Nondestructive Measurement of Water Content in Hardwood Leaves Using Near-infrared Spectroscopy

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Near infrared (NIR) spectroscopy was applied to conduct nondestructive measurements of water content in hardwood leaves. The authors developed a prediction method using a partial least squares regression (PLSR) analysis of NIR spectra data of six hardwood species. The pretreated spectra were compared by the full spectral range (1200 nm to 2500 nm) and short spectral ranges (1300 nm to 1600 nm [short range 1 (S1)] and 1800 nm to 2100 nm [S2]). Good prediction results were obtained for the full spectral range with six species. The correlation coefficient for prediction of each of the species ranged from 0.94 to 0.97, and the root mean standard error of prediction ranged from 1.59 to 7.72. Compared with the full spectral analysis, predictions based on S1 and S2 were less accurate. However, leaf water content could be predicted based on measurements in the S1 and S2 ranges. It was worth comparing the wavelengths in a preliminary experiment. In this research, NIR spectroscopy was a powerful nondestructive technique for determining the moisture content of tree leaves.

Keywords: Hardwood leaf; Water absorption; Near infrared spectroscopy; Partial least squares regression

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INTRODUCTION

Tree growth is affected by numerous factors such as the air conditions, soil, temperature, water, and so on. In particular, it is important to know the water condition of the leaves for tree growth. Water stress and transpiration speed can be estimated based on a leaf's water content. Leaf water content is directly related to tree health. For example, pine wilt disease causes significant damage to pine forests due to tree death that results from cavitation associated water shortage (Kuroda 1991, 2004). The degree of leaf death caused by water shortage can be used to determine whether a pine tree is affected by wilt disease (Fukuda 1997; Fukuda *et al.* 2007). Because water conditions of the tree are indicated by observing the leaves, tree growth can be predicted and factors affecting biomass production can be efficiently manipulated. Thus, a nondestructive measurement system for monitoring the water content of tree leaves is needed.

Near infrared spectroscopy (NIRS) is widely used to analyze the water content of food such as vegetables, fruits, or agricultural commodities (Rodriguez-Otero *et al.* 1997; Büning-Pfaue 2003; Andres *et al.* 2007). The use of NIRS is a great advantage for a nondestructive measurement of water content. The measurement principles between the near infrared (NIR) region of spectra and the water absorption band have been extensively discussed (Langford *et al.* 2001; Segtnan *et al.* 2001). However, NIRS has been used to analyze the water content in vegetables and fruit trees (Creccato *et al*. 2001), but has not been applied to tree breeding to increase the biomass efficiently.

In this study, the authors employed a multivariate analysis of NIR spectra to nondestructively measure the leaf water content of six hardwood tree species. The prediction accuracy of spectra collected over ranges around the water absorption band was compared. If the leaf water content could be accurately predicted based on the analysis of a limited spectral range, it might be possible to develop a small, portable NIRS instrument for field use.

EXPERIMENTAL

Materials

Leaf sample preparation and water content measurement

Leaves from six species, *Castanopsis sieboldii* (chinquapin species), *Cercidiphyllum japonicum* (Japanese Judas tree), *Cinnnamomum camphora* (camphor tree), *Quercus myrsinifolia* (oak species), *Fagus crenata* (Japanese beech), and *Quercus serrata* (Japanese beech species), were prepared from November 2014 to May 2015 at the Nihon University Forest in Fujisawa, Japan. Sampling were performed on a sunny or cloudy day from morning to evening to obtain the various moisture content of leaves. After NIR spectroscopy measurements, the leaf weight (W) was determined, and the leaves were dried at 70 °C for 24 h. The leaf dry weight (DW) was then measured. The water content was determined according to Eq. 1:

$$\text{Moisture content (\%)} = \frac{W - DW}{DW} \times 100 \quad (1)$$

The water content data for six leaves are shown in Table 1. The data were divided into two groups (calibration set and prediction set).

Table 1. Number of Calibration and Prediction Sets for Leaf Samples from Six Different Tree Species

Species	Calibration Set					Prediction Set				
	n	Min. (%)	Max. (%)	Avg. (%)	SD	n	Min. (%)	Max. (%)	Avg. (%)	SD
Castanopsis sieboldii	169	91.5	141.1	109.7	8.4	30	93.3	128.9	110.6	6.1
Cercidiphyllum japonicum	169	191.7	286.9	232.2	19.2	30	209.8	256.4	224.8	12.2
Cinnnamomum camphora	120	51.9	77.8	64.2	9.1	30	52.5	66.5	62.9	4.2
Quercus myrsinifolia	112	70.9	119.3	94.5	9.4	30	74.0	96.5	83.7	4.8
Fagus crenata	69	65.6	108.8	87.6	10.1	30	68.2	102.3	82.2	10.0
Quercus serrata	170	129.5	212.4	166.8	21.8	30	128.0	180.9	155.6	17.2
All samples	809	51.9	286.9	136.5	61.0	180	52.5	256.4	119.2	57.2

Note: n- number of sample; SD- standard deviation

The calibration set was used to build the regression model and the prediction set was used as the prediction. The calibration set included the high and low water content values for each species. The average and standard deviation values were almost the same as the calibration set and prediction set at each sample.

Methods

NIR measurement

A photograph of the NIR spectrometer used in the study (S-7100; Soma Optics Ltd., Tokyo, Japan) is shown in Fig. 1. A tungsten halogen lamp was used as the light source with a Czerny Turner spectroscope equipped with two concave mirrors and one grating, producing monochromatic light over the wavelength range 1200 nm to 2500 nm (1 nm resolution). Samples were irradiated with the monochromatic light; the resulting reflectance was measured using a detector, and the data were transferred to a personal computer for analysis. The NIR spectra were collected at 1 nm resolution over the wavelength range 1200 nm to 2500 nm.

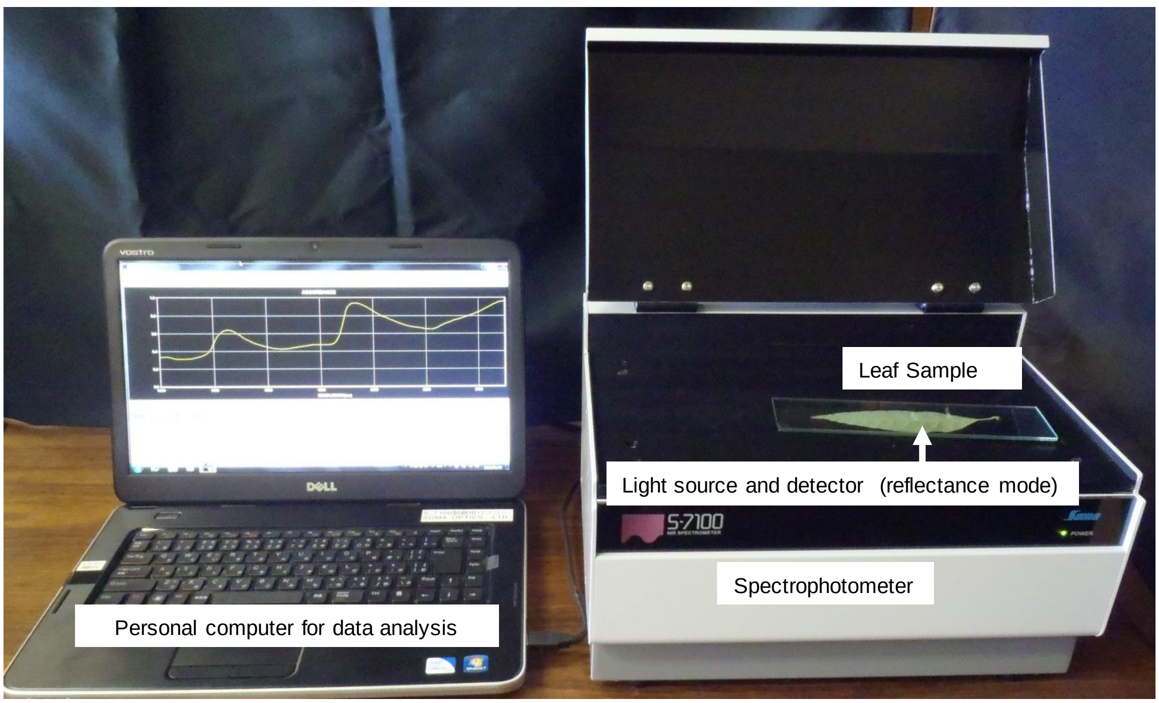

Fig. 1. NIR spectroscopy system used in this study

Leaves differ in size, color, nervures (veins or ribs), and surface characteristics, such as roughness. The opposite surfaces of leaves also naturally differ. Both surfaces (abaxial and adaxial surfaces) were measured and the data averaged to minimize measurement errors associated with leaf thickness and surface roughness. Furthermore, to control for signal stability, the data for five scans were averaged for each measurement.

Data pre-processing

Raw NIR spectra often exhibited a baseline shift or drift due to variations in the measurements. Moreover, different factors could affect the spectra, such as instrument stability, temperature, humidity, or the surface condition of the sample (Candolfi *et al.* 1999). Therefore, data pre-treatment was an important step in data analysis to eliminate such errors. Although pre-treatment for NIR was varied and complex, some basic pre-treatments that were moving average (the method to remove random noise by taking spectral average), multiplicative scatter correction (MSC), and the second derivative was used for data. Pre-treated NIR spectra (moving average smoothing with a segment size 13; MSC; and second derivative) were used for this analysis (Fujimoto *et al.* 2010a). Second derivative spectra were processed using the Norris Gap algorithm with a gap size of 13 (Meza-Marquez *et al.* 2010). Moving average eliminated random noise of the NIR spectra. The MSC compensated for both the multiplicative effect and additive effect with spectra. Second derivative also reduced the multiplicative effect and additive effect with spectra and further amplified the peak that was buried in spectra (Kurata 2017).

Regression analysis

Partial least squares regression (PLSR) was performed as the regression analysis. The final number of factors used for each calibration was recommended by Unscrambler software (Version 9.6, Camo Software, Oslo, Norway). First, the spectral range from 1200 nm to 2500 nm (full spectral range) was applied for prediction accuracy of leaf water content with pretreatment data for PLSR. Furthermore, the regulating spectral range of 1300 nm to 1600 nm (short range 1 [S1]) and 1800 nm to 2100 nm (short range 2 [S2]), the pretreatment spectra, were used to predict the leaf water content *via* PLSR. The wavelength range for S1 and S2 was selected by considering the water absorption band in whole-wavelength analyses (Büning-Pfaue 2003; Nicolaï *et al.* 2007). If the leaf water content could be accurately predicted using only short-wavelength analyses, a small, portable NIR spectroscopy instrument could be developed for field use.

Data were evaluated based on the coefficient of determination (R^2 for calibration set and R_p^2 for prediction set) between the predicted and measured values. In addition, the root mean square error of cross validation (RMSECV), the root mean square of prediction (RMSEP), and the ratio of performance to deviation (RPD) were statistically evaluated and compared for calibration or prediction (Williams and Sobering 1993; Schimilec *et al.* 2001; 2003; Fujimoto *et al.* 2010b).

RESULTS AND DISCUSSION

Figure 2 shows changes in the raw NIR spectra (a) and pretreated spectra (b) with the change in water content for *Cercidiphyllum japonicum*. The intensity of the raw NIR spectra increased with increasing water content in Fig. 2(a). Several peaks were detected around the water absorption band, as shown in Fig. 2(b). Table 2 shows the PLSR results for the water content of the six species using the pretreated NIR spectra.

In the calibration results of PLSR for each species, the R^2 values ranged from 0.95 to 0.98, and the RMSECV ranged from 1.42 to 7.43. In the prediction results of PLSR for each species, the R_p^2 values ranged from 0.94 to 0.97, and the RMSEP ranged from 1.59 to 7.72. The RPD was in the range of 2.16 to 3.44. An RPD value greater than 2.5 would indicate that the method is suitable for screening (Williams and Sobering 1993). However, the RPD value for two regressions was not greater than 2.5. In all of the samples, R_p^2 value was 0.94, and RPD was 2.52.

Figure 3 shows the regression coefficients for the PLSR models that predicted water content based on the pretreated spectra.

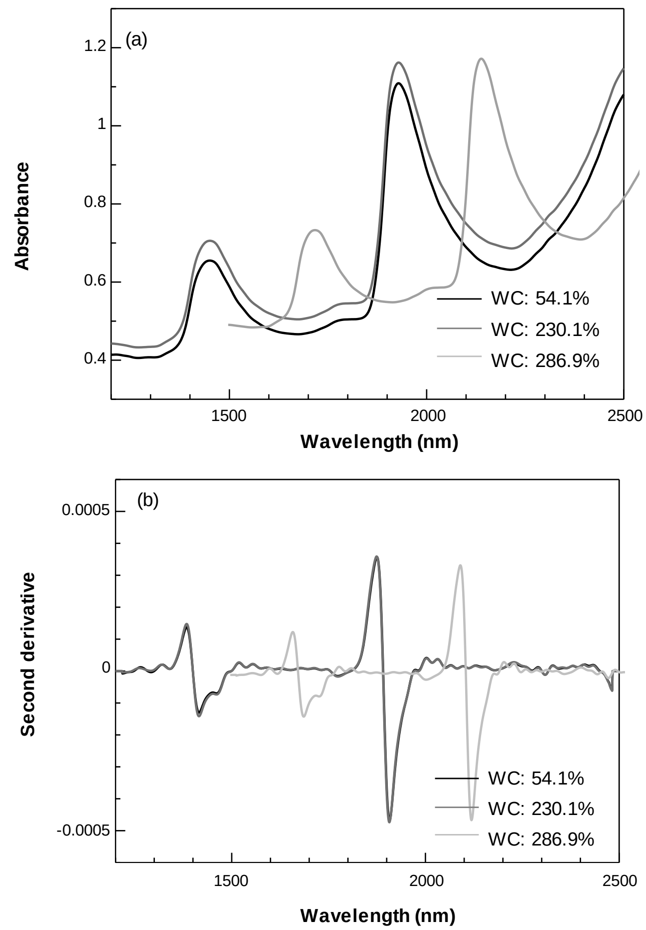

Fig. 2. Changes in NIR spectra with changing water content in *Cercidiphyllum japonicum* leaves; (a) raw spectra; (b) pretreated spectra; WC: Water content

Table 2. Results of PLSR Modeling for Predicting Leaf Water Content

Species	Calibration Set			Prediction Set		
	Factors [a]	R^2	RMSECV [b]	R_p^2	RMSEP [c]	RPD [d]
Castanopsis sieboldii	3	0.98	2.01	0.97	2.27	2.69
Cercidiphyllum japonicum	2	0.97	5.23	0.95	5.65	2.16
Cinnnamomum camphora	3	0.96	1.42	0.96	1.59	2.64
Quercus myrsinifolia	3	0.97	1.7	0.96	1.84	2.61
Fagus crenata	3	0.97	2.59	0.96	2.9	3.44
Quercus serrata	3	0.95	7.43	0.94	7.72	2.23
All samples	5	0.96	21.4	0.94	22.69	2.52

[a]: Number of partial least squares components; [b]: root mean standard error of cross validation; [c]: root mean standard error of prediction; [d]: ratio of performance to standard deviation

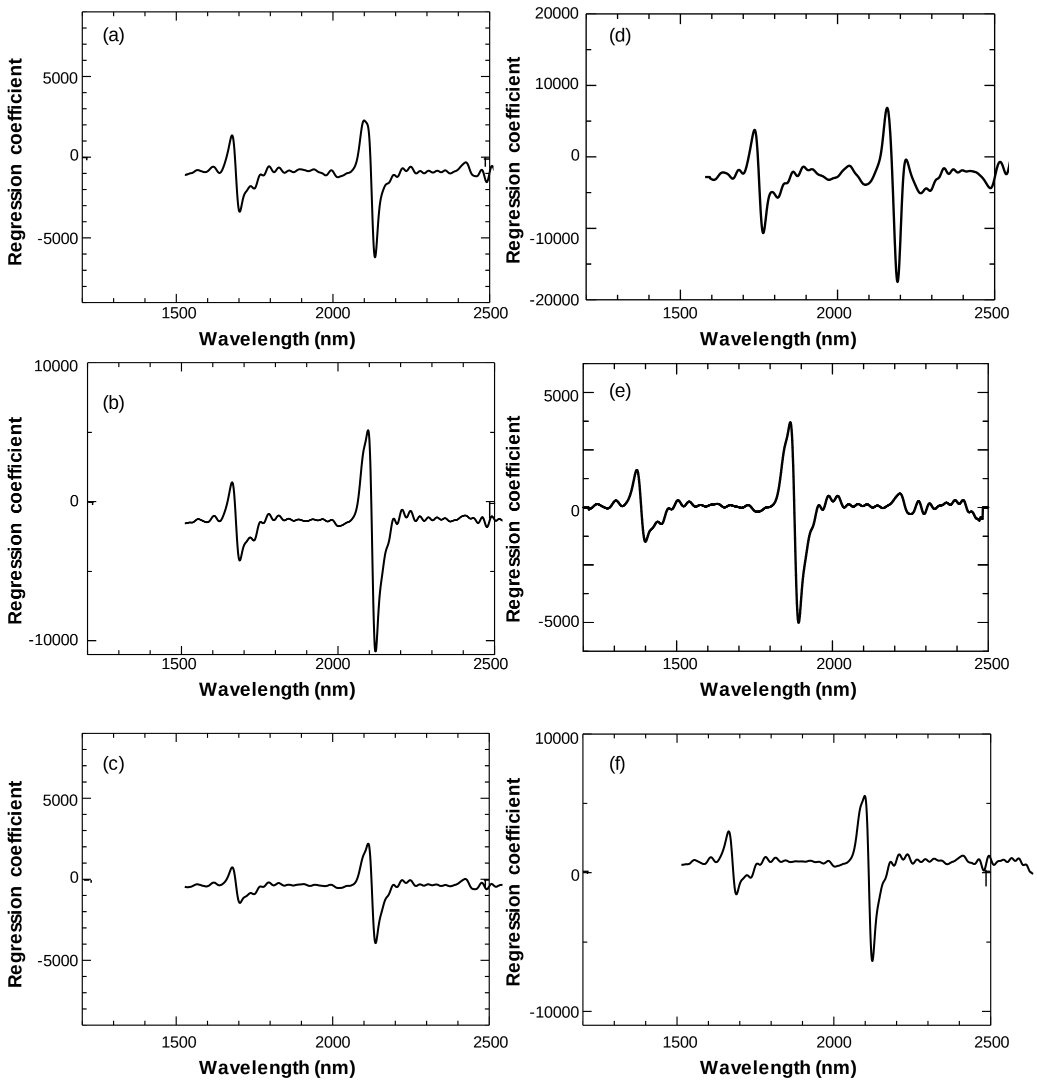

Fig. 3. Regression coefficient for the PLS models predicting with water content of six leaves with pretreated NIR spectra: (a) *C. sieboldii*, (b) *C. japonicum*, (c) *C. camphora*, (d) *Q. myrsinifolia*, (e) *F. crenata*, and (f) *Q. serrata*

There were no clear differences in the regression coefficients between the six samples. The regression coefficients changed around 1450 nm and 1950 nm. The regression coefficients varied considerably around the water absorption band. Tables 3a and 3b show the PLSR results for the water content of six species using S1 and S2. Both S1 and S2 gave poor prediction results compared with the full spectral range. In particular, the RPD values for using S1 and S2 were severely decreased.

As the S1 results were superior to the S2 results, it was worthwhile to compare the wavelengths range. Thus, a smaller, portable NIR spectrometer capable of measurements in the S1 ranges might be developed in the future for use in the field. The use of NIR spectroscopy represents a powerful nondestructive technique for determining the moisture content of tree leaves.

Table 3a. Results of PLSR Modeling for Predicting Leaf Water Content Using Partial Spectral Ranges S1 (1300 nm to 1600nm)

S1 (1300 nm to 1600 nm)						
Species	Calibration Set			Prediction Set		
	Factors [a]	R^2	RMSECV [b]	R_p^2	RMSEP [c]	RPD [d]
Castanopsis sieboldii	3	0.95	3.57	0.95	3.82	1.6
Cercidiphyllum japonicum	2	0.94	8.2	0.93	8.82	1.38
Cinnnamomum camphora	2	0.93	2.4	0.93	2.63	1.6
Querus myrsinaefolia	2	0.94	3.62	0.94	3.95	1.22
Fagus crenata	3	0.92	4.54	0.93	4.71	2.12
Quercus serrata	2	0.93	10.12	0.94	10.8	1.59
All samples	4	0.93	26.42	0.91	27.05	2.11

Table 3b. Results of PLSR Modeling for Predicting Leaf Water Content Using Partial Spectral Ranges S2 (1800 nm to 2100 nm)

S2 (1800 nm to 2100 nm)						
Species	Calibration Set			Prediction Set		
	Factors [a]	R^2	RMSECV[b]	R_p^2	RMSEP [c]	RPD [d]
Castanopsis sieboldii	2	0.94	3.79	0.94	3.92	1.56
Cercidiphyllum japonicum	2	0.92	8.56	0.92	8.78	1.39
Cinnnamomum camphora	2	0.92	2.52	0.92	2.69	1.56
Querus myrsinaefolia	2	0.93	3.84	0.92	4.01	1.2
Fagus crenata	3	0.94	4.68	0.92	4.78	2.09
Quercus serrata	2	0.96	11.4	0.96	12.04	1.43
All samples	4	0.92	27.14	0.9	27.67	2.07

[a]: Number of partial least squares components; [b]: root mean standard error of cross validation; [c]: root mean standard error of prediction; [d]: ratio of performance to standard deviation

CONCLUSIONS

1. As water content of hardwood leaves was predicted using near infrared (NIR) spectroscopy from 1300 nm to 2500 nm, the coefficient of determination for the prediction set (R_p^2) values ranged from 0.94 to 0.97, and the root mean square of prediction (RMSEP) ranged from 1.59 to 7.72.

2. Two spectra regions that were around water absorption band (S1 and S2) were compared with the full spectral range. As S1 results were superior to the S2 results, S1 and S2 results was not superior to the full spectral range.

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