of determination (R\(^2\)), low standard error of calibration (SEC), low standard error of prediction (SEP) and low bias. The ratio of standard error of prediction to standard deviation (RPD) value was the other parameter used for evaluating the performance of calibration model. For good prediction model, it is clearly understood that high RPD value is highly required (Williams, 1987). Calibration model of original spectra at all wavelengths range resulted high coefficient of determination (R\(^2\) = 0.86 - 0.88). However, the calibration model for original spectra resulted high standard error of prediction (SEP = 0.089 - 0.100). The number of factor for original spectra at all wavelengths range is high (F = 9 - 10) and for this reason it should be considered as a case of over-fitting. In the second derivative spectra at all wavelengths range the number of factor was de-

![Fig. 3 Second derivative of tomato leaf spectra in the near infrared wavelength with high and low leaf water potential values.](image-url)
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Table 2 Calibration and validation results for leaf water potential of tomato.

<table>
<thead>
<tr>
<th>Preprocessing</th>
<th>Wavelength range (nm)</th>
<th>Factor</th>
<th>R²</th>
<th>SEC</th>
<th>SEP</th>
<th>Bias</th>
<th>RPD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>700–950</td>
<td>9</td>
<td>0.86</td>
<td>0.073</td>
<td>0.100</td>
<td>–0.004</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>700–960</td>
<td>9</td>
<td>0.88</td>
<td>0.069</td>
<td>0.097</td>
<td>–0.007</td>
<td>2.06</td>
</tr>
<tr>
<td></td>
<td>700–970</td>
<td>9</td>
<td>0.88</td>
<td>0.071</td>
<td>0.097</td>
<td>–0.014</td>
<td>2.06</td>
</tr>
<tr>
<td></td>
<td>700–980</td>
<td>10</td>
<td>0.86</td>
<td>0.074</td>
<td>0.090</td>
<td>–0.015</td>
<td>2.22</td>
</tr>
<tr>
<td></td>
<td>700–990</td>
<td>9</td>
<td>0.88</td>
<td>0.068</td>
<td>0.089</td>
<td>–0.008</td>
<td>2.25</td>
</tr>
<tr>
<td>2nd Derivative</td>
<td>700–950</td>
<td>7</td>
<td>0.85</td>
<td>0.079</td>
<td>0.099</td>
<td>–0.007</td>
<td>2.02</td>
</tr>
<tr>
<td>(d² Log (1/T))</td>
<td>700–960</td>
<td>7</td>
<td>0.83</td>
<td>0.086</td>
<td>0.102</td>
<td>–0.002</td>
<td>1.96</td>
</tr>
<tr>
<td></td>
<td>700–970</td>
<td>8</td>
<td>0.81</td>
<td>0.086</td>
<td>0.102</td>
<td>–0.005</td>
<td>1.96</td>
</tr>
<tr>
<td></td>
<td>700–980</td>
<td>9</td>
<td>0.85</td>
<td>0.080</td>
<td>0.099</td>
<td>–0.008</td>
<td>2.02</td>
</tr>
<tr>
<td></td>
<td>700–990</td>
<td>7</td>
<td>0.86</td>
<td>0.076</td>
<td>0.088</td>
<td>–0.012</td>
<td>2.27</td>
</tr>
</tbody>
</table>

creased ($F = 7–9$) and coefficient of determination was also decreased ($R^2 = 0.81–0.86$). Thus, the best calibration model was identified at wavelength range of 700–990 nm for second derivative spectra with $R^2 = 0.86$ and $SEC = 0.076$. This wavelength range has low factor ($F = 7$) and low standard error of prediction ($SEP = 0.088$). For this wavelength range the ratio of standard error of prediction to standard deviation (RPD) value is relatively high (RPD = 2.27).

In order to clarify the behavior of the calibration model the regression coefficient was plotted against the wavelength (Fig. 4). The wavelength of 760 nm and 970 nm significantly contributed to build the calibration model. These wavelengths correspond with the absorbance band due to water (H₂O) in the second and third overtone (Osborne et al., 1993). The good result of water stress detection using NIR spectroscopy can be clearly understood since that water absorption band center at 760 nm, 970 nm, 1200 nm, 1450 nm, 1780 nm and 1940 nm (Ben-Gera and Norris, 1968; Curran, 1989).

**Validation of calibration model**

The validation of calibration model resulted in low SEP and low bias. Scatter plot between actual and predicted values is depicted in Fig. 5. By a 95% confidence pair $t$-test, there were no significant differences between the LWP measured using the pressure chamber method and that predicted by near infrared spectroscopy. This result showed that a calibration model for nondestructive determination of LWP using near infrared spectroscopy could be developed.

**Effect of leaf thickness**

As reported by Ourcival et al. (1999) the spectral characteristic was influenced by leaf thickness and for this reason different position of tomato leaf result in different spectral characteristics. In the LWP point of view, the different position of tomato leaf should be considered since anatomy of the tomato leaves vary with position (Bertin et al., 1999). This will result in different response to water stress. The different position of tomato leaves result variation in the LWP values of about

Fig. 4 Regression coefficient plot for leaf water potential calibration in wavelength range 700–990 nm.
5 to 20% (Araki, 1993). In this study, a young tomato leaf on the same position was used as the sample. Hence, the influence of the leaf thickness to both the spectral characteristics and the LWP values is very low and can be negligible. This assumption could be accepted since the calibration model for the LWP determination resulted in a high accuracy. However, for practical use in the field, it is important to develop a global calibration model of the LWP determination regardless of the leaf thickness and leaf position on plants.

REFERENCES


