Chapter 6

Nondestructive Evaluation of Inoculation Effects of AMF and *Bradyrhizobium japonicum* on Soybean under Drought Stress from Reflectance Spectroscopy

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Additional information is available at the end of the chapter

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Abstract

Precise estimation of leaf chlorophyll content (LCC) and leaf water content (LWC) of soybean, using remote sensing technology, provides a new avenue for the nondestructive evaluation of inoculation effects of arbuscular mycorrhizal fungi (AMF) and *Bradyrhizobium japonicum* (BJ) on soybean growth condition. In this study, a series of pot experiments were conducted in the greenhouse, soybean inoculated with *Glomus intraradices* (G.i, one of AMF species), G.i and BJ, and non-inoculation were planted under drought stress (DS) and normal irrigation (NI) conditions. Leaf spectra and LCC and LWC were measured on the 28th and 56th days after inoculation. Two new simple ratio (SR) indices, derived from the first derivative spectral reflectance at λ1 nm ($D_{\lambda1}$) and the raw spectral reflectance at λ2 nm ($R_{\lambda2}$), were developed to estimate LCC and LWC. The results indicate that under DS, plants inoculated with G.i had higher LCC and LWC than the non-inoculated plants, followed by the counterparts co-inoculated with G.i and BJ. Linear estimation models, established by the $D_{650}/R_{red\ edge}$ and D_{1680}/R_{680}, achieved great improved accuracy for quantifying LCC and LWC of soybean under inoculation and drought stress treatments, with determination of coefficient of 0.63 and 0.76, respectively.

Keywords: leaf chlorophyll, leaf water, remote sensing, soybean, inoculation effects

1. Introduction

Drought is one of the major abiotic stress factors, which reduces plant growth, productivity, and other physiological processes of soybean around the world. For the leguminous plants, there are a lot of AMF and rhizobium in their rhizosphere micro-ecosystem [1]. More than 80% of land plants' roots are known to establish a mutualistic association with AMF [2, 3]. An increasing number of studies have indicated that the symbiosis is beneficial to both the host plants and AMF. The host plants provide the fungi directly with needed carbohydrates including glucose and sucrose to maintain their life cycle [4]. In turn, the fungi can expand the absorption range and area of plant roots and improve plant uptake of water and nutrients in soil [5], greatly contributing to enhance the resistance of plants to drought stress [6].

Over recent years, substantial efforts have focused on understanding the mechanisms of inoculation effects on moisture and nutrient uptake of host plants and the enhanced stress tolerance (e.g., drought stress). Numerous studies have shown the positive effects of the inoculation on plant growth. The symbiotic effects of AMF and BJ on leguminous plants were tested; studies reported that AMF and rhizobium were both beneficial and constrained to each other [7, 8]. The results of Auge et al. [6] and Abdel-Salam et al. [9] provide evidences that AMF improved drought tolerance of rose plants. Many researchers have found that a series of leaf parameters including chlorophyll a and chlorophyll b contents, LCC, LWC, and leaf area of AMF inoculated plants had increased, compared to non-inoculated plants under abiotic stresses [10–12]. Given the sensitivity of LCC and LWC to the inoculation, they are considered as the main biochemical parameters to indicate the inoculation effects of AMF and BJ on plant growth. However, these indicators are almost measured with traditional chemical methods in the laboratory, which is time-consuming and destructive sampled. An accurate, rapid, and nondestructive method is urgently needed for the direct or indirect evaluation of inoculation effects.

Because leaf spectral reflectance is predominantly influenced by LCC and leaf cell structures in visible to near-infrared (NIR) regions (400–900 nm) and by LWC in NIR to shortwave infrared (SWIR) regions (900–2500 nm), the estimation of LCC and LWC with nondestructive optical method, i.e., remote sensing technology, has been developed, suggesting that it would be possible to offer a new avenue to indirectly monitor the effects of AMF and BJ inoculation. Several approaches have been applied to assess LCC and LWC of plants from their optical properties, such as spectral indices or spectral transformations. Chappelle et al. [13] suggested that combinations of band ratios can minimize the effects of spectral convolution; lots of simple ratio (SR) type of spectral indices were proposed in the literatures. Indices, such as pigment-specific simple ratio (PSSR), red-edge spectral parameter (RES), water index (WI), reciprocal of moisture stress index (RMSI), moisture stress index (MSI), etc., were sensitive to LCC and LWC of plants and widely used for the estimation [14–18]. In addition, the first derivative transformation of the apparent absorbance was found to show a great potential for leaf biochemical parameter monitoring [19]. Several derivative spectral indices were derived from the first derivative spectra [20, 21].

So far, only limited information is available on remote estimation of LCC and LWC of soybean inoculated with AMF and/or BJ and on nondestructive and indirect evaluation of the inoculation effects of AMF and/or BJ using remote sensing data. The objectives of this study were (i) to investigate the effects of inoculation on LCC and LWC of soybean under the inoculation and drought stress treatments; (ii) to identify the optimum bands and develop spectral indices for the precise estimation of LCC and LWC, from the first derivative spectra and the raw spectra of soybean leaves; and (iii) to establish the estimation models for LCC and LWC in leaves with AMF inoculation and AMF and BJ co-inoculation, respectively.

2. Materials and methods

2.1. Experimental design

The experiment was conducted in the microbial remediation greenhouse at China University of Mining and Technology (Beijing) (116°21.3′ E, 40°00′ N), from the middle of May to the beginning of July, in 2014. A cultivar of soybean seeds (*Zhonghuang* 35), provided by the Chinese Academy of Agricultural Sciences, were selected and sowed in the pots. The tested strains were arbuscular mycorrhizal *Glomus intraradices* and *Bradyrhizobium japonicum*. 1.1 kg of sandy soil was filled in per pot after sieving, autoclaving, and air-drying. The pot size was 12 cm (top diameter) × 9 cm (bottom diameter) × 15 cm (height). The basic characteristics of sandy soil were as follows: 7.62 of pH, 35.4 μs cm^{-1} of electrical conductivity, 26% of maximum water holding capacity, 13.75 mg kg^{-1} of available phosphorus, and 49.23 mg kg^{-1} of available potassium. Before sowing, the nutrient solution containing NH_4NO_3, KH_2PO_4, and KNO_3 was applied prior to seeding as basal fertilizer, which made the mass fraction of nitrogen, phosphorus, and potassium in the soil reach to 100×10^{-6}, 10×10^{-6}, and 150×10^{-6}, respectively. After 3 weeks of emergence, two healthy and uniformed soybean seedlings were retained per pot, half of the pots were subjected to drought stress (DS), and the other half were irrigated normally (NI), with the maximum water holding capacity of 35 and 75%, respectively. There were G.i inoculation, G.i and BJ inoculation (G.i + BJ), and non-inoculation (CK) treatments under each water gradient. For two inoculation treatments, 50 g of G.i strains soil was added to each pot, while 50 g autoclaved sandy soil was added to the pots of CK treatment. Additionally, for the G.i + BJ treatment, 10 ml BJ solution was poured around the plant roots on the first day of emergence. Each treatment had four replications and was harvested two times, on the 28th and 56th days of inoculation, respectively. All treatments were randomly arranged in the greenhouse.

2.2. Data acquisition

Leaf spectral reflectance and leaf biochemical parameters were measured on the 28th day and 56th day after inoculating, the 28th day of inoculation was exactly the 7th day after drought stress. Forty-five datasets were obtained after removing the outliers.

2.2.1. Leaf spectral reflectance measurement

All leaf spectral reflectance of soybean measurements was made with an ASD FieldSpec4 spectrometer (Analytical Spectral Devices, Boulder, CO, USA) in a dark room. The instrument was fitted with a 25° field of view fiber optics, recording reflectance between 350 nm and 1050 nm with a sampling interval of 1.40 nm and a resolution of 3 nm and reflectance between

1000 and 2500 nm with a sampling interval of 2 nm and a resolution of 10 nm. For each pot, two leaves were cut from the top, middle, and bottom layers of the plants, respectively. Spectral measurement was made at the height of 20 cm above each leaf from four angles rotated with a step of 90°. Each spectral measurement was preceded by an optimization measurement, and a white reference measurement was taken before leaf spectral measurement using a white Spectralon® (Labsphere, Inc., New Hampshire, USA) reference panel. Ten scans were performed for each angle, then 40 scans were averaged as the spectral reflectance of each leaf. The averaged reflectance of six leaves was used to represent the plants' spectrum of each pot.

2.2.2. Leaf biochemical parameter measurement

Immediately following spectral reflectance measurement, LCC and leaf fresh weight (LFW) were measured. LCC was represented by values obtained by SPAD-502 (Minolta Camera Co., Osaka, Japan), due to the good correlation with the extractable chlorophyll content [22]. Fifteen to Twenty points were selected randomly on the leaf; their average value was considered as the LCC.

LFW and leaf dry weight (LDW) were measured using the analytical balance. After weighting LFW, leaves were dried at 105°C for 30 min in an oven and subsequently dried at 80°C until constant weight to measure LDW. LWC (%) was calculated as.

$$\mathrm{LWC}\,(\%) = (FW - DW)/FW \times 100\% \tag{1}$$

2.3. Spectral first derivative transformation

Spectral first derivative transformation was used to process the raw spectral reflectance from 400 nm to 2500 nm. This was computed by Eq. (2), allowing for suppression of the effects from illumination, background reflectance, atmospheric scattering, and absorption on leaf spectral reflectance and inversely highlighting spectral absorption features of leaf biochemical parameters [23]:

$$\mathrm{D}_{\lambda} = (\mathrm{R}_{\lambda\text{-}1} + \mathrm{R}_{\lambda+1})/2\Delta\lambda \tag{2}$$

where D_{λ} is the first derivative spectral reflectance at wavelength λ, $\mathrm{R}_{\lambda-1}$ and $\mathrm{R}_{\lambda+1}$ are the raw spectral reflectance before and after wavelength λ, and $\Delta\lambda$ is the interval between wavelength $\lambda - 1$ and wavelength $\lambda + 1$.

2.4. The construction of spectral indices

SR is one of the primary types of indices for vegetation biochemical parameters. An SR index is conventionally defined as a ratio of the raw spectral reflectance at two wavelengths ($\mathrm{R}_{\lambda1}$ and $\mathrm{R}_{\lambda2}$); its formulation is as Eq. (3). The derivative reflectance has shown to be more sensitive to leaf biochemical parameters at corresponding absorption wavebands than the raw spectral reflectance [19]. Inspired, we inserted a first derivative spectral reflectance ($\mathrm{D}_{\lambda1}$) into SR and constructed the new SR indices with a raw reflectance ($R_{\lambda 2}$) to quantify LCC and LWC for the inoculated soybean, shown as Eq. (4).

$$SR = R_{\lambda 1}/R_{\lambda 2} \tag{3}$$

$$SR_{D_\lambda} = D_{\lambda 1}/R_{\lambda 2} \tag{4}$$

2.5. Statistical analysis

Correlation analysis was used to analyze the relationship between reflectance and LCC and LWC of soybean. Linear regression was applied to model the relationship between spectral indices and LCC and LWC. A leave-one-out cross-validation procedure was used to validate the behavior of spectral indices in LCC and LWC estimation, due to the limited number of sample datasets in our case. The coefficient of determination (R^2), root mean square error (RMSE), and mean absolute error (MAE) were employed as accuracy indicators to evaluate the performances of estimation models, as well as the accuracy of reflection of inoculation effects. The higher the R^2 and the lower the RMSE and MAE, the better accuracy of the models. RMSE and MAE were computed as follows:

$$RMSE = \sqrt{\sum_{i=1}^{n} (\hat{y}_i - y_i)^2/n} \tag{5}$$

$$MAE = \frac{1}{n}\sum_{i=1}^{n}\left|\hat{y}_i - y_i\right| \tag{6}$$

3. Results and discussion

3.1. The effects of inoculation on leaf chlorophyll content and leaf water content

The changes of LCC of soybean under different treatments at two inoculation periods are shown in **Table 1**. LCC of inoculated soybean under NI (i.e., G.i + BJ-NI and G.i-NI) increased, whereas LCC under all other treatments decreased as time went on. After 28 days of inoculation, comparison of LCC of G.i + BJ-DS and G.i-DS with lower content of CK-DS revealed a significant difference due to the effects of inoculation. However, under NI treatment, the difference of LCC between inoculation and CK had not reached the significant level. After 56 days, drought stress resulted in a reduced LCC of soybean, since the stressed plants all had lower LCC than the corresponding normal irrigated plants. LCC of soybean co-inoculated with G.i + BJ and G.i was further significantly higher than that CK under NI, reaching as high as 43.63 and 43.23, respectively.

LWC of soybean under different treatments was measured at two inoculation periods; they were highly affected by the inoculation and application of drought stress (**Figure 1**). After 28 days of inoculation, the LWC was relatively high, with the maximum of 82.3% under CK-NI treatment. Similar to LCC, drought also led to a strong decline in LWC during the beginning of stress. This was reflected by the lower LWC of three treatments under DS compared to the corresponding treatments under NI. After 56 days of inoculation, LWC of all treatments sharply declined along the plant growth, especially for the G.i + BJ-NI and G.i-NI treatments (more than 11%). The growth process of plants markedly increased in July, with the improvement of nutritional and moisture uptake and transport, the growth of more leaves, and bigger leaf area. The stomatal opening and plant transpiration became extremely enhanced, due to the bigger leaf area as well as considerable high temperature in the greenhouse caused by the high outdoor temperature and the plant respiration, consequently resulting in the reduction of LWC. Such decrease in LWC was more pronounced in G.i + BJ/G.i plants than CK plants under NI, partly because the former had much bigger leaf area and more transpiration than the latter.

Treatments	28 days	56 days
G.i + BJ-DS	39.88a	36.73b
G.i-DS	38.28a	36.37b
CK-DS	34.26b	34.15b
G.i + BJ-NI	37.63a	43.63a
G.i-NI	38.79a	43.23a
CK-NI	38.41a	36.27b

Note: G.i + BJ, G.i, and CK indicate treatments of G.i and BJ co-inoculation, G.i inoculation, and non-inoculation, respectively; DS and NI indicate drought stress and normal irrigation, respectively. G.i + BJ-DS indicates soybean co-inoculated with G.i and BJ under drought stress, etc. The letters (a and b) indicate significant differences at $p < 0.05$ among six treatments at each inoculation period.

Table 1. Soybean leaf chlorophyll content under different treatments at two inoculation periods.

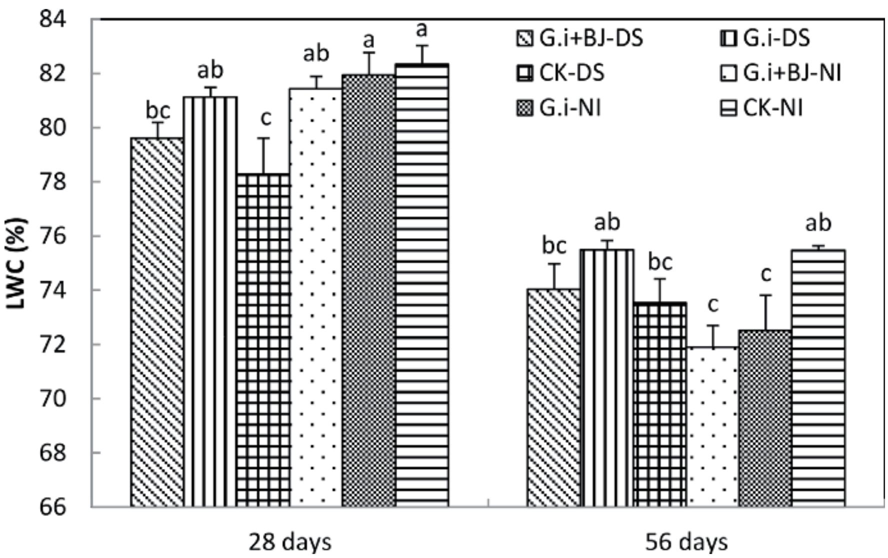

Figure 1. Soybean leaf water content (LWC) under different treatments at two inoculation periods. G.i + BJ, G.i, and CK indicate treatments of G.i and BJ co-inoculation, G.i inoculation, and non-inoculation, respectively; DS and NI indicate drought stress and normal irrigation, respectively. G.i + BJ-DS indicates soybean co-inoculated with G.i and BJ under drought stress, etc. The letters (a–c) indicate significant differences at $p < 0.05$ among six treatments at each inoculation period.

For the LCC and LWC, it was worth noting that the G.i and G.i + BJ plants achieved higher LCC and LWC than CK plants under DS during the two inoculation periods, which was in accordance with the finding of Aliasgharzad et al. [24]. This often benefits from the a mutualistic association formed by AMF and soybean roots (or AMF, soybean roots, and BJ), which could help plants to alleviate the suffering of drought stress directly [9, 25]. Another reason for enhancing drought tolerance in the inoculated plants might be the improvement of phosphorus absorption [26]. In comparison to G.i treatment, LCC and LWC of G.i + BJ plants were slightly lower, because the relationship of mutual restriction between G.i and BJ might play a dominant role compared to that of mutual promotion [7].

3.2. Identifying optimum bands for spectral indices

We analyzed the correlation between first derivative reflectance and LCC and LWC from 400 to 2500 nm, respectively. The results are presented in **Figures 2a** and 3**a**. Generally, the derivative of the green to NIR region (around 550–800 nm) appeared to correlate well with LCC (**Figure 2a**), whereas LWC showed relative high correlation with the derivative reflectance in the NIR to shortwave region (around 1400–2000 nm) compared to the visible region. Specifically, it was evident that LCC got higher coefficients ($-0.5 < r < 0.5$) at three spectral regions, i.e., 610–620, 645–665, and 730–760 nm. LWC showed high r values ($-0.5 < r < 0.5$) with derivative spectra of 1410–1420, 1675–1685, 1875–1880, 1900–1910, and 2240–2280 nm, since they are the absorption bands of leaf water. It was interesting to note that LWC also obtained good correlation in the region between 720 and 750 nm, probably due to its covariance with LCC.

The red-edge position (REP) is defined as the wavelength which has the maximum reflectance of the derivative spectrum within the range of 680–760 nm [27]; it is the transition between low reflectance in the red region and high reflectance in the NIR of the raw spectrum [28]. The reflectance of red-edge region was found to be frequently impacted by chlorophyll absorption [29], affecting many spectral indices (e.g., SR). We, therefore, chose the raw spectral reflectance of REP (i.e., R_{REP}) as the denominator of SR and $SR_{D\lambda}$ indices for soybean LCC estimation. To identify the optimal band of $D_{\lambda 1}$ in the $SR_{D\lambda}$ index, we calculate linear regression of the $SR_{D\lambda}$ ($D_{\lambda 1}/R_{REP}$) model vs. LCC, with λ1 changed from 610 to 760 nm; the result is shown in **Figure 2b**. It was pronounced in LCC estimation where bands between 640 and 660 nm show the higher coefficients of determination (R^2), with peak at 650 nm, near the band of leaf chlorophyll maximum absorption. Then the $SR_{D\lambda}$ index composed of D_{650} and R_{REP} held a promising potential for LCC assessment.

Previous studies have indicated that the significant spectral indices for LWC estimation tend to link to LCC, because of the covariation between the two leaf biochemical parameters [30]. Our result showed the similarity with such finding, with correlation coefficient of −0.4 (not shown for brevity). As a result, 680 nm, where the maximum absorption of chlorophyll, was selected, and R_{680} was used in $SR_{D\lambda}$ index to estimate LWC. Similar to LCC, the R^2 of $SR_{D\lambda}$ ($D_{\lambda 1}/R_{680}$) linear models vs. LWC was computed, with λ1 changed from 1410 to 2280 nm. As shown in **Figure 3b**, three peaks with higher R^2 values were achieved where λ1 was centered around 1410, 1680, and 1900 nm. It was evident that the $SR_{D\lambda}$ index employed the derivative reflectance of 1680 nm (D_{1680}) as the numerator was the most suitable for LWC estimation.

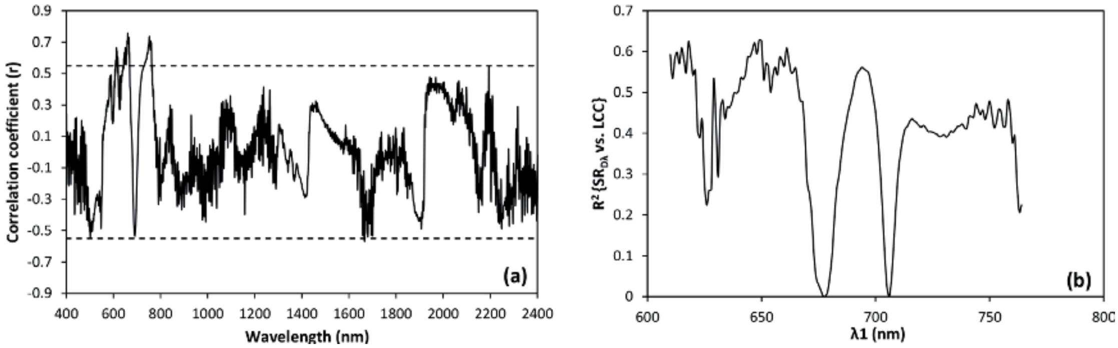

Figure 2. (a) Correlation coefficient (r) between first derivative reflectance and LCC, the dotted lines indicate r = 0.55 or r = −0.55; (b) the R^2 of linear estimation models between $SR_{D\lambda}$ and LCC with λ1 in $D_{\lambda 1}$ changed from 610 to 760 nm.

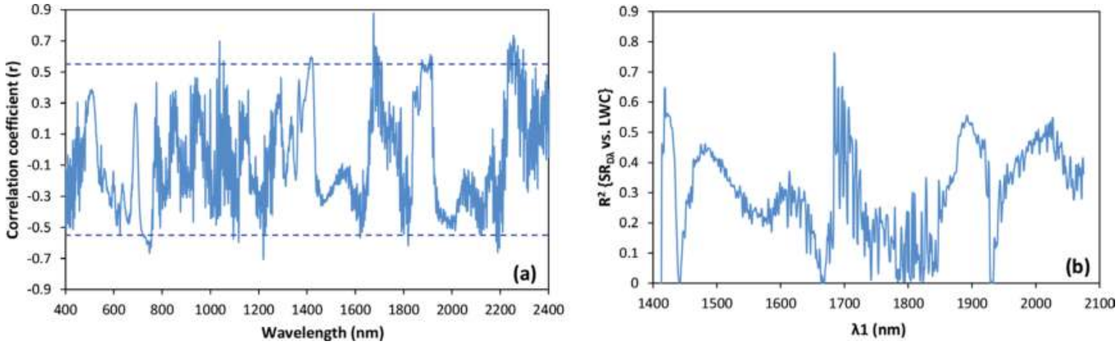

Figure 3. (a) Correlation coefficient (r) between first derivative reflectance and LWC; the dotted lines indicate r = 0.55 or r = −0.55; (b) the R^2 of linear estimation models between $SR_{D\lambda}$ and LWC with λ1 in $D_{\lambda 1}$ changed from 1410 to 2280 nm.

3.3. Leaf chlorophyll content estimation and validation

To indirectly and nondestructively evaluate the inoculation effects on the growth of soybean under all treatments, we estimated LCC and LWC using remote sensing data. Empirical relationships between spectral indices and LCC and LWC were proposed. Based on the analyses presented above, the SR and $SR_{D\lambda}$ indices, developed in this study, for LCC and LWC estimation, were formulated as Eqs. (7)–(10); they were referred as SR-LCC, $SR_{D\lambda}$-LCC, SR-LWC, and $SR_{D\lambda}$-LWC, respectively.

$$\text{SR-LCC} = R_{650}/R_{REP} \tag{7}$$

$$SR_{D_\lambda}\text{-LCC} = D_{650}/R_{REP} \tag{8}$$

$$\text{SR-LWC} = R_{1680}/R_{680} \tag{9}$$

$$SR_{D_\lambda}\text{-LWC} = D_{1680}/R_{680} \tag{10}$$

Forty-five datasets were used to build the estimation models of LCC under different treatments; the results are shown in **Figure 4**. From the distribution of scattering points of the two models, the new index $SR_{D\lambda}$-LCC was strongly and linearly related to LCC variation, whereas the SR-LCC was less sensitive to LCC with the points scattering around the fitted line.

On the other hand, the $SR_{D\lambda}$-LCC model greatly outperformed the SR-LCC model to assess the changes of LCC, achieving the higher value of the coefficient of determination ($R^2 = 0.63$), compared to the R^2 value of 0.11. Furthermore, these results provide a definite proof that spectral first derivative transformation plays an important role in LCC estimation.

To evaluate the general applicability of spectral indices and the prediction precision of models, we analyzed the validation results of LCC (**Figure 5**). As expected from the model calibration, the SR-LCC yielded the weaker prediction accuracy than the $SR_{D\lambda}$-LCC, with a lower R^2 and higher RMSE and MAE values (R^2, RMSE, and MAE of 0.02, 4.25, and 3.58%). Nevertheless, the $SR_{D\lambda}$-LCC model was superior to the SR-LCC model, with the R^2 of 0.59 and RMSE and MAE of 2.68, and 2.15%, demonstrating that it was the best model for the estimation of LCC inoculated with BJ and/or G.i.

3.4. Leaf water content estimation and validation

The linear relationship between LWC of soybean and spectral indices were plotted in **Figure 6**. As seen, the $SR_{D\lambda}$-LWC had a significant linear relationship with LWC; 76% of variation was

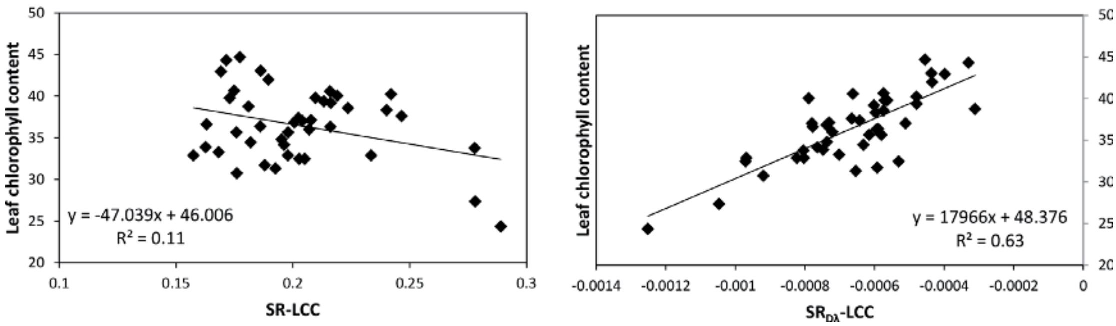

Figure 4. Relationship between leaf chlorophyll content and spectral indices under inoculation and drought stress treatments.

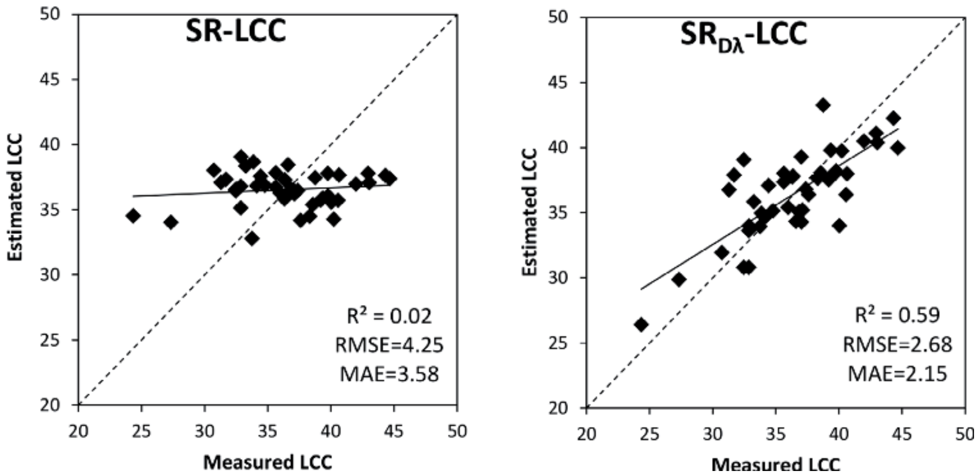

Figure 5. Relationship between measured LCC and estimated LCC using the SR-LCC and $SR_{D\lambda}$-LCC indices under inoculation and drought stress treatments.

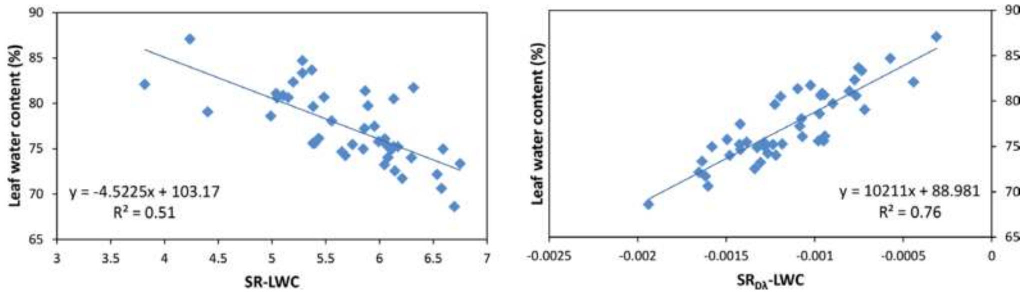

Figure 6. Relationship between leaf water content and spectral indices under inoculation and drought stress treatments.

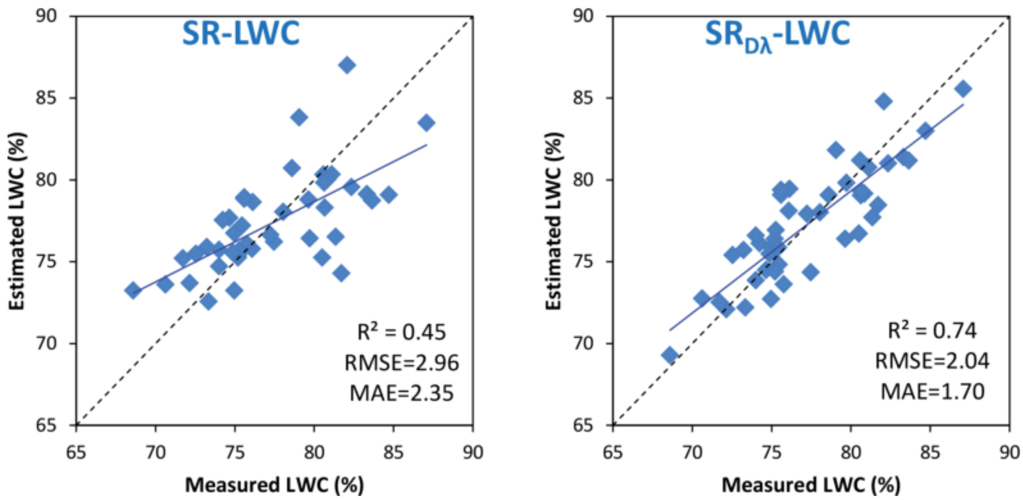

Figure 7. Relationship between measured LWC and estimated LWC using the SR-LWC and $SR_{D\lambda}$-LWC indices under inoculation and drought stress treatments.

explained by the estimation model. Compared to the SR-LWC, the $SR_{D\lambda}$-LWC index yielded a significant increase in R^2 by 49%, greatly improving the accuracy of estimation of LWC under inoculation and drought stress treatments. Meanwhile, it should be noted that the SR index performed better in modeling LWC than its behavior in estimating LCC (**Figure 4**).

Also in this case, the predictive power of spectral indices for LWC estimation were tested (**Figure 7**). The scattering plot showed that a consistent agreement between LWC measured in the laboratory and those estimated by $SR_{D\lambda}$-LWC index, with significant R^2, RMSE, and MAE values of 0.74, 2.04, and 1.70%, respectively, showing its strong stability and predictability. The result indicated that the $SR_{D\lambda}$-LWC model was reliable to predict LWC in this study.

4. Conclusion

The analyses of this study indicated the potential of remote sensing technology in the evaluation of inoculation effects of arbuscular mycorrhizal fungi (AMF) and *Bradyrhizobium japonicum* on soybean growth condition. Drought reduces nutrient uptake, plant growth, and other physiological processes of most crops. Given the higher leaf chlorophyll content (LCC) and leaf water content (LWC) in G.i and BJ-co-inoculated and G.i-inoculated plants than non-inoculated plants under drought stress after 28 and 56 days of inoculation in this study, it can be concluded that rhizobium and/or mycorrhizal fungi could contribute to increase drought tolerance ability of plants, thus leading to alleviation of drought stress in soybean. The changes of LCC and LWC could reflect the inoculation effects on soybean growth condition. Spectral first derivative transformation produced more effective spectral indices for LCC and LWC assessment. The two $SR_{D\lambda}$ indices, using the reflectance of first derivative spectra and the raw spectra of leaves, have been proposed and applied. We found that the $SR_{D\lambda}$-LCC (D_{650}/R_{REP}) and $SR_{D\lambda}$-LWC (D_{1680}/R_{680}) were highly sensitive to LCC and LWC, as compared to the indices that have analogous forms using two reflectances corresponding to the same wavebands of raw spectra, showing their advantages over other spectral indices. Accordingly, quantifying LCC and LWC of crops using remote sensing data would offer a nondestructive and indirect way to monitor the inoculation effects; meanwhile, our results could also expand the present capabilities and applications of vegetation remote sensing.

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Conflict of interest

The authors declare no conflict of interest.

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