

Measurements and analysis of water content in winter wheat leaf based on terahertz spectroscopy

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Abstract: Wheat is a major grain crop in China. Water is one of the most important factors which influence the lifecycle and yield of wheat. It is of great significance to study the water content at the key stages of wheat growth in order to make irrigation decision to raise its yield. As Terahertz (THz) spectroscopy is a brand new sensing technology and sensitive to water absorption, the relationship between terahertz spectra and water content in winter wheat leaf was investigated and a preliminary result was presented in this paper. Forty winter wheat leaves samples with diverse range of water content (42.8%-72.5%) were collected. The Terahertz time domain spectra (THz-TDS) were first obtained and then transformed into Frequency-domain amplitude with the Fast Fourier Transformation (FFT) method. The absorption and refractive index spectra were then calculated. The spectra were linearly fitted to obtain the slope and intercept used for building a calibration model. The partial least squares (PLS) method and linear regression were employed to establish models to determine leaf water content in the winter wheat. The predicted correlation coefficient and the root mean square error of the optimal model established with the Frequency-domain amplitude parameter at 0.3 THz by linear regression were 0.812% and 4.4%, respectively. The results showed that terahertz spectroscopy performed well in water content prediction and could be an effective and potential method for leaf water content measurement in winter wheat.

Keywords: terahertz spectroscopy, winter wheat, gravimetric water content (GWC), partial least squares method

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1 Introduction

Water plays an important role in plant's life cycle. Photosynthesis and respiration take place in plant's leaf continuously and they are closely related to leaf water content. The behavior of plants under drought stress is of interest for plant phenotyping as well as for agricultural plant production. Shortage of water will cause a terrible effect on plant morphology^[1]. Research on transpiration response to water availability for winter wheat has also been conducted^[2]. In some areas, water deficit is one of the main limiting factors in cereal production^[3,4]. Water content in wheat leaf was up to 40%-80% that has a great influence on its growing period^[5]. Since water stress is one of the most important abiotic stress for crop, leaf water content monitoring is of great importance for irrigation guiding^[6]. Reasonable irrigation can be helpful to promote the growth of wheat and raise the yield. A commonly used traditional method for leaf water content measurement is the gravimetric water content (GWC) method. It is destructive and time-consuming^[7]. And there are many other techniques for crop water stress measurement in literature, such as

delayed chlorophyll fluorescence^[8], prompt chlorophyll fluorescence^[9,10]. A review on irrigation strategy was presented by Jones et al.^[11]. However, the determination of leaf water content is still difficult, especially if the measurements shall be noninvasive and continuous over a long period of time.

Terahertz, an electromagnetic radiation wave, lies in the range of the microwave and infrared bands^[8]. With the rapid development of the terahertz emitting and detection technologies, it has been applied to many fields, such as agricultural products and food^[9], moisture and liquid detection^[10] and so on. An effective medium theory (EMT) has been used to predict the water content in a coffee leaf^[11]. The permittivity of the leaf was described as a function of water content in the leaf. The theory was also used to predict an Arabidopsis thaliana leaf water dynamics^[12]. The grapevine water status was monitored contactless by terahertz time domain spectroscopy (THz-TDS)^[13]. The quality of wheat grain with various degrees of deterioration (normal, worm-eaten, moldy, and sprouting wheat grains) was determined by THz-TDS^[14]. The leaves water content have been assessed using leaf reflectance ratios in the visible, near-, and short-wave-infrared. Leaf water indices can be utilized for qualitative assessment of leaf water content^[15]. The changes of THz transmission of coffee plants with watering and without watering were monitored and the THz transmission decreased with the water content in the leaves^[16].

Wheat is an important grain crop and the objective of this work is to try to develop a novel sensing and analysis method for monitoring leaf water content in winter wheat using THz spectra. THz time domain spectra in 0.1-2.0 THz were obtained and preprocessed to obtain the frequency-domain amplitude, absorption and refractive index spectra respectively. Partial least squares and

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linear regression were used to establish a steady model to predict the water content in winter wheat.

2 Materials and methods

2.1 Leaf sampling and water content measurements

As the THz instruments were large laboratory equipment, the experiment was conducted in Agricultural THz Spectroscopy and Imaging Laboratory at Beijing Nongke Building. 40 winter wheat (Jingdong 24) leaves in ripening stage, with diverse range of water content, were collected from the wheat field in Beijing Research Center for Information Technology in Agriculture. They were put into self-sealing plastic bags once they were taken off from the plant. Then all of the leaves were placed in the incubator (4°C) in order to decrease water evaporation and were tested in the laboratory immediately: Firstly, the leaf was wiped clean with sterile cotton, then the leaf sample was cut into circle to fit the sample-holder for collecting its THz spectra and the thickness of each leaf was measure by vernier caliper. Each leaf repeats the above steps. In order to explore the feasibility of this novel sensing technology for leaf water content measurement in winter wheat, forty leaf samples in total were prepared in this experiment.

The water content of all wheat leaf samples were measured with the gravimetric water content (GWC) method. Each fresh wheat leaf round sample mass was weighed before acquiring its THz spectra. Then the thickness of sample was measured and recorded. After the THz spectra collection, the wheat leaf sample

was put into an oven under 90°C for 30 min until the mass of the leaf reaching a constant value. Then each sample was weighed again to get its dry mass's weight. The GWC was calculated according to the following formula.

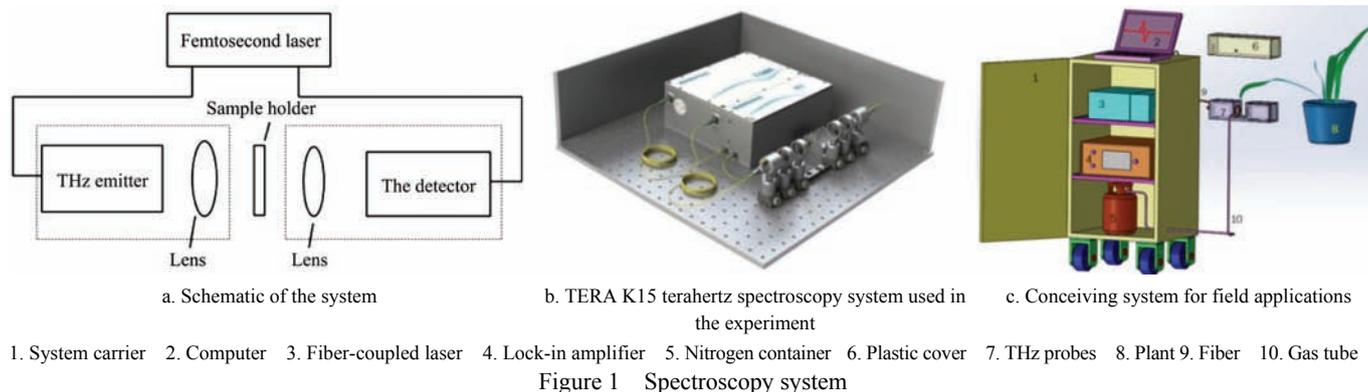
$$GWC=(FM-DM)/FM \tag{1}$$

where, GWC is the gravimetric water content; FM is the fresh leaf mass, g; DM is the dry leaf mass, g.

2.2 Terahertz spectroscopy instrument

Terahertz spectroscopy instrument used in the experiments was a fiber-coupled TERA K15 made by Menlo Systems Company in Germany. Its spectral range was greater than 5 THz. THz frequency resolution was less than 1.2 GHz. The wavelength of it laser was 1560 nm. The THz emitter and detector optical power were 33 mW. The system is shown in Figure 1. The terahertz spectra were gathered on the sample by the lens in front of the THz emitter, and then the THz spectra were detected by the THz detector.

The wheat leaf samples were fixed onto the sample-holder and then scanned to collect the spectra data. Nitrogen gas was pumped in continuously to reduce THz absorption influence caused by the moisture in the air. The time domain spectra from 0 to 8 ps were acquired. Terahertz spectra without any sample were measured as reference spectra at the beginning. Then each sample spectra were collected in the same way. In order to get detailed information of each sample, it usually took 5 min to obtain the whole spectra of each sample.



2.3 Data analysis

The original spectra data were in the time domain. In order to get the frequency domain amplitude, the time domain spectra were processed with the Fast Fourier Transform (FFT) method. Then the absorption spectra and the refractive spectra were calculated with the standard algorithm^[17]:

$$n(\omega) = \frac{\phi(\omega)c}{\omega d} + 1 \tag{2}$$

$$\alpha(\omega) = \frac{2}{d} \ln \left\{ \frac{4n(\omega)}{\rho(\omega)[n(\omega)+1]^2} \right\} \tag{3}$$

where, $n(\omega)$ is the refractive spectra; $\alpha(\omega)$ is the absorption spectra; d is the thickness of the sample; $\rho(\omega)$ is the amplitude; $\phi(\omega)$ is the phase.

The linear regression method and the partial least squares were used to build calibration model for water content in the winter wheat respectively. The partial least squares (PLS) method has the advantage of multiple linear regression, principal component analysis and correlation analysis while using the whole spectra^[18]. With the THz spectra and the water content, the models were obtained using different mathematical method. The correlation

coefficient (R_c and R_p) and the root mean square error ($RMSEC$ and $RMSEP$) were used to evaluate the accuracy of the model. An optimal model should have higher correlation coefficient and lower root mean square error. RMSE for calibration and prediction was used, respectively:

$$RMSEC = \sqrt{\frac{1}{N-P-1} \sum_{i=1}^N (y_i - \hat{y}_i)^2} \tag{4}$$

$$RMSEP = \sqrt{\frac{1}{N_p-1} \sum_{i=1}^{N_0} (y_{i_0} - \hat{y}_{i_0})^2} \tag{5}$$

where, N is the samples number of calibration set; N_p is the samples number of prediction set.

3 Results and discussion

3.1 Water content in winter wheat

Forty wheat leaf samples in total were measured in the experiment. The samples were divided into calibration set (30 samples) and prediction set (10 samples) randomly. The calibration set was used to establish the model and the prediction set was used to verify the accuracy of the model. The gravimetric water content (GWC) was ranged from 42.8% to 72.5%. The

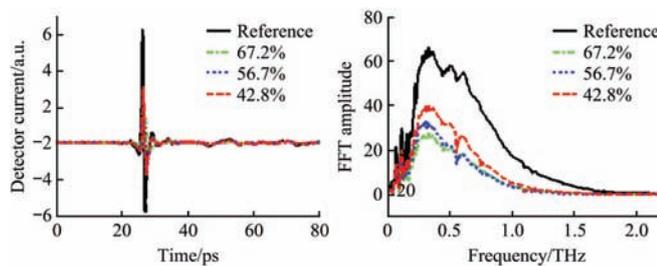
average and standard deviations of the water content are 58.4% and 6.9%, respectively. The results of the water content were listed in Table 1.

Table 1 Results of water content

	Maximum	Minimum	Average	Standard deviation
Gravimetric water content (GWC)	72.5%	42.8%	58.4%	6.9%

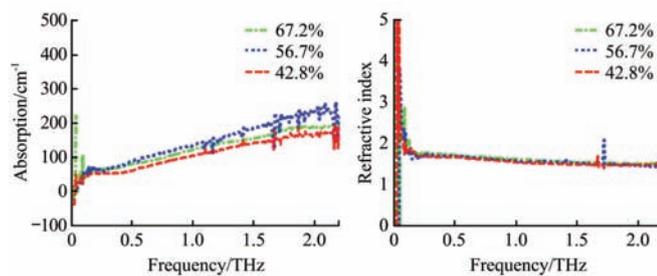
3.2 Original terahertz spectra

The original terahertz time domain spectra with water content at 67.2%, 56.7% and 42.8% were selected to show in Figure 2a. Then the time domain spectra were processed by Fourier transformation to get the frequency domain spectra which were shown in Figure 2b. Compared with the time domain spectra of the samples and the reference, the sample time has been delayed and the phase has changed. After transforming into frequency domain spectra, the FFT amplitude of selected samples revealed a distinct attenuation when comparing these samples with the reference. It was assumed that it is a result of the leaf water absorption of the terahertz spectra. Due to different water content in the leaves, the attenuation is also various. Moreover, the more water content the leaf has, the more attenuated the FFT amplitude has. Also, the signal was infinitely close to 0.1 after when the frequency larger is higher than 1.5 THz. It showed that the effective range was shorter than 1.5 THz.



a. THz time domain spectra b. Frequency domain spectra
Figure 2 Original spectra of the winter wheat leaf

With the wheat leaf thickness, corresponding reference and time domain spectra, the absorption and the refractive spectra were calculated by the standard algorithm, respectively^[17]. The spectra were shown in Figure 3. The low frequency region which indicates the lower signal-to-noise ratio (SNR) should be considered with caution. Data were removed in further steps. According to the analysis above, the effective spectra range is at 0.3-1.5 THz. The absorption spectra showed that there were no obvious absorption peaks in THz range and the different absorption spectra approximate a straight line. The slope and intercept was different from each other. The refractive index was ranged at 1.5-1.8.



a. THz absorption spectra b. THz refractive index spectra
Figure 3 THz spectra

The correlation coefficient between the FFT amplitude,

absorption, refractive index and the water content were shown in Figure 4. The correlation coefficient between the FFT amplitude and water content was negative and the absolute value at 0.3 THz was the maximum one. The correlation coefficient between the absorption, refractive index and the water content were positive.

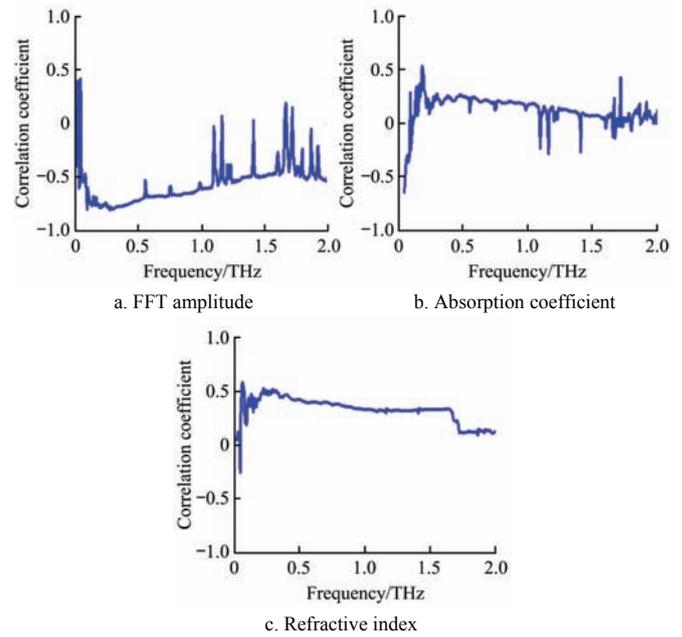
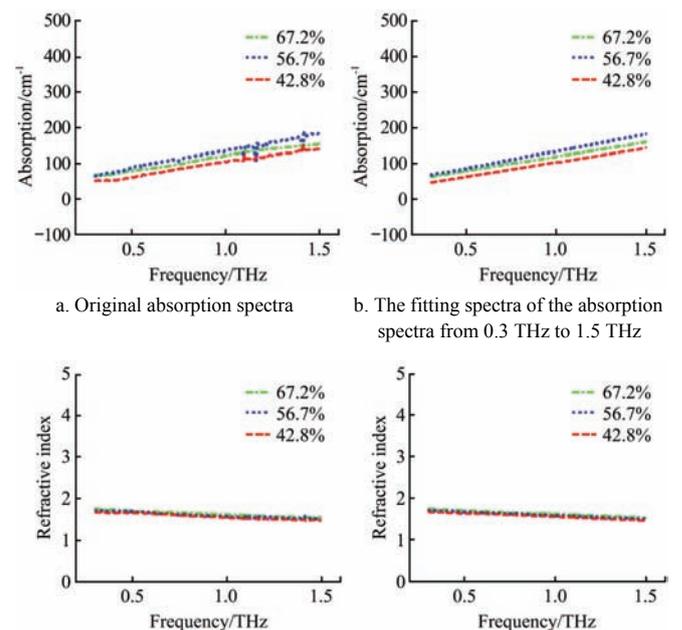


Figure 4 Correlation coefficient of the THz spectra

3.3 Fitting curves of THz spectra

As for the influence of the system noise, the frequency of 0.3-1.2 THz spectra was selected for linear fitting to describe the relationships between the slope and intercept of the spectra line and water content. The fitting curves were shown in Figure 5. Also the correlation and the standard deviation of the curves were calculated. The fitting curve can retain the most information about the original spectra. The correlation coefficients and the standard deviation between the original absorption spectra and fitting curve were ranged from 0.999 to 0.980 and 1.418 to 6.827, respectively. The correlation coefficients and the standard



a. Original absorption spectra b. The fitting spectra of the absorption spectra from 0.3 THz to 1.5 THz
c. Original refractive spectra d. The fitting spectra of the refractive spectra from 0.3 THz to 1.5 THz
Figure 5 Original spectra and fitting spectra

deviation between the refractive index spectra and fitting curve were ranged from 0.714 to 0.994 and 0.006 to 0.029 respectively. The slope and intercept values (k , b) were also acquired according to the fitting curve. The linear regression method can be used to build a model with the slope and intercept (k , b).

3.4 Model evaluation

3.4.1 Model based on the time domain spectra and the FFT amplitude spectra

As for water absorption of the terahertz spectra, the amplitude of the time domain and the FFT amplitude have been attenuated. The maximum and the minimum time domain amplitude (T_{max} and T_{min}) were used to establish a prediction model. The FFT amplitude spectra reflected the attenuation of leaf from the 0.3 THz to 1.5 THz when THz spectra penetrating the sample. The correlation coefficient of the FFT amplitude and water content was negative. The maximum absolute value was at 0.3 THz. Therefore, the FFT amplitude spectra at 0.3 THz were used to build the optimal model. The correlation coefficient and the root mean square error of the prediction set were 0.812 and 0.044, respectively. The result was shown in Table 2. The prediction accuracy of the model established with the FFT amplitude spectra at 0.3 THz was the best. It indicated that the model built with the FFT amplitude spectra at 0.3 THz was more stable and it was more suitable for predicting the water content.

Table 2 Parameters model results of different parameters with linear regression

Parameters	R_c	RMSEC	R_p	RMSEP
Tmax	0.877	0.034	0.686	0.059
Tmin	0.869	0.036	0.791	0.055
Tmax-Tmin	0.876	0.035	0.737	0.057
0.3 THz	0.809	0.043	0.812	0.044

The partial least squares method was also used to predict the water content with the FFT amplitude. The correlations coefficient between the FFT amplitude and water content were negative and the absolute values were under 0.8. Different principal components (PCs) were selected to get the best one to establish the model. The results were shown in Table 3. The correlations coefficients of the models established with the partial least squares method were negative and the absolute values were under 0.8. The best model was the one built with linear regression method at 0.3 THz. The scatter plots were shown in Figure 6.

Table 3 Results of FFT amplitude with the partial least squares method

PCs	R_c	RMSEC	R_p	RMSEP
1	-0.781	0.199	-0.720	0.128
2	-0.532	0.156	-0.774	0.116
3	-0.276	0.123	-0.458	0.096

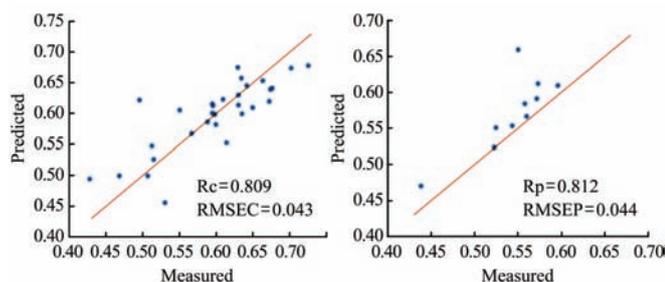


Figure 6 Scatter plot of the optimal model based on FFT amplitude spectra in 0.3 THz

3.4.2 Model based on absorption spectra and refractive spectra

As for the spectra data, the partial least squares method was used to build the model. The THz absorption and refractive spectra from 0.3 THz to 1.5 THz were selected as the input variable to establish a water content prediction model. Different principle components were used in the model. The results were shown in Table 4. When the principle component 7 was used in the partial least squares, prediction correlation of best the absorption spectra model was 0.6. However, the prediction correlation of the refractive spectra was 0.796 which the principle component was 9. According to the result of the Table 4, the prediction correlation and the root mean square error of the optimal model built refractive index was 0.796 and 0.048, respectively. The scatter plots were shown in Figure 7.

Table 4 Partial least squares results of the absorption spectra and refractive spectra

PCs	Absorption spectra				Refractive spectra			
	R_c	RMSEC	R_p	RMSEP	R_c	RMSEC	R_p	RMSEP
3	0.701	0.050	0.309	0.108	0.630	0.052	0.592	0.051
4	0.784	0.046	0.387	0.111	0.657	0.049	0.507	0.043
5	0.819	0.043	0.455	0.112	0.710	0.047	0.570	0.048
6	0.867	0.038	0.573	0.101	0.751	0.045	0.570	0.048
7	0.900	0.032	0.600	0.095	0.774	0.043	0.645	0.051
8	0.918	0.029	0.540	0.109	0.840	0.040	0.753	0.055
9	0.925	0.028	0.506	0.120	0.891	0.035	0.796	0.048
10	0.940	0.025	0.417	0.136	0.912	0.030	0.723	0.045

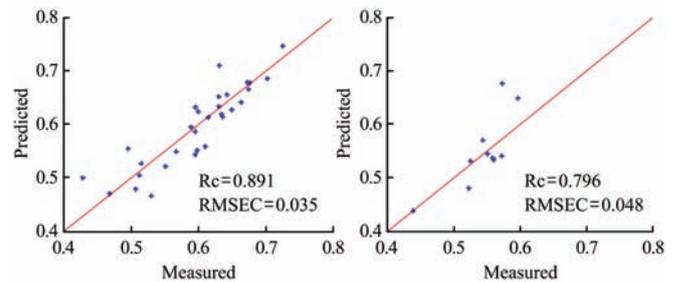


Figure 7 Scatter plot of the optimal model based on absorption spectra and refractive spectra

3.4.3 Model based on slope and intercept

The slope and intercept of the fitting curve can represent the property of the fitting spectra. The multiple linear regression method^[19] was used to establish the model with the slope and intercept (k and b). The results were shown in Table 5. The prediction accuracy of the refractive spectra was higher than that of the absorption spectra. However, the calibration set accuracy of the refractive spectra was lower than the absorption spectra. The correlation coefficients were under 0.8. It was concluded that the model built with slope and intercept of the fitting curve was not fit for predicting the water content.

Table 5 Parameters model result of the absorption spectra and refractive spectra

Parameters	Absorption spectra				Refractive spectra			
	R_c	RMSEC	R_p	RMSEP	R_c	RMSEC	R_p	RMSEP
k, b	0.668	0.055	0.497	0.067	0.429	0.067	0.729	0.017

Compared all the above models built with different methods, the correlation coefficients of the model built with frequency domain amplitude spectra at 0.3 THz showed the best prediction

ability. The correlation coefficient and the root mean square error of the calibration set were 0.809 and 0.043, respectively. And the correlation coefficient and the root mean square error of the prediction set were 0.812 and 0.044, respectively.

3.4.4 Discussion

The above result reveals that the water content in plant leaves can be detected effectively by terahertz technology, which provides a reference for on-line, non-destructive and rapid detection of water content in plant leaves. However, the high cost of the terahertz equipment, the limited detection efficiency is hindering the development of this new technology and its application. It is necessary to further reduce the cost of equipment and improve the detection efficiency. In our future study, more samples in different complex conditions will be tested, and more universal prediction model will be designed to improve the accuracy of detection.

4 Conclusions

In this study, terahertz spectroscopy was employed to detect the water content of winter wheat leaves to provide guidance for irrigation and raise grain yield. The time domain spectra were obtained and transformed into the frequency domain with the Fourier transform method. Also the absorption and the refractive spectra were obtained with the standard method. The linear regression and the partial least squares were used to predict water content. Different principle components were used when the partial least squares and linear regression were applied to establish models. The optimal model was the one established with the spectra at 0.3 THz, which would be significant for further exploring portable single frequency terahertz device.

This study explored a brand new sensing method for evaluating leaf water content of winter wheat using THz spectra. Results indicated that THz-TDS can be a new technology and has great potential for evaluating winter wheat leaf water content. Compared with the traditional detection methods, terahertz spectroscopy has great potential in plant water content detection. With the rapid development of THz equipment manufacture, portable and contactless detection will be realized in the near future. As this development continues, it is expected to gain more and new insight into water dynamics of plants in a way that has not been possible before with previous technologies. However, further studies should be made to improve detection accuracy when determining the leaf water content in winter wheat based on terahertz spectroscopy in the future.

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