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Estimating Moisture Content of Cucumber Seedling Using Hyperspectral Imagery

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Abstract

Purpose: This experiment was conducted to detect water stress in terms of the moisture content of cucumber seedlings under water stress condition using a hyperspectral image acquisition system, linear regression analysis, and partial least square regression (PLSR) to achieve a non-destructive measurement procedure. **Methods:** Changes in the reflectance spectrum of cucumber seedlings under water stress were measured using hyperspectral imaging techniques. A model for estimating moisture content of cucumber seedlings was constructed through a linear regression analysis that used the moisture content of cucumber seedlings and a normalized difference vegetation index (NDVI). A model using PLSR that used the moisture content of cucumber seedlings and reflectance spectrum was also created. **Results:** In the early stages of water stress, cucumber seedlings recovered completely when sub-irrigation was applied. However, the seedlings suffering from initial wilting did not recover when more than 42 h passed without irrigation. The reflectance spectrum of seedlings under water stress decreased gradually, but increased when irrigation was provided, except for the seedlings that had permanently wilted. From the results of the linear regression analysis using the NDVI, the model excluding wilted seedlings with less than 20% (n=97) moisture content showed a precision (R^2 and R_a^2) of 0.573 and 0.568, respectively, and accuracy (RE) of 4.138% and 4.138%, which was higher than that for models including all seedlings (n=100). For PLS regression analysis using the reflectance spectrum, both models were found to have strong precision (R^2) with a rating of 0.822, but accuracy (RMSE and RE) was higher in the model excluding wilted seedlings as 5.544% and 13.65% respectively. **Conclusions:** The estimation model of the moisture content of cucumber seedlings showed better results in the PLSR analysis using reflectance spectrum than the linear regression analysis using NDVI.

Keywords: Hyperspectral imagery, Moisture content, Image processing, Non-destructive analysis, Water stress

Introduction

Seedlings are central to crop production because they influence its success or failure (Yoon et al., 1995; Yu et al., 2002). Good and sturdy seedlings of any crop ensure high quality and productivity (Byun et al., 2012). The size of the domestic plant nursery market has increased by 30%, from 187,000 million won in 2010 to 242,000 million won in 2013, and the number of seedling suppliers has increased by six times, from 50 in 1997 to 292 in 2014. In addition, the area for seedling production has increased by eight times from 20 ha in 1997 to 159 ha in 2010. Therefore, production of seedlings is expected to be a future growth engine (MAFRA, 2014).

Fruits and vegetables such as peppers, tomatoes,

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cucumbers, and watermelons are the common plants cultivated in domestic plant nurseries (Han et al., 2012). These plants show physiological or biochemical changes when they encounter various stresses such as temperature and water during the typically hot days of summer (Kang et al., 2007). Cucumbers grow fast; therefore, many physiological changes occur during the nursery period. The ratio of female to male flower production of cucumbers differs depending on temperature and day length, and it affects cucumber yield after planting (GBA, 2011).

Various studies about environmental management for seedlings have been in progress, and some studies have been conducted to develop rapid and non-destructive measurement methods. Spectroscopic techniques, in particular, have been gaining attention as a non-destructive analysis technique (Kim et al., 2012). A water stress measurement method for red pepper leaves was developed using a hyperspectral short wave infrared imaging technique (Park and Cho, 2014), and non-destructive sorting techniques (viable vs. non-viable) for radishes were developed using hyperspectral imaging techniques (Ahn et al., 2012). In addition, the hyperspectral reflectance spectrum imaging technique was used in predicting internal quality of cherry tomatoes (Kim et al., 2011) and in estimating the main properties in potherb mustards (Ryu et al., 2014).

Thus, this experiment was conducted to detect water stress in terms of the moisture content of cucumber seedlings under water stress conditions using a hyperspectral image acquisition system, linear regression analysis, and partial least square regression (PLSR), to achieve a non-destructive procedure.

Materials and Methods

Sample

Cucumber seedlings were provided by Namji-purun Nursery, Gyeongnam Province. The cultivar of "Heukjong" was used for the rootstocks of cucumber seedlings, and the cultivar of "Hanganmatdadagi" was used for scions after grafting with "Heukjong" cultivar. Each 10 seedlings were placed in 10 trays with 40 cells in an interval of 2-3-3-2 in order not to have overlap on the 11th day after grafting.

Methods

The experiments were conducted at a greenhouse in Gyeongsang University from October 2-4, 2015. The inside temperature of the greenhouse was measured by a thermometer (SK-L200TH-II, SATO KEIRYOKI MFG., Japan) which showed a mean daytime temperature of 33.9°C, the highest daytime temperature of 44.1°C, a mean nighttime temperature of 13.4°C, and the lowest nighttime temperature of 8.9°C. Peat moss was used to grow the seedling in-tray. Sub-irrigation, bottom watering for 10 s, was repeated three times a day at intervals of 3 h for 3 days as shown in table 1. Bottom watering was applied to all tray before the experiment date to equalize initial moisture

Table 1. Irrigation schedule to cause water stress in seedlings

Tray No.	Day 1 1st 10:30	Day 1 2nd 13:30	Day 1 3rd 16.30	Day 2 1st 8:30	Day 2 2nd 11:30	Day 2 3rd 14:30	Day 3 1st 8:30	Day 3 2nd 11:30	Day 3 3rd 14:30
No.1									
No.2									
No.3									
No.4									
No.5									
No.6									
No.7									
No.8									
No.9									
No.10									

Gray: irrigated, White: not irrigated

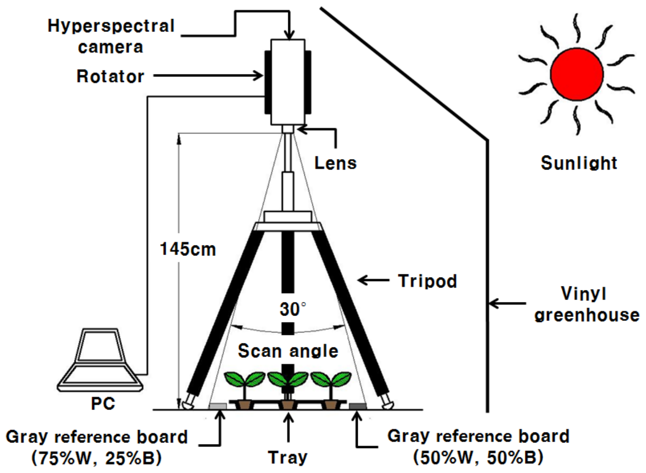

Figure 1. Schematic diagram of the hyperspectral imagery acquisition system.

conditions of the seedlings. The timing of sub-irrigation on the 2nd and 3rd days was rescheduled to adjust the radiance condition in green house. Trays received varying numbers of irrigations to provide variation for testing. The 1st irrigation on day 1 was applied to tray 1, and the 2nd irrigation was applied to tray 1 and 2. In the same way, the 3rd irrigation was applied to tray 1, 2, and 3. The 3rd irrigation on day 3 was applied to all the trays from 1 to 9, and tray 10 was not watered during the experiment.

Figure 1 depicts the hyperspectral imagery acquisition system used in the greenhouse to measure the spectral characteristics of cucumber seedlings under natural daylight condition. A hyperspectral camera (VNIR spectral camera PS, Specim Spectral imaging Ltd., Finland) with a spectral range of 400-1,000 nm and a spectral resolution of 2 nm was used for this study, along with a lens (OLE 23 C-Mount, Specim, Finland) having a 23 mm focal length, a F2.3 aperture, and a 21.1° viewing angle. A rotator was installed on a tripod, and the height of the camera was 145 cm. The scan angle of the camera (the rotation angle of the rotator) was 30°. Two reference boards (50% black-50% white and 25% black-75% white) were placed on both sides of the sample to correct images for changes of sunlight, and an image acquisition program (Spectral DAQ, Specim Spectral Imaging Ltd., Finland) was used to acquire the images.

Images were acquired 30 min after each irrigation (11:00 am, 2:00 am, and 5:00 am on day 1 and 9:00 am, 12:00 am, and 3:00 am on day 2 and day 3, respectively). The fresh weights of leaves were measured after the 3rd experiment on day 3. Dry weights of those leaves were also measured after drying in a dry oven (JSON-050, JS Research Inc., Korea) for 72 h at 60°C. The moisture content was calculated using the measured fresh and dry weights.

Data analysis

Dedicated software (ENVI 4.7, Exelis Visual Information Solution, USA) was employed to separate the seedlings from the background by applying GreenNDVI. The seedling separated from the background was assigned as a region of interest (ROI), and the mean spectral values of the seedlings were extracted. The reflectance spectrum of the seedlings was calculated by dividing the mean spectral value of the seedlings ($Ref_{seedling}$) by the mean spectral value of the assigned reference board (Ref_{board}) to minimize

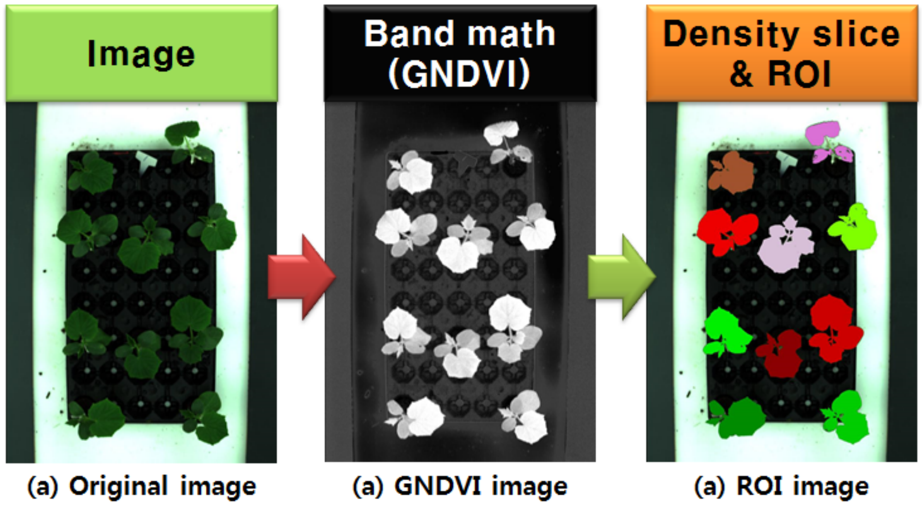

Figure 2. Course of the image processing.

solar-induced disturbances, as shown in equation (1) (Ryu et al., 2014).

$$Ref_{cucumber} = \frac{Ref_{seedling}}{Ref_{board}} \tag{1}$$

A statistical program (Unscramble ver 10.3, CAMO software AS, Norway) was used to create a model to estimate the moisture content. A linear regression model was created by the NDVI (NIR: 852.8 nm, RED: 637.7 nm) that used the reflectance spectrum value of the specific band and moisture content, and the performance of the model was evaluated using the correlation coefficient (r), coefficient of determination (R^2), adjusted coefficient of determination (R_a^2), and relative error (RE). A PLSR model used the moisture content and mean reflectance spectrum of cucumber seedlings, and the performance was evaluated through full-cross validation, coefficient of determination (R^2), root mean square error (RMSE), and relative error (RE).

Results and Discussion

Measurement of the moisture content

Table 2 provides the moisture content of each tray after the 3rd irrigation of day 3 after water stress, in terms of mean and standard deviations. There was no significant change in the mean and standard deviation of the moisture content for trays 1 to 5, but the mean was decreased and the standard deviation increased in trays 6 to 10. There was no change in the moisture content in trays 1 to 5 when sub-irrigation was applied to the seedlings having water stress. Wilting was observed in tray 6 on the 3rd irrigation of day 2, and three seedlings (30%) out of 10 met the wilting point. The number of the wilted seedlings increased in tray 7, and all the seedlings in tray 10 were wilted. The seedlings recovered 100% when irrigation was applied within 42 h (2nd irrigation on day 2 after water stress). However, the seedlings suffering from initial wilting did not recover when more than 42 h passed without irrigation.

Table 2. Moisture content of cucumber seedlings after water stress on the 3rd irrigation of day 3

Tray No.	Mean ± S.D. [%]
No.1	82.49 ± 0.89
No.2	83.50 ± 1.83
No.3	83.54 ± 0.93
No.4	83.86 ± 0.56
No.5	83.12 ± 2.11
No.6	78.34 ± 6.28
No.7	74.81 ± 11.15
No.8	59.68 ± 27.95
No.9	66.05 ± 12.52
No.10	42.14 ± 13.56

Hyperspectral data analysis

The reflectance spectrums of all seedlings (n = 100) were analyzed and it was observed that the reflectance spectrum value among the seedlings differed in the range between 400 nm to 900 nm. The reflectance spectrum was high in seedlings with high moisture content, and low in seedlings with low moisture content (Figure 3).

In tray 6, 3 seedlings wilted early because of less vigorous and less vegetative growth than 7 other seedlings even after applying homogenous irrigation. The wilted seedlings in tray 6 is denoted as 6′ (No.6′). The mean reflectance spectrum of three seedlings randomly selected in tray 5 (No.5), three seedlings randomly selected among the non-wilted seedlings in tray 6 (No.6), and three seedlings selected among wilted seedlings in tray 6′ (No.6′) were compared to examine the changes in the reflectance spectrum of the seedlings, depending on the water stress treatment. Figure 4(a) shows the reflectance spectrum of the seedlings measured after the 1st irrigation of day 1, and no difference was observed during early water stress. Figure 4(b) presents the reflectance spectrum of the seedlings measured after the 1st irrigation of day 2 in tray

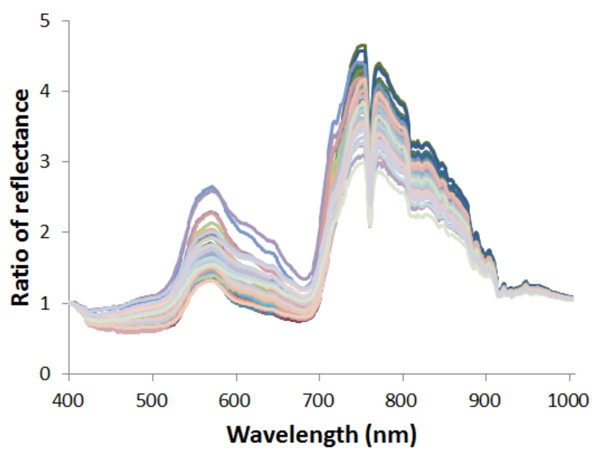

Figure 3. Reflectance spectrum graphs of all seedlings on the 3rd irrigation of day 3.

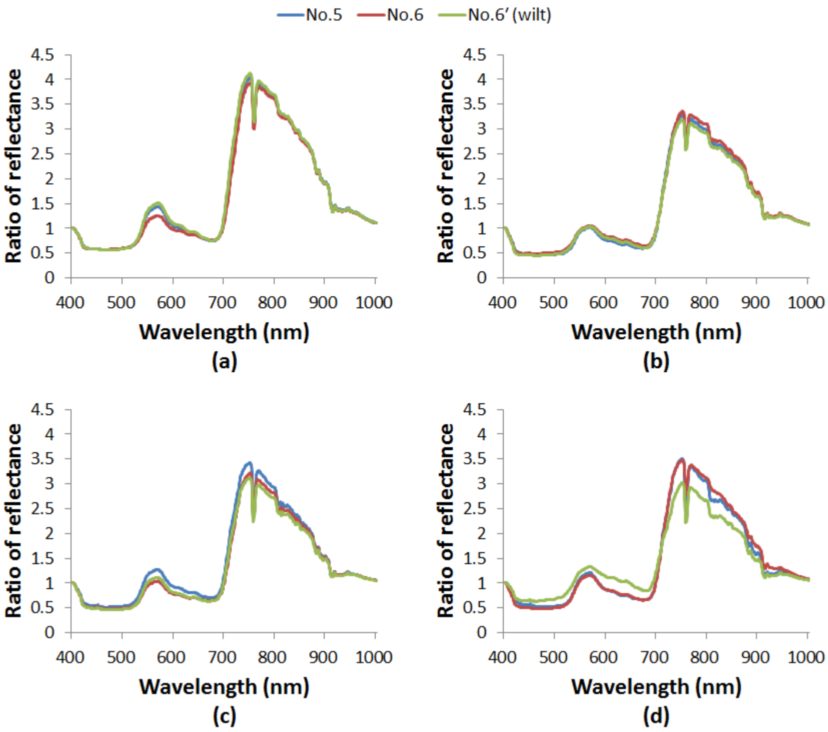

Figure 4. Reflectance spectrum graphs of seedlings after 1st irrigation on day 1 (a), after 1st irrigation- on day 2 (b), after 2nd irrigation on day 2 (c), after 3rd irrigation on day 3 (d).

5, tray 6 containing non-wilted seedlings, and tray 6′ containing wilted seedlings. The reflectance spectrum decreased in three trays, which was due to water stress. The same results were found by Mo et al. (2015), namely, that reflectance spectrum decreased with increasing water stress. Figure 4(c) shows the reflectance spectrum of the seedlings measured after the 2nd irrigation of day 2.

The reflectance spectrum of the seedlings in the tray 5 remained higher due to the sub-irrigation, but the reflectance spectrum of both wilted and non-wilted seedlings in tray 6 decreased as it was under water stress after the 2nd irrigation of day 2. Figure 4(d) presents the reflectance spectrum as measured after the 3rd irrigation of day 3. The reflectance spectrum after irrigation of the seedlings in tray 5 and non-wilted seedlings in tray 6 were distinctly higher than the reflectance spectrum of the wilted seedlings in tray 6′. This result could be attributed to the use of the spectral band ranging from 400-900 nm, where the reflectance spectrum shows differences between non-wilted and wilted seedlings. However, the reflectance spectrum of the seedlings that did not recover after irrigation continued to decrease. Further studies to analyze the relationship between water stress and the number of irrigations may be useful in identifying the wilting point of cucumber seedlings. From the result of T-test, it can be observed that figure 4(a) significantly differs from figure 4(b). The reflectance spectrums of tray 5, tray 6 with non-wilted seedlings, and tray 6′ with wilted seedlings show statistical separation between 400 nm and 751 nm (figure 4[c]) and between 705 nm and 716 nm (figure 4[d]). Compared to stressed and non-stressed seedlings, this result implies that there is a statistically significant difference between treatment and control groups.

The estimation of the moisture content using NDVI (linear regression analysis)

Table 3 presents the results of the linear regression

Table 3. Results of linear regression analysis of cucumber seedlings

Parameters	All sample(n=100)	All sample except 3 seedlings(n=97)
Moisture content [%] (Mean ± S.D.)	73.75 ± 17.12	75.69 ± 13.20
r	0.683	0.757
R^2	0.467	0.573
R_a^2	0.462	0.568
RE [%]	3.876	4.138

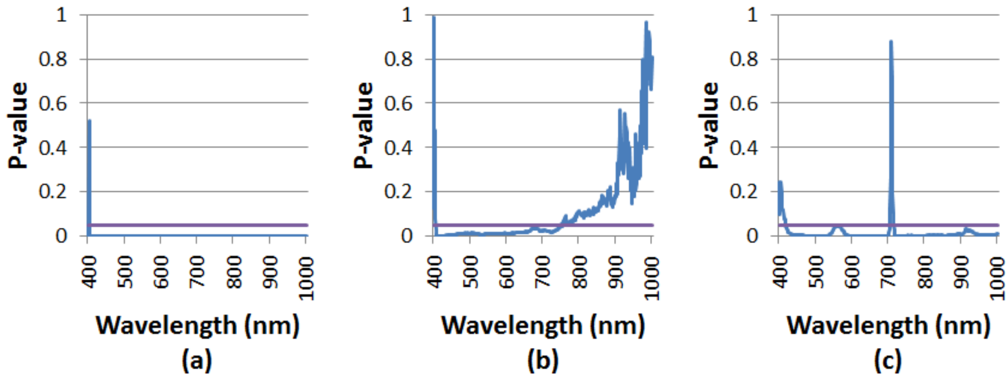

Figure 5. P-value of significant difference between (a) fig. 4(a) and fig. 4(b), (b) tray 5 and 6, 6′ (tray 6 was not applied water at day 2 in fig. 4[c]) and (c) tray 6′ and 5, 6 (tray 6 was applied water at day 3 in fig. 4[d]).

analysis performed for the cucumber seedlings (n=100) using the NDVI and the seedlings moisture content. The model established including all samples (n=100) was not strong enough to estimate moisture content of cucumber seedlings compared with the model including the 97 unwilted seedlings, as after the removal of 3 wilted seedlings, both the precision and accuracy increased. The three wilted seedlings with moisture content below 20% were excluded because the NDVI values of those seedlings were not calculated. The correlation coefficient, precision, and accuracy of the regression model excluding the wilted seedlings (n=97) were higher than the ones of the model including all seedlings. Wilted seedlings were the cause of decrease in the correlation coefficient, precision, and accuracy of the model. Figure 5 shows the linear regression analysis model using 97 seedlings. The NDVI predicted more than 57% moisture content using reflectance spectrum, and the accuracy (RE) was 4.138%. However, less than 20% moisture content was not predicted by the NDVI using reflectance spectrum.

Table 4. Results of the PLSR analysis of cucumber seedlings

Parameters		All sample (n=100)	All sample except 3 seedlings (n=97)
Moisture content [%] (Mean ± S.D.)		73.75 ± 17.12	75.69 ± 13.20
Latent variables		5	4
Cal.	R^2	0.822	0.822
	RMSE [%]	7.187	5.544
	RE	10.26	13.65
Val.	R^2	0.748	0.783
	RMSE [%]	8.557	6.120
	RE	8.619	12.37

The estimation of the moisture content using reflectance spectrum (PLSR analysis)

The results of the PLSR analysis using the reflectance spectrum of all seedlings (n=100) showed a precision of 0.822 and accuracy (RMSE and RE) of 7.187% and 10.26%, respectively, for calibration. They also showed a precision of 0.748 and accuracy (RMSE and RE) of 8.557% and 8.619%, respectively, for validation. The results of the PLSR analysis excluding the three wilted seedlings (n=97) showed a precision of 0.822 and accuracy (RMSE and RE) of 5.544% and 13.65%, respectively, for calibration. They also showed a precision of 0.783 and accuracy (RMSE and RE) of 6.120% and 12.37%, respectively, for validation (Table 4).

The latent variables decreased in the PLSR model that excluded the wilted seedlings. Even as the precision was unchanged, the accuracy of the model increased and the error decreased. The precision and accuracy of the model improved in full-cross validation. Error becomes close to zero by using many latent variables, however, the model becomes unstable with the excessive use of variables. Therefore, using the minimum number of variables while maintaining model precision improves the model (Kim et al., 2015).

There were significant differences in the precision and accuracy of the model using the NDVI with seedlings containing less than 20% moisture content. However, accuracy improved while the precision was maintained in the PLSR model (Figure 6). Therefore, precision and accuracy were improved when using values of all wavelengths.

Wavelengths or combinations of wavelengths related to water stress should be selected to estimate the moisture content of seedlings accurately. In addition, the

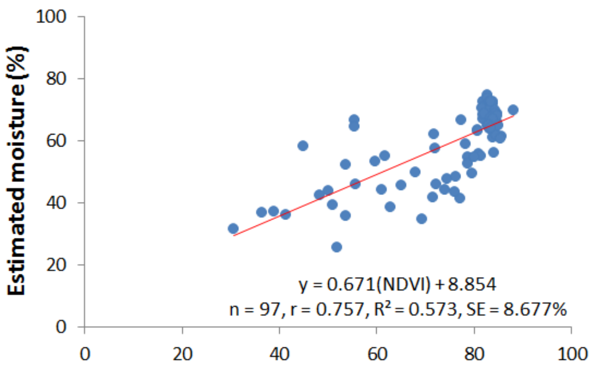

Figure 6. Linear regression graph comparing the estimated moisture content and the measured one from 97 seedlings (excluding three wilted seedlings).

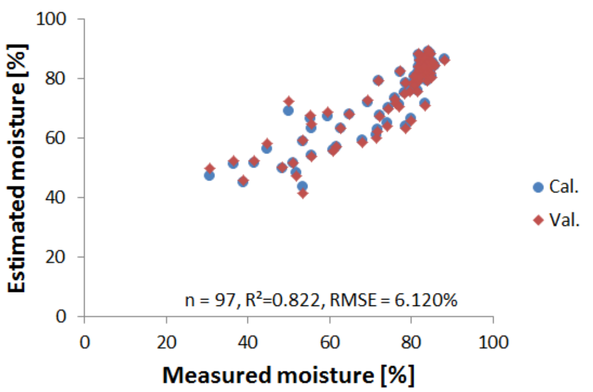

Figure 7. PLSR between estimated moisture content and measured one from 97 seedlings.

wilting point of cucumber seedlings should be identified using the relationship between moisture and reflectance spectrum. Further studies that estimate water stress, moisture content, and wilting point regardless of the type of seedlings should be conducted by repeated experiments with various seedlings that have different ages and environmental and cultivation conditions.

Conclusions

This study estimated the moisture content of cucumber seedlings by using hyperspectral imagery. The reflectance spectrum decreased when the seedlings were under water stress, and it increased when irrigation was provided at initial wilting point of seedling. The reflectance spectrum of the seedlings continued to decrease after permanent wilting even after applying irrigation. From the results of the linear regression analysis using the NDVI, the model excluding wilted seedlings that had moisture content less than 20% (n=97) showed a precision (R^2 and R_a^2) of 0.573 and 0.568, respectively, and accuracy (RE) of 4.138%, which was higher than that model including all seedlings (n=100). For PLS regression analysis using the reflectance spectrum, a strong precision (R^2) of 0.822 for both models was observed, but the accuracy (RMSE and RE) was higher, being 5.544% and 13.65% respectively, in the model excluding wilted seedlings. The model estimating moisture content of cucumber seedlings showed better results in the PLSR analysis using reflectance spectrum than in the linear regression analysis using NDVI. Thus, the results reveal that by using the relationship between water stress and reflectance spectrum it might be possible to detect the initial and permanent wilting points of seedlings.

Conflict of Interest

The authors have no conflicting financial or other interests.

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