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Technical Paper

Developing A Calibration Model of Leaf Water Potential Determination in Tomato Plants Using Nir Spectroscopy with Temperature Compensation

Pengembangan Model Kalibrasi untuk Penentuan Leaf Water Potential Tanaman Tomat Menggunakan NIR Spectroscopy dengan Kompensasi Suhu

Diding Suhandy[1] and Takahisa Matsuoka[2]

Abstract

In this study a calibration model with temperature compensation for leaf water potential (LWP) determination in tomato plants was successfully developed. A number of 150 tomato plants (Lycopersicon esculentum cv. Momotaro T-93) were used as samples. The plants were cultivated under same EC level (0.8 dSm^{-1}) in Deep Flow Technique (DFT) using Wagner's pot. For each leaf, the "on-plant" six spectral acquisitions from six positions were conducted. The measuring condition for spectral acquisitions was 10 ms for scanning time and 50 scans for averaging. Immediately after the "on plant" spectral acquisition, leaf was cut and its LWP value was measured using the pressure chamber method. The Partial Least Squares (PLS) Regression was used to develop a calibration model. The result showed that the best calibration model with temperature compensation was identified for the original spectra in the wavelength range of 700-1040 nm. This calibration model had R^2 = 0.83, SEC = 0.091 and SEP = 0.120. This calibration model resulted in low SEP and bias for prediction of LWP for samples having temperature 15, 25 and 35°C. This study has shown the superiority of using calibration model with temperature compensation to that without temperature compensation. This result opened the possibility of using NIR spectroscopy as a tool for nondestructive on-plant LWP determination on the field.

Keywords: *calibration model, temperature compensation, leaf water potential, pressure chamber method, on-plant spectral acquisition, on-plant LWP determination*

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Introduction

In the previous study, it has been showed that NIR spectroscopy can be used as a rapid and nondestructive method for LWP determination in tomato plants (Suhandy et al., 2006). However, in the previous study the effect of leaf temperature did not take into account since that during the measurement, the leaf temperature is conditioned between ranges 25-30°C. Actually in the greenhouse environment, the leaf temperature could fluctuate from 15 to 35°C. Therefore, in order to be used practically on the field it is highly required to develop a calibration model of LWP determination that could take into account the variation of leaf temperature.

Kawano et al. (1995) had successfully developed a calibration model with temperature compensation for intact peaches. The sample temperatures were controlled by dipping each sample into a water bath controlled at 21, 26 and 31°C. Then, a similar method was conducted for a development of calibration model with temperature compensation for tomato fruits at sample temperature 15, 25 and 35°C (Khuriyati and Matsuoka, 2005).

However, due to the characteristic of leaves such as leaf mass and leaf thickness, it is not recommended to use a water bath for leaf temperature control. The heat capacity of leaf is very small compared to that of fruit. With only a small energy input leaf temperature will raise. As a result temperature control could be difficult to be obtained. The temperature fluctuation in a leaf surface can be expressed mathematically as follow:

$$\Delta T = \frac{Q}{m \times c} \qquad (1)$$

Where :

Q = heat (kJ)

m = mass (kg)

c = specific heat capacity (kJ/kg·K)

ΔT = temperature changes (K)

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From the equation, it is clearly understood that if mass and specific heat capacity parameter is small, the parameter of temperature changes will be higher. With only a small energy input it is enough to promote the temperature changes on leaf surface.

Based on the limitation of using water bath, in this study a relatively leaf temperature control was achieved by placing the all plants into the growth chamber with a controlled temperature setting.

In this study, two kinds of calibration and validation sample sets were prepared, calibration and validation with temperature compensation and that without temperature compensation. Based on these sample sets, a calibration model with and without temperature compensation was developed and then the performance of the calibration models in predicting the LWP was evaluated.

Materials and Methods

Plant Material

A number of 150 tomato plants (*Lycopersicon esculentum cv.* Momotaro T-93) were used as samples. The plants were cultivated under same EC level (0.8 dSm^{-1}) in Deep Flow Technique (DFT) using Wagner's pot. To support the optimum growth, an aeration system was added using an air pump. The plants were divided into three groups of temperature control, 15, 25 and 35°C, respectively. After 67 days from seedling, sampling was conducted. Firstly, the fifth leaf from the apex was selected for sampling since that this leaf could be useful as indicator for tomato plants under stress condition (Araki, 1993). Then, three leaflets from this leaf were used as samples. For sample temperature of 15°C, 136 tomato leaves were used as samples, out of which 69 leaves were used for developing a calibration model and 67 leaves were used for performing a validation test. Then for sample temperature of 25°C, 136 tomato leaves were used as samples, out of which 70 leaves were used for developing a calibration model and 66 leaves were used for performing a validation test. Finally in the sample temperature of 35°C, 133 leaves were used as sample, out of which 66 leaves were used for developing a calibration model and 67 leaves

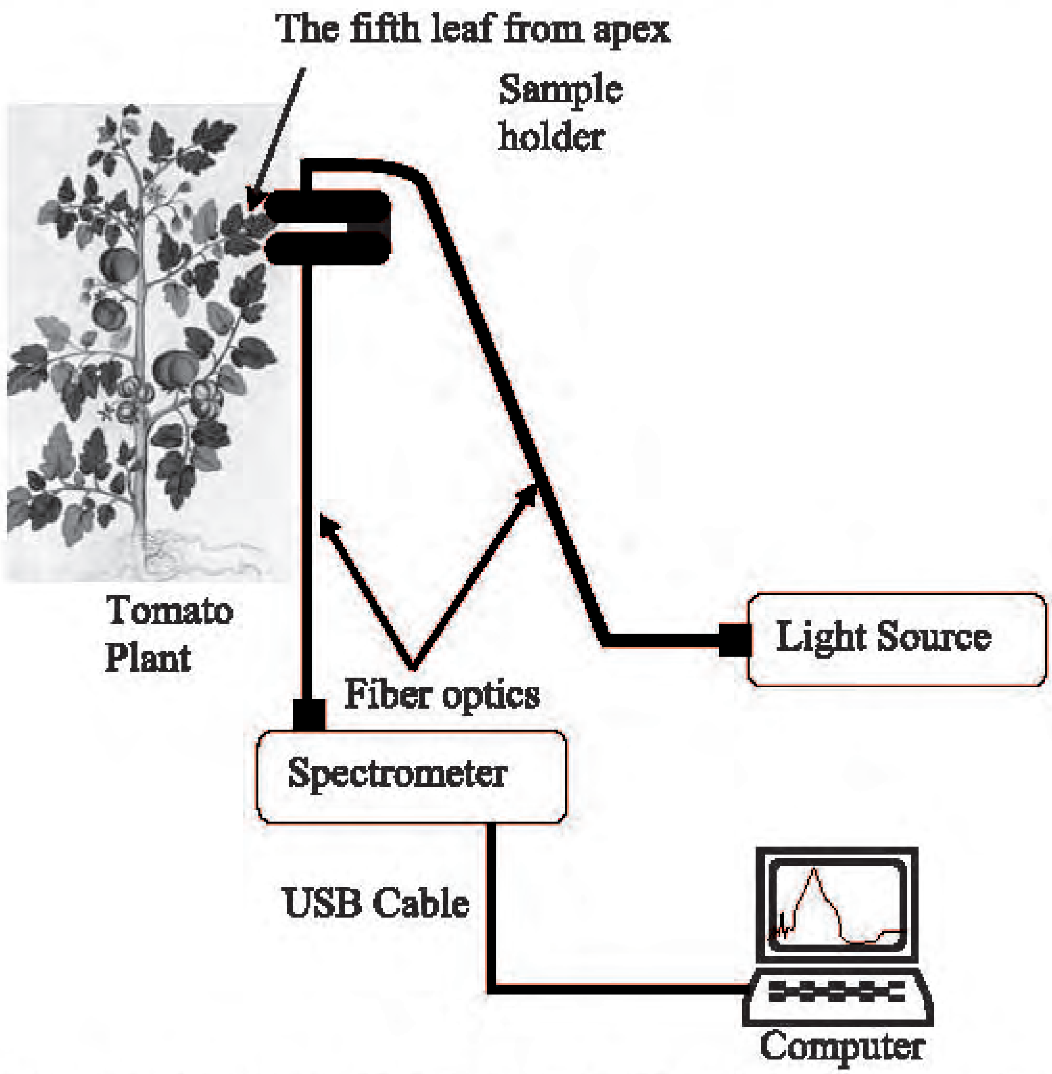

Figure 1. The configuration of on-plant NIR measurement for LWP determination in transmittance mode.

Table 1. Characteristics of uncombined sample used for developing calibration and validation model for LWP determination in 15, 25 and 35°C.

Temperature	15°C		25°C		35°C	
Item	Calibration set	Validation set	Calibration set	Validation set	Calibration set	Validation set
Number of samples	69	67	70	66	66	67
Range^z	-0.90 ~ -0.19	-0.89 ~ -0.19	-0.97 ~ -0.24	-0.97 ~ -0.25	-1.13 ~ -0.30	-1.12 ~ -0.29
Mean^z	-0.59	-0.59	-0.62	-0.62	-0.70	-0.69
Standard deviation^z	0.20	0.20	0.21	0.21	0.23	0.23

^{z} Leaf water potential is expressed in MPa unit.

Table 2. Characteristics of combined sample used for developing calibration and validation model for LWP determination in 15,25 and 35°C.

Item	Calibration set	Validation set
Number of samples	205	200
Range^z	-1.13 ~ -0.19	-1.12 ~ -0.19
Mean^z	-0.64	-0.64
Standard deviation^z	0.22	0.21

^{z} Leaf water potential is expressed in MPa unit.

were used for performing a validation test. To gain the broad range of LWP values, during the experiment, a water stress was induced by draining nutrient solution from the roots.

Method of Leaf Spectral Acquisition

Spectral acquisition method was similar to that in the previous study. Using the same instrumentation, the relative absorbance of leaves to that of ceramic plate was measured. However, in order to be used for LWP monitoring in the field, the "on plant" spectral acquisition was conducted instead of cut the leaf. Figure 1 showed the "on plant" spectral acquisition in the transmittance mode. Spectral acquisition was conducted in the growth chamber (TOMY, Cultivation Chamber model CU-158).

For each leaf, six spectra from six positions were acquired. 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. The measuring condition for spectral acquisitions was 10 ms for scanning time and 50 scans for averaging. A ceramic plate with 1.0 mm thickness was used as a reference. The intensity of light transmitted through the ceramic plate was measured, and then the 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.

Method of LWP Measurement

LWP was measured using the pressure chamber method similar to that in the previous study. Immediately after the "on plant" spectral acquisition, leaf was cut and its LWP value was measured using PMS instrument model 600 (Oregon, USA). Characteristics of uncombined sample used for developing calibration and validation for LWP determination in 15, 25 and 35°C is shown in Table 1. The characteristic of combined sample used for developing calibration and validation for LWP determination in 15, 25 and 35°C is shown in Table 2.

Data Analysis

The average spectra from six positions were processed using Savitzky-Golay second derivative (left and right averaging: 33 nm, polynomial order: 2) and moving average of smoothing. The number of point on which to average in the moving average were 5. The partial least squares (PLS) regression was used to develop a calibration model for original, second derivative and smoothing spectra. Two analyses were carried out. *First*, the calibration model without temperature compensation was developed using uncombined calibration sample set for each sample temperature of 15, 25 and 35°C. For each sample temperature of 15, 25 and 35°C, a validation was performed using uncombined validation sample set. *Second*, the calibration model with temperature compensation was developed using combined calibration sample set in 15, 25 and 35°C then the validation process was performed using combined validation sample set. The prediction performance of the calibration model with and without temperature compensation was evaluated using uncombined and combined validation sample set. All of these analyses were performed using The Unscrambler® version 7.01 (CAMO, AS, 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.

Table 3. Calibration and validation results for LWP determination in 15°C.

Preprocessing	Wavelength range (nm)	Factor	R^2	SEC	SEP	Bias	RPD
Original	700-1000	11	0.92	0.060	0.092	-0.003	2.17
Log (1/T)	700-1010	13	0.96	0.044	0.095	-0.007	2.11
	700-1020	13	0.94	0.048	0.092	-0.002	2.17
	700-1030	13	0.94	0.051	0.097	0.004	2.06
	700-1040	15	0.96	0.038	0.106	0.000	1.89
	700-1050	15	0.96	0.038	0.096	-0.006	2.08
Second Derivative	700-1000	19	0.94	0.049	0.131	-0.013	1.53
d^2 Log (1/T)	700-1010	20	0.96	0.044	0.124	-0.010	1.61
	700-1020	19	0.94	0.046	0.135	-0.015	1.48
	700-1030	19	0.94	0.045	0.133	-0.025	1.50
	700-1040	20	0.94	0.046	0.132	-0.022	1.52
	700-1050	20	0.96	0.043	0.117	-0.024	1.71
Smoothing	700-1000	13	0.83	0.085	0.123	-0.015	1.63
Log (1/T)	700-1010	13	0.83	0.087	0.124	-0.013	1.61
	700-1020	18	0.96	0.043	0.123	-0.012	1.63
	700-1030	14	0.83	0.083	0.128	-0.009	1.56
	700-1040	14	0.85	0.082	0.129	0.003	1.55
	700-1050	17	0.92	0.056	0.128	-0.005	1.56

Results and Discussion

Analysis of Tomato Leaf Spectra

Immediately after spectral acquisitions, the LWP values were measured. Since that the LWP measurement using the pressure chamber method was destructive, it is impossible to compare the effect of leaf temperature to the behavior of spectra with using only one leaf and one LWP value. For this reason, a comparison was made by selecting three leaves from three different leaf temperatures of 15, 25 and 35°C having same LWP values. Figure 2 and 3 showed the spectra of three samples having same LWP values (LWP = -0.30 MPa and LWP = -0.90 MPa) at different leaf temperature of 15, 25 and 35°C.

It is clearly identified that the spectra was different in absorbance level. However, because we use three different leaves, it is possible that the different in the spectra were not only due to the effect of leaf temperature but also due to another factor such as leaf anatomical and morphological. As reported by Ourcival et al. (1999) the spectral characteristic was influenced by leaf thickness. Therefore to clarify the effect of leaf temperature to the behavior of tomato leaf spectra, it needs to develop a calibration model with and without temperature compensation and evaluate its performance by using uncombined and combined validation sample sets.

Developing a Calibration Model without Temperature Compensation

For each sample temperature of 15, 25 and 35°C, the best calibration model for LWP determination was evaluated. Using the PLS regression method

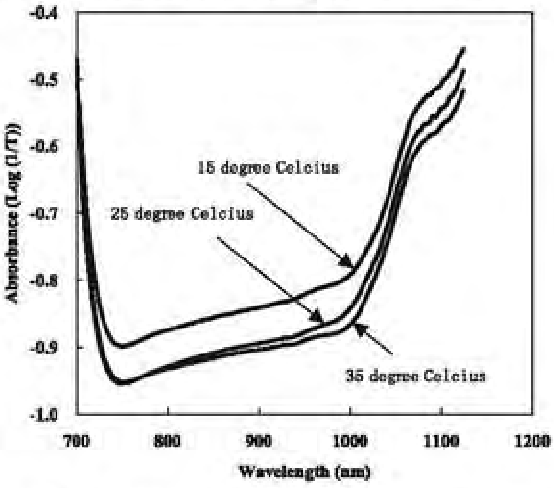

Figure 2. Original tomato leaf spectra having LWP of –0.30 MPa at different leaf temperature 15, 25 and 35°C.

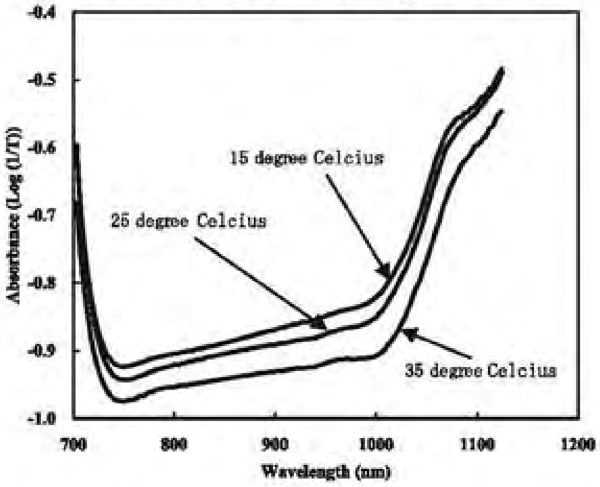

Figure 3. Original tomato leaf spectra having LWP of –0.90 MPa at different leaf temperature 15, 25 and 35°C.

Table 4. Calibration and validation results for LWP determination in 25°C.

Preprocessing	Wavelength range (nm)	Factor	R^2	SEC	SEP	Bias	RPD
Original Log (1/T)	700-1000	14	0.88	0.072	0.135	-0.002	1.56
	700-1010	15	0.90	0.064	0.120	-0.005	1.75
	700-1020	14	0.88	0.069	0.111	-0.012	1.89
	700-1030	15	0.90	0.068	0.101	-0.011	2.08
	700-1040	17	0.92	0.056	0.114	0.001	1.84
	700-1050	16	0.90	0.066	0.088	-0.004	2.39
Second Derivative d^2Log (1/T)	700-1000	7	0.37	0.167	0.170	-0.007	1.24
	700-1010	6	0.35	0.170	0.171	-0.001	1.23
	700-1020	6	0.35	0.170	0.168	0.001	1.25
	700-1030	11	0.62	0.129	0.155	-0.027	1.35
	700-1040	19	0.85	0.082	0.143	0.006	1.47
	700-1050	20	0.86	0.075	0.136	0.000	1.54
Smoothing Log (1/T)	700-1000	4	0.29	0.177	0.170	-0.001	1.24
	700-1010	10	0.52	0.145	0.160	-0.019	1.31
	700-1020	9	0.44	0.158	0.155	-0.019	1.35
	700-1030	8	0.38	0.165	0.157	-0.008	1.34
	700-1040	8	0.40	0.163	0.160	-0.008	1.31
	700-1050	4	0.31	0.175	0.167	-0.002	1.26

Table 5. Calibration and validation results for LWP determination in 35°C.

Preprocessing	Wavelength range (nm)	Factor	R^2	SEC	SEP	Bias	RPD
Original Log (1/T)	700-1000	13	0.88	0.077	0.125	-0.018	1.84
	700-1010	14	0.90	0.072	0.120	-0.015	1.92
	700-1020	13	0.88	0.079	0.110	-0.007	2.09
	700-1030	18	0.96	0.040	0.085	0.000	2.71
	700-1040	15	0.94	0.053	0.090	0.004	2.56
	700-1050	17	0.96	0.046	0.089	-0.005	2.58
Second Derivative d^2Log (1/T)	700-1000	19	0.86	0.082	0.125	-0.028	1.84
	700-1010	19	0.88	0.080	0.125	-0.020	1.84
	700-1020	19	0.88	0.076	0.133	-0.028	1.73
	700-1030	20	0.92	0.066	0.118	-0.027	1.95
	700-1040	18	0.90	0.075	0.125	-0.029	1.84
	700-1050	17	0.86	0.083	0.136	-0.031	1.69
Smoothing Log (1/T)	700-1000	19	0.88	0.078	0.141	-0.007	1.63
	700-1010	15	0.79	0.104	0.146	-0.025	1.58
	700-1020	13	0.67	0.131	0.155	-0.023	1.48
	700-1030	17	0.90	0.075	0.139	-0.004	1.65
	700-1040	13	0.69	0.128	0.151	-0.014	1.52
	700-1050	13	0.71	0.125	0.144	-0.013	1.60

the calibration and validation was performed for original, second derivative and smoothing spectra (Table 3, 4 and 5). Then the quality of the calibration model was quantified by standard error of calibration (SEC), standard error of prediction (SEP) and multiple coefficient of determination (R^2) between the predicted and measured parameters. A good model should have a low SEC, a low SEP, a high multiple coefficients of determination but also a small difference between SEC and SEP. A large difference indicates that too many latent variables are used in the model and noise is modeled (Gómez et al., 2006). In Table 3, original spectra in the wavelength range of 700–1000 nm gave the best calibration model with R^2 = 0.92, SEC = 0.060 and SEP = 0.092. The number of factor (latent variables) was relatively high (F = 11) compared to the previous study. However, a relatively high RPD value (RPD = 2.17) could be obtained.

For the sample temperature of 25°C, original spectra in the wavelength range of 700–1050 nm resulted in best calibration model performance with

Table 6. The performance of the calibration model without temperature compensation for LWP prediction using uncombined and combined validation sample set.

Wavelength Range	Sample Set	Sample Temperature	F	R^2	SEC	SEP	Bias	RPD
700-1000 nm	Calibration	15°C	11	0.92	0.060			
	Prediction	15°C				0.092	-0.003	2.17
		25°C				0.308	-0.016	0.68
		35°C				0.485	0.166	0.47
		15, 25 and 35°C				0.344	0.050	0.61
700-1050 nm	Calibration	25°C	16	0.90	0.066			
	Prediction	15°C				0.253	0.149	0.79
		25°C				0.088	-0.004	2.39
		35°C				0.237	-0.020	0.97
		15, 25 and 35°			C	0.220	0.042	0.95
700-1040 nm	Calibration	35°C	15	0.94	0.053			
	Prediction	15°C				0.315	0.034	0.63
		25°C				0.265	0.059	0.79
		35°C				0.090	0.004	2.56
		15, 25 and 35°C				0.243	0.032	0.86

R^2 = 0.90, SEC = 0.066 and SEP = 0.088 (Table 4). The relatively high of RPD value (RPD = 2.39) could be obtained. However the number of factor was very high (F = 16). The similar trend was founded in the sample temperature of 35°C. The number of factor varied between 11 and 20. In this temperature the best calibration model was identified for original spectra in the wavelength range of 700–1040 nm with number of factor = 15, R^2 = 0.94, SEC = 0.053 and SEP = 0.090 and a relatively high of RPD value (RPD = 2.56) (Table 5).

In order to know the structure of the calibration model, a plot between wavelength and regression coefficient was presented in Figure 4 for sample temperature of 15,25 and 35°C, respectively. There are so many peaks observed in all temperature. It could be understood since that the high number of factor for all calibration models was occurred. However, the peak of water absorbance was identified for all sample temperature. In the sample temperature of 15 and 25°C, a peak of 760, 839 and 970 was identified. In the sample temperature of 35°C, a peak of 760 and 970 nm was identified.

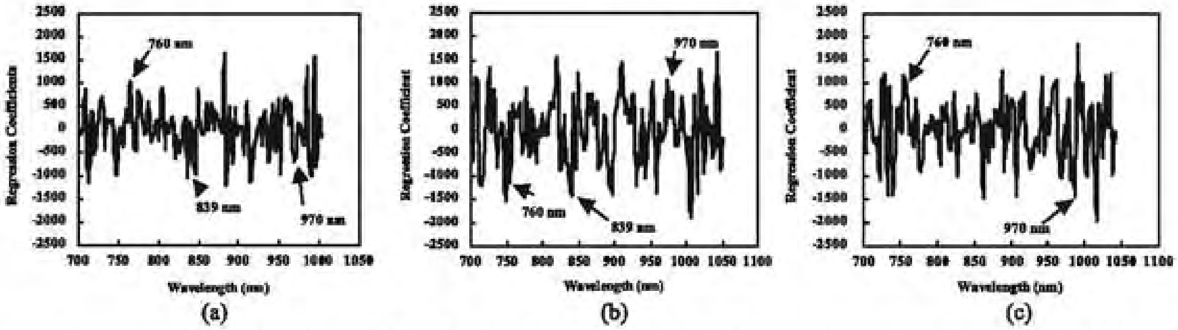

Figure 4. Regression coefficient plot for leaf water potential calibration in wavelength range (a) 700–1000 nm in 15°C (b) 700–1050 nm in 25°C, and (c) 700–1040 nm in 35°C.

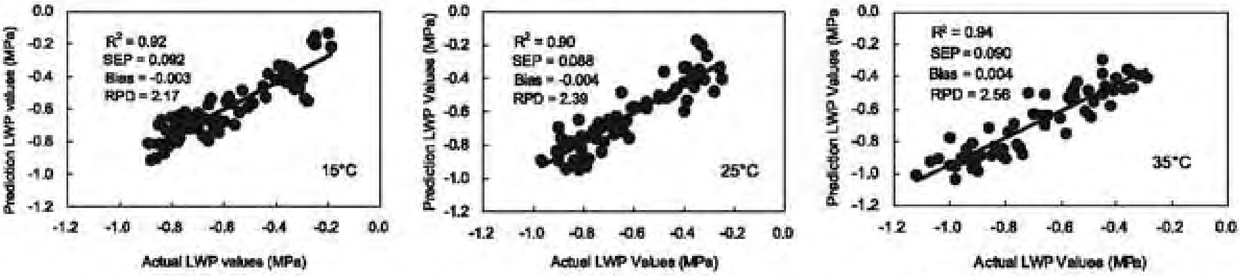

Figure 5. Scatter plot between actual and predicted LWP values using uncombined calibration sample set measured at 15, 25 and 35°C.

Table 7. Calibration and validation results for LWP determination using combined sample set in 15, 25 and 35°C

Preprocessing	Wavelength range (nm)	Factor	R^2	SEC	SEP	Bias	RPD
Original Log (1/T)	700-1000	17	0.74	0.112	0.147	-0.005	1.43
	700-1010	16	0.74	0.113	0.139	-0.003	1.51
	700-1020	17	0.77	0.104	0.130	0.001	1.62
	700-1030	18	0.81	0.096	0.123	0.002	1.71
	700-1040	20	0.83	0.091	0.120	0.004	1.75
	700-1050	20	0.81	0.094	0.126	0.006	1.67
Second Derivative d^2Log (1/T)	700-1000	17	0.58	0.143	0.154	-0.010	1.36
	700-1010	18	0.58	0.142	0.155	-0.009	1.35
	700-1020	20	0.62	0.134	0.154	-0.008	1.36
	700-1030	20	0.66	0.127	0.154	-0.002	1.36
	700-1040	19	0.62	0.132	0.154	-0.006	1.36
	700-1050	6	0.36	0.175	0.171	-0.015	1.23
Smoothing Log (1/T)	700-1000	17	0.59	0.139	0.163	-0.014	1.29
	700-1010	9	0.32	0.180	0.171	-0.016	1.23
	700-1020	18	0.66	0.129	0.158	-0.009	1.33
	700-1030	20	0.67	0.124	0.153	-0.010	1.37
	700-1040	20	0.67	0.126	0.153	-0.009	1.37
	700-1050	6	0.29	0.183	0.174	-0.014	1.21

Table 8. The performance of the calibration model with temperature compensation for LWP prediction using uncombined and combined validation sample set.

Wavelength Range	Sample Set	Sample Temperature	F	R^2	SEC	SEP	Bias	RPD
700-1000 nm	Calibration	15, 25 and 35°C	20	0.83	0.091			
	Prediction	15°C				0.111	0.011	1.80
		25°C				0.121	0.004	1.74
		35°C				0.128	-0.001	1.80
		15, 25 and 35°C				0.120	0.004	1.75

The scatter plot between actual and predicted LWP values for each sample temperature is shown in Figure 5. By a 95% confidence paired *t-test*, there were no significant differences between the LWP measured using the pressure chamber method and that predicted by NIR spectroscopy for all sample temperature.

However the performance of the calibration model decreased when it was used to predict the LWP values from spectra having temperature that is different with the temperature used for developing the calibration model. The prediction resulted in low SEP and low bias when the calibration and validation sample set was in same temperature (Table 6). When the sample temperature of the calibration and validation sample set was different, the prediction resulted in high SEP and bias for all sample temperature 15, 25 and 35°C. The result showed that the performance of the calibration model in predicting the LWP values would be influenced by the variation of sample temperature. However, in order to be used practically in the field it is highly required to develop a calibration model whose performance is not influenced by the variation of sample temperature.

Developing a Calibration Model with Temperature Compensation

According to the second analysis, a calibration model using a combined calibration sample set from 15, 25 and 35°C was developed for original, second derivative and smoothing spectra. Then the calibration model was evaluated using the combined validation sample set from 15, 25 and 35°C. The result is shown in Table 7.

The best calibration model was identified for the original spectra in the wavelength range 700–1040 nm. This calibration model had R^2 = 0.83, SEC = 0.091 and SEP = 0.120. The RPD of 1.75 and bias = 0.004 could be obtained. The regression coefficient plot is depicted in Figure 6. The peak of water absorbance was identified at wavelength of 760 nm, 839 nm and 970 nm.

The scatter plot between actual and predicted LWP values in this calibration model was depicted in Figure 7. By a 95% confidence paired *t-test*, there were no significance differences between the LWP measured using the pressure chamber method and that predicted by NIR spectroscopy.

To evaluate the performance of the calibration model with temperature compensation, a prediction of LWP values using uncombined validation sample set at 15, 25 and 35°C was performed. The result is shown in Table 8. Using calibration model with temperature compensation, the LWP prediction had SEP 0.111, 0.121 and 0.128 for 15, 25 and 35°C respectively. The prediction also resulted in low bias 0.001, 0.004 and –0.001 for 15, 25 and 35°C respectively. While in using the calibration model without temperature compensation the SEP was relatively high (0.237–0.485). It was also relatively high for bias (-0.016– 0.166). It is clear that the SEP and bias predicted by the calibration model with temperature compensation is better than that predicted by the calibration model without temperature compensation. This result showed that using a

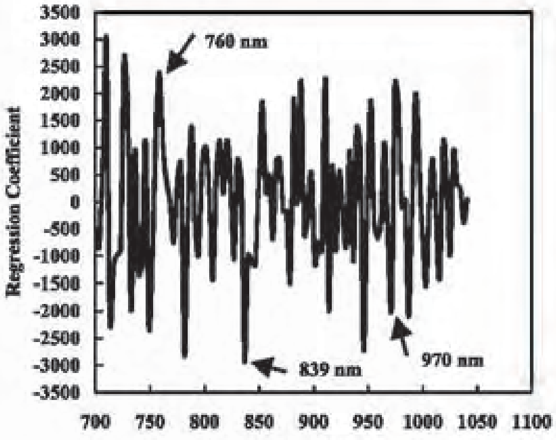

Figure 6. Regression coefficient plot for leaf water potential calibration with temperature compensation in wavelength range of 700-1040 nm in 15, 25 and 35°C.

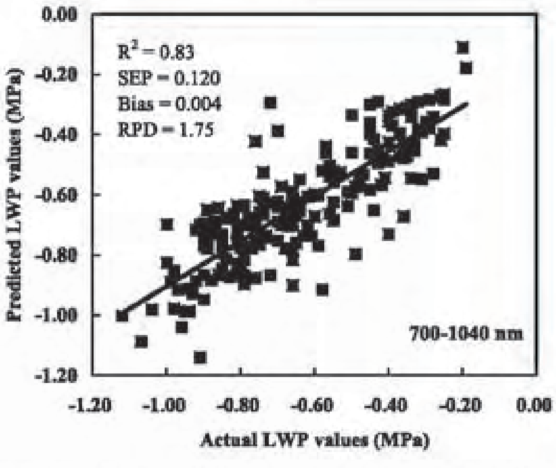

Figure 7. Scatter plot between actual and predicted LWP values using combined calibration sample set measured at 15, 25 and 35°C.

calibration model developed from a combined sample set is better than used an uncombined sample set. The performance of the calibration model could be influenced by the sample temperature. This study has shown the superiority of using calibration model with temperature compensation to that without temperature compensation. This result opened the possibility of using NIR spectroscopy as a tool for nondestructive on-plant LWP determination on the field.

Conclusions

The calibration model developed from uncombined calibration sample set was not stable since that the performance of the calibration model was influenced by the variation of sample temperature. The use of calibration model developed from combined calibration sample set was better. This calibration model could take into account the variation of the sample temperature. This is a promising method since that we can obtain the LWP information of the plant in real time. This bioinformation can be used as a feedback for water stress management in the soilless culture.

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