Plant and Soil **227**: 47–58, 2000.

A proposal for universal formulas for estimating leaf water status of herbaceous and woody plants based on spectral reflectance properties

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Received 7 October 1999. Accepted in revised form 27 June 2000

Key words: herbaceous plants, reflectance ratio, spectral reflectance, water status of plant leaves, woody plants

Abstract

The present study deals with the relationships between water status parameters of plant leaves and reflectances (R_λ) at characteristic wavelengths, between 522 and 2450 nm, as well as reflectance ratios, R_λ/R_{1430}, R_λ/R_{1650}, R_λ/R_{1850}, R_λ/R_{1920}, and R_λ/R_{1950}, based on the air-drying experimental results of soybean (*Glycine max* Merr.), maize (*Zea mays* L.), tuliptree (*Liriodendron tulipifera* L.) and viburnum (*Viburnum awabuki* K. Koch.) plants. The water status parameters include leaf water content per unit leaf area (LWC), specific leaf water content (SWC), leaf moisture percentage of fresh weight (LMP), relative leaf water content (RWC) and relative leaf moisture percentage on fresh weight basis (RMP). Effective spectral reflectances and reflectance ratios for estimating the LWC, SWC, LMP, RWC and RMP were identified. With these spectral indices, approaches to estimating LWC, RWC and RMP were discussed. Eventually, an attempt on universal formulas was made for estimating the leaf moisture conditions of both herbaceous and woody plants as mentioned above. Moreover, applicability of these formulas was checked with the field experimental results of soybean and maize grown under water and nutrient stresses.

Introduction

Parameters indicating water and nutritional status of leaves are usually viewed as important information of living plants since they are frequently used to explain various physiological and ecological phenomena or problems related to the plants. Unfortunately, it is very difficult to obtain dynamics of an identical leaf, because the measurements concerned are commonly made with destructive methods. In the last two decades, numerous researchers have attempted to relate these parameters to reflectance spectral properties of leaves by remote sensing measurements. For example, Woolley (1970), Gupta and Woolley (1971) and Gausman et al. (1971a,b) investigated the difference of reflectance spectra among leaf positions and among leaf ages. Al-Abbas et al. (1974), Milton et al. (1989,1991), Adams et al. (1993), Blackmer et al. (1994,1996), Masoni et al. (1996) and Schepers et al. (1996) studied the influence of nutrient-deficiency on reflectance spectral properties, and presented a diagnosis method of nutrient-deficiency of plants. Thomas and Gausman (1977), Aoki et al. (1986), Mass and Dunlap (1989), Chappelle et al. (1992), Gitelson and Merzlyak (1996) and Datt (1999) associated vegetable pigments, such as leaf chlorophyll etc., to reflectance spectral properties.

On the other hand, many researches estimated leaf water status by measuring reflectance spectra. Carlson et al. (1971), Gausman et al. (1971), Thomas et al. (1971), Tucker (1980) and Hunt et al. (1987) analyzed the relationships between reflectance spectra and leaf water status in numerous crop species, and pointed out a possibility to estimate relative leaf water content (RWC) by reflectances at some specific wavelengths

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in the range of near infrared. Aoki et al. (1988) reported that there is a close relationship between the leaf water content per unit leaf area (LWC) and the reflectance ratio R_{1650}/R_{1430} for woody plants, while the work of Inoue et al. (1993) showed an approach to estimate LWC and RWC for herbaceous plants using the reflectance ratios R_{1200}/R_{1430} and R_{800}/R_{1650}. When specific parameters of the leaf water status are used to analyze various physiological and ecological phenomena of plants, the parameters have different physiological meanings and readiness in data collection. Therefore, to establish effective reflection index and to develop methods for each parameter, it is necessary to conduct corresponding comparative studies. Also, in the situation of employing environmental remote sensing, it is necessary to develop some universal models that can be used for effectively evaluating the leaf water status for both herbaceous and woody plants.

Therefore, this research focused on the relationships between reflectances at characteristic wavelengths and leaf water status parameters, such as leaf water content per unit leaf area (LWC), specific leaf water content (SWC), leaf moisture percentage on fresh weight (LMP), relative leaf water content (RWC) and relative leaf moisture percentage on fresh weight basis (RMP), and between reflectance ratios and the leaf water status parameters as well. Based on the air-drying experimental results of four plant species, universal evaluation formulas for estimating the leaf water status parameters (LWC, RWC and RMP) were presented, and verified by using field measured data for soybean and maize plants stressed in soil water and nutrients.

Materials and methods

Air-drying experiment of collected leaves

The experiments used two soybean (*Glycine max* Merr.) cultivars (Enray and Sayamusume, abbreviated as GMa and GMb, respectively), maize (*Zea mays* L., cv. Haniebantamu), tuliptree (*Liriodendron tulipifera* L.) and viburnum (*Viburnum awabuki* K. Koch.) plants. Seven layers of leaves for each species were taken from the different heights of the plants without environmental stresses for measurement. The sampled leaves were full-grown and in normal physiological status. Among them, four leaves were made to absorb water for 2 hours, then the reflectance spectra and the fresh weight were measured hourly. The remaining three leaves were used for measuring the reflectance spectra and the fresh weight of water saturated leaves. The water-saturated leaves were obtained by watering them in the dark until there was no further fresh weight change. The water status parameters were calculated with the fresh weight at the time of measuring reflectance spectra. The dry weight and leaf area were obtained after the whole measurement.

Processing of water and fertilizer

At the end of June 1998, soybean (GMb) and maize (cv. Haniebantamu) were sown in pots (diameter 16 cm, height 19 cm, area 1/50 m^2). On July 24th, fertilizer (888 compound fertilizer, 8% NH_4–N, 8% soluble P_2O_5, 8% soluble K_2O) was added to the pots. Amount of applied fertilizer was designed into three levels as lower (F1, 0 g pot^{-1}), medium (F2, 3.0 g pot^{-1}) and higher (F3, 9.0 g pot^{-1}), equivalent to 0, 120 and 360 kg ha^{-1} of nutrients of NH_4–N, soluble P_2O_5 and soluble K_2O, respectively. Additionally, in August, by weighing pots with an electronic scale, six levels of moisture stresses from field capacity (W1) to plant wilting point (W6) were imposed on the plants with specific level of fertilizer treatments. The plants in the 18 pots treated by combining water stresses to fertilizer processing for each crop were investigated under the research objectives.

The measurements were performed twice in the morning and in the afternoon on 21st for soybean and on 22nd for maize in August of 1998. Two leaves in each pot were collected for reflectance spectral measurements. The measurements were made in the order of water stress for each fertilizer treatment. Then the leaves were cut off for determining water contents. The water status parameters were calculated as mentioned in the above paragraph. Simultaneously, using a pressure chamber (Model 3005, SMEC), measurements of leaf water potential, Ψ_L (MPa), were performed for the leaves whose reflectance spectra was just measured. For soil water content, it was calculated according to weighs of pot and dry soil in it. Net assimilation rate, transpiration rate and stomatal conductance were measured by means of portable photosynthesis system (LI-6200, Li-Cor Tnc.). The measurements were performed twice in the morning and in the afternoon on 21st and on 22nd August 1998 for maize and soybean.

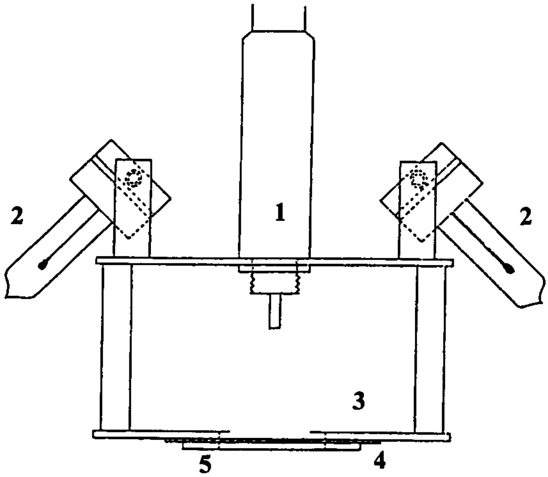


Figure 1. Schematic diagram of a unit for measurement of reflectance spectra of a leaf at natural condition. 1: Probe of Field Spec FR with fiber optics, 2: tungsten-halogen, 3: sample stage, 4: leaf sample, 5: metal fittings for holding a leaf.

Measurement of reflectance spectra

The reflectance spectra were recorded from 350 to 2500 nm wavelength for the upper surfaces of single leaf with a resolution 1 nm, using a spectrophotometer (Analytical Spectral Devices Inc., Field Spec FR) positioned in an exclusive measurement unit, a box with artificial illumination provided by two halogen lamps (Figure 1). When the reflectance spectra were *in situ* measured outdoors, the leaf sample was set on the sample stage, and then the box was wrapped with light-tight curtain. The reflectance spectra were measured against the white reference standard (Labsphere, SRT-99-020). Also, to avoid damage of the leaves exerted by heat of the lamps, the fixation stand was cooled with ice before fixing the measured leaves on it.

Results and discussions

Reflectance spectra of leaves in the process of air-drying

Figure 2 shows the change of reflectance spectra in the process of air-drying (from SWC=2.948 to SWC=0.707) for soybean leaves. The spectral ranging from 350 to 2500 nm wavelength can be characterized by three categories: (1) visible light absorption region 500 – 750 nm, dominated by pigments (primarily chlorophylls *a* and *b*, carotene and xanthophylls); (2) short wavelength near infrared (SWNI) region 750 – 1350 nm, a region of high reflectance and low absorption considerably affected by internal leaf structure; and (3) middle- and long-wavelength near infrared (MWNI and LWNI) of interval 1350 – 2500 nm, a region influenced to some degree by leaf structure, but mainly by the amount of water in the tissue (Gausman et al., 1969,1971a,b; Thomas et al., 1971). Figure 2 shows that the reflectance in visible range is small, while having high reflectance in near infrared, this coordinates with the common view as metioned above. Moreover, in the visible region, the biggest absorption appeared near 680 nm, and the maximum reflectance near 550 nm. In the near infrared region, as reported by many researchers (Aoki et al., 1988, Carlson et al., 1971; Inoue et al., 1993; Thomas et al., 1971; Tucker, 1980; Woolly, 1971), the characteristic wavelength bands for strong absorption by water are near 1430 and 1950 nm, and reflectance peaks are near 1100, 1650 and 2200 nm within the region of SWNI, MWNI and LWNI, respectively. In addition, some smaller valleys of reflectance occurred near 950, 1150, 1750, 2150 and 2300 nm, and some smaller peaks were observed near 1300 and 1850 nm.

From Figure 2, it is easily seen that the reflectance in visible and near infrared regions increased with the progress of air-drying process of soybean leaf. The valleys of reflectance near 1430 and 1950 nm became shallow, in contrast that the peaks of reflectance near 1100, 1650 and 2200 nm became high. Similar results were also observed for the other three plant species. This implies that there exists a close relationship between the water status parameters of the plant leaves and the spectral reflectance.

Effective spectral reflectance for estimating leaf water status

Thomas et al. (1971), Carlson et al. (1971), Aoki et al. (1988) and Inoue et al. (1993), in the studies of numerous plants species, have made it clear that the spectral reflectance at 1430, 1650 and 1920 nm increased in an exponential function with the decrease of relative leaf water content, RWC. In addition, to specify the sensitive wavelengths capable of indicating change of leaf water content, Inoue et al., (1993) computed the first derivative (FD) and the coefficients of variation (CV) of reflectance at each wavelength with variation of leaf water content. They found that the peak values of FD were at 522, 570, 708, 1121, 1367, 1483, 1860 and 2010 nm, and the peak values of CV at 650, 1430,

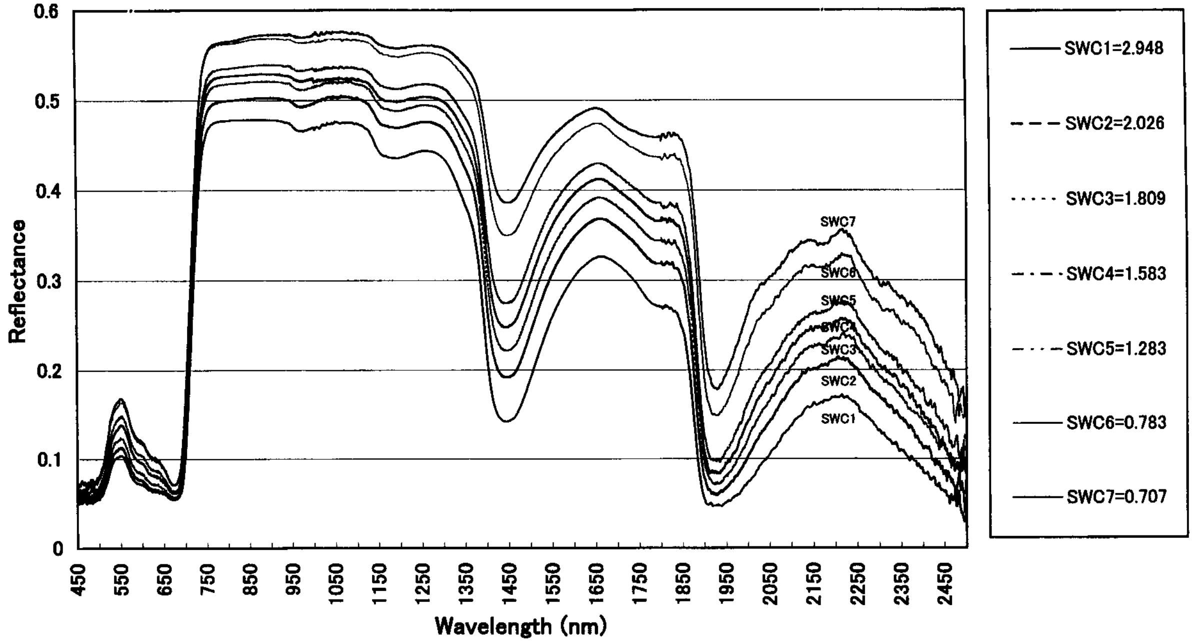


Figure 2. Changes of reflectance spectra of single soybean leaf with different specific leaf water content (SWC) in air-drying experiments.

Table 1. Effective reflectances (R_λ) and coefficient of determination (R^2) of the regression formulas for remote sensing of leaf water contents of soybean (GM), maize (ZM.), tuliptree (LT) and viburnum (VA), and all of the four plant species (ALL).

Leaf water content	R_λ	GM	ZM	LT	VA	ALL
	R_{1367}	0.867	0.642	0.936	0.802	0.594
Leaf water content per unit	R_{1430}	0.965	0.755	0.951	0.904	0.832
leaf area	R_{1483}	0.959	0.759	0.955	0.874	0.838
(LWC, $gH_2O\ m^{-2}$)	R_{1850}	0.963	0.723	0.940	0.868	0.771
$R_\lambda = -a\ \ln(LWC)+b$	R_{1860}	0.948	0.653	0.941	0.908	0.712
	R_{2010}	0.949	0.759	0.904	0.757	0.810
	R_{1367}	0.856	0.754	0.916	0.642	0.640
Specific leaf water content	R_{1430}	0.828	0.906	0.945	0.828	0.533
(SWC, $gH_2O\ g^{-1}$ dry mass)	R_{1483}	0.807	0.909	0.950	0.821	0.522
$R_\lambda = -a\ \ln(SWC)+b$	R_{1850}	0.813	0.884	0.934	0.778	0.553
	R_{1860}	0.823	0.764	0.937	0.792	0.192
	R_{2010}	0.787	0.776	0.917	0.745	0.418
	R_{1367}	0.856	0.717	0.914	0.641	0.647
Leaf moisture percentage	R_{1430}	0.832	0.912	0.947	0.803	0.562
on fresh weight basis	R_{1483}	0.812	0.907	0.952	0.794	0.548
(LMP,%)	R_{1850}	0.817	0.819	0.935	0.764	0.574
R_λ=a LMP +b	R_{1860}	0.827	0.731	0.938	0.822	0.221
	R_{2010}	0.789	0.810	0.921	0.750	0.451

The free degree is 67 for GM and 33 for ZM, LT and VA, and 166 for ALL.

1910 and 2450 nm. In our study, based on the results made before, we analyzed the relation between leaf water status and spectral reflectance, R_λ, at characteristic wavelengths λ selected as 522, 550, 570, 650, 680, 708, 800, 1100, 1121, 1200, 1367, 1430, 1483, 1600, 1650, 1850, 1860, 1910, 1920, 1950, 2010, 2150, 2200, 2250, 2300 and 2450 nm. The moisture conditions of leaf are represented by specific leaf water content (SWC), leaf water content per unit leaf area (LWC), and leaf moisture percentage on fresh weight basis (LMP), and they were calculated with:

$$SWC=(FW-DW)/DW\ (g\ H_2O\ g^{-1}\ \text{dry mass}) \quad (1)$$

$$LWC=(FW-DW)/SL=\rho_L SWC\ (g\ H_2O\ m^{-2}) \quad (2)$$

$$LMP=100(FW-DW)/FW\ (\%) \quad (3)$$

where FW, DW, SL and ρ_L denote fresh weight (g), dry weight (g), leaf area (m^2) and leaf specific mass (g m^{-2}), respectively.

Through analyzing the relationship between reflectances (R_λ) at characteristic wavelengths λ and leaf water parameters (SWC, LWC and LMP), we found that R_λ has a logarithm relation with SWC and LWC across the whole wavelength band, i.e.:

$$R_\lambda=a\ \ln(SWC\ \text{or}\ LWC)+b \quad (4)$$

For LMP, the relationship can be expressed as a linear function like:

$$R_\lambda=a+b\ LMP \quad (5)$$

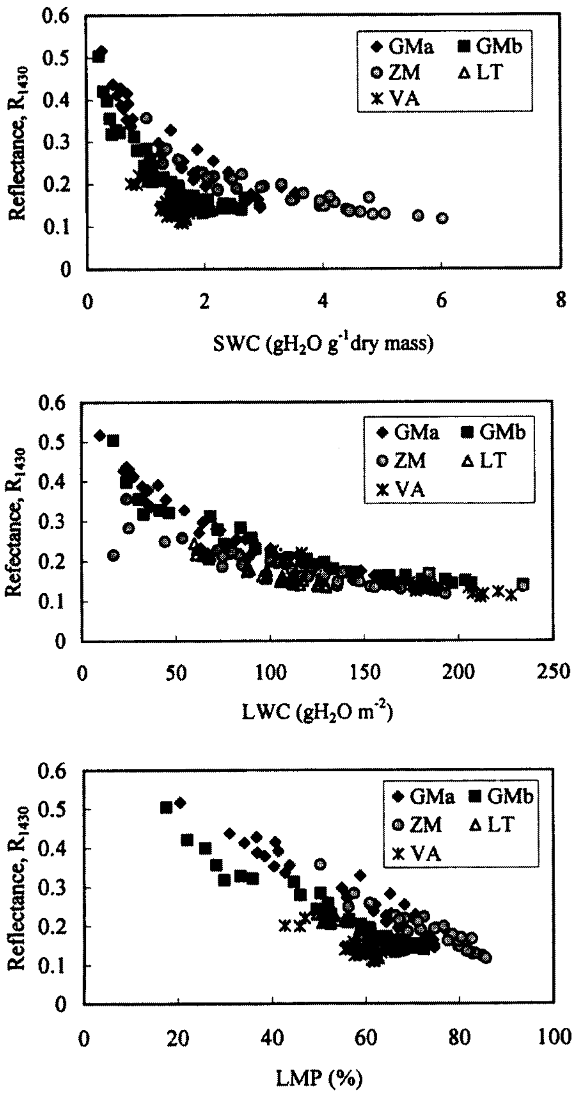


Figure 3. Relationships between reflectance (R_{1430}) and leaf water content per unit leaf area (LWC), specific leaf water content (SWC) as well as leaf moisture percentage on fresh weight (LMP) for soybean (cv, GMa and GMb), Maize (ZM), tuliptree (LT) and viburnum (VA).

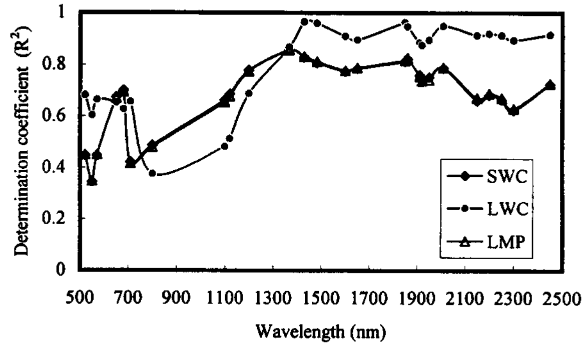


Figure 4. Coefficient of determination (R^2) of the regression formulas for the relationships between reflectances (R_λ) and leaf water content per unit leaf area (LWC), specific leaf water content (SWC) as well as leaf moisture percentage on fresh weight basis (LMP). The diagram is based on the data set of cultivars GMa and GMb of soybean. The degree of freedom is 67.

The relationships between R_λ and SWC, LMP and LWC are plotted in Figure 3 by taking characteristic wavelengths λ =1430-nm as an example.

Figure 4 lists the coefficients of determination (R^2) of the regression formulas between R_λ and SWC, LMP and LWC for soybean. Similar results were also observed for the other three plant species. As shown in Figure 4, R^2 values for the near infrared band were higher than those for the visible band. This implies that using the reflectance near infrared band could make effective estimation on plant leaf water status. This conclusion corresponds with conventional reports of a lot of studies (Aoki et al., 1988; Thomas et al., 1971; Tucker, 1980).

Table 1 shows the R^2 values of the regression formulas for estimating SWC, LMP and LWC using the most effective wavelength (1367, 1430, 1483, 1850, 1860 and 2010 nm). The higher R^2 values suggest a potential for these formulas to be used for quantitatively evaluation of the leaf water status in the four species with sufficient accuracy. Another result that can be seen from Figures 3 and 4 and Table 1 is that the R^2 values for LWC are higher than those for SWC and LMP.

However, when utilizing the data of the four plant species as one set, no satisfactory estimations of SWC and LMP were obtained (Table 1). This is presumably attributable to differences of the plants in ρ_L and saturated water content of the leaves (Table 2). But the relationships between R_λ and LWC were regular. Therefore, universal formulas were developed for estimating LWC of the four plant species. The empirical formulas (Equations 9.1 to 9.3) ranking first three in effectiveness are listed in Table 3.

Figure 3 demonstrates that larger unevenness is with the relationships between R_λ and SWC as well as LMP of the plant species. Table 1 shows that the R^2 values of the regression formulas are lower than 0.64 for estimating SWC or LMP of the four plant species. Therefore, in looking for the universal formulas for estimating SWC or LMP of the four plant species, the work was impeded. In this study, a detail examination was made using the relative leaf water

Table 2. Maximum and the standard deviation (STD) of water status parameters of saturated leaves

Plant species	SWC ($gH_2O\ g^{-1}$)		LMP (%)		LWC ($gH_2O\ m^{-2}$)	
	Maximum	STD	Maximum	STD	Maximum	STD
Glycine max(GMa)	3.54	0.32	77.97	2.44	161.6	14.9
Glycine max(GMb)	2.70	0.14	72.94	1.08	234.4	26.5
Zea mays	6.02	0.83	85.75	2.13	234.4	26.5
Liriodendron tulipifera	2.14	0.05	68.13	0.53	129.9	22.0
Viburnum awabuki	1.73	0.05	63.41	0.65	213.3	36.0

SWC, LMP and LMC are identical to those in Table 1.

Table 3. Empirical equations for estimating leaf water content per unit leaf area (LWC) relative specific leaf water content (RWC) and relative leaf moisture percentage on fresh weight basis (RMP) using reflectances (R_λ) based on the data set of all of the four plant species

Leaf water content	Empirical equation	Equation number	R^2	RMSE ($g\ m^{-2}$ or%)	RRMSE (%)
LWC ($gH_2O\ m^{-2}$)	$LWC = 497.2 \exp(-7.1936\ R_{1483})$	(9.1)	0.838	26.9	22.9
	$LWC = 404.1 \exp(-6.8534\ R_{1430})$	(9.2)	0.832	28.1	23.8
	$LWC = 277.3 \exp(-8.0455\ R_{2010}$	(9.3)	0.810	32.5	27.6
RWC (%)	$RWC = 204.9 \exp(-6.5103\ R_{1430})$	(10.1)	0.903	10.4	16.7
	$RWC = 246.5 \exp(-6.7819\ R_{1483})$	(10.2)	0.896	10.8	17.4
	$RWC = 144.0 \exp(-7.663\ R_{2010})$	(10.3)	0.889	10.8	17.5
RMP (%)	$RMP = -191.2\ R_{1430} + 121.68$	(11.1)	0.896	5.6	7.2
	$RMP = -198.7\ R_{1483} + 127.00$	(11.2)	0.884	6.8	7.5
	$RMP = -222.9\ R_{1850} + 147.76$	(11.3)	0.864	6.8	8.2

R^2 is the coefficient of determination of the regression formulas. RMSE denotes the root mean square error and RRMSE the relative root mean square error. The free degree is 166 for all regression formulas.

content (RWC,%) and relative leaf moisture percentage on fresh weight basis (RMP,%), were calculated, respectively, with:

$$\begin{aligned} RWC &= 100\,(FW\text{-}DW)/(SW\text{-}DW) \\ &= 100\,SWC/SWCs = 100\,LWC/LWCs \quad (6) \end{aligned}$$

$$RMP = 100\,LMP/LMPs \quad (7)$$

where SWCs, LWCs and LMPs represent saturated values of SWC, LWC and LMP (Table 2). Using these relative values, unevenness among the plant species in the relations of R_λ and SWC as well as LMP was reduced to some extent. The results indicate that the empirical formulas effective for RWC were produced as Equations 10.1 to 10.3 and the ones for RMP are given as Equations 11.1 to 11.3 in Table 3. Table 3 also shows the R^2 values, root mean square error (RMSE) and relative root mean square error (RRMSE), which were calculated with:

$$RMSE = \left[\frac{1}{N-1}\Sigma(Y_{est} - Y_{mea})^2\right]^{1/2} \quad (8)$$

$$RRMSE = 100\ RMSE/\overline{Y}_{mea} \quad (9)$$

where Y_{est} represents the estimated values, Y_{mea} and $\overline{Y}_{mea}$ are the measured values and their mean value, respectively. N is number of the data pairs.

From Table 3, it is understood that RMSE value was 26.9–32.5 g m^{-2} for LWC, 10.4–10.8% for RWC, and 5.9–6.8% for RMP. RRMSE values for the LWC, RWC and RMP are 22.9–27.6%, 16.7–17.5% and 7.2–8.2%, respectively. Moreover, there is no obvious difference in the R^2 values between LWC, RWC and RMP. However, with regard to the RRMSE, the R^2

Table 4. The functions for expressing the relationships between some reflectance ratios and water status parameters of the leaves

Reflectance ratio	X=LWC and SWC or RWC Function	Band	LMP or RMP Function	Band
$Y=R_\lambda/R_{1430}$	Y=b X+a	Whole band	Y=a exp(bX)	Whole band
$Y=R_\lambda/R_{1650}$	Y=b X+a	$\lambda \leqq 1367$	Y=b X+a	Whole band
	Y=b ln(X)+a	$\lambda \geqq 1430$		
$Y=R_\lambda/R_{1850}$	Y=b X+a	$\lambda \leqq 1367$	Y=a exp(bX)	$\lambda \leqq 1367$
	Y=b ln(X)+a	$\lambda \geqq 1430$	Y=b X+a	$\lambda \geqq 1430$
$Y=R_\lambda/R_{1920}$	$Y=c X^2+b X+a$	Whole band	Y=a exp(bX)	$\lambda \leqq 1367$
$Y=R_\lambda/R_{1950}$			Y=b X+a	$\lambda \geqq 1430$

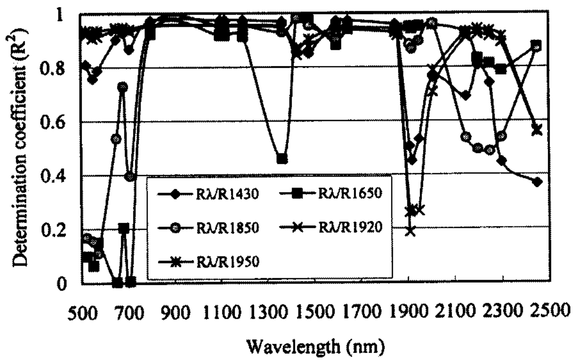


Figure 5. Coefficient of determination (R^2) of the regression formulas in Table 4 expressing the relationships between leaf water content per unit leaf area (LWC) and reference ratios (R_λ/R_{1430}, R_λ/R_{1650}, R_λ/R_{1850}, R_λ/R_{1920}, R_λ/R_{1950}). The diagram is based on the data set of cultivars GMa and GMb of soybean. The degree of freedom is 67.

value for RMP is the smallest, while that for LWC is the biggest (Table 3).

Thus, it can be seen from above that the most effective reflectances (R_λ) for estimating LWC and RWC are R_{1430}, R_{1483} and R_{2010}, and for estimating RMP are R_{1430}, R_{1483} and R_{1850}. That is the effective reflectances, R_{1430} and R_{1438}, are suitable for estimating either of LWC, RWC or RMP. Figure 2 shows that 1430 nm is a characteristic wavelength band for strong absorption by water in the near infrared region. As reported by Inoue et al. (1993), at 1430 nm there exists peak value of the coefficients of variation (CV) of reflectance at each wavelength with variation of leaf water content. Moreover, 1483 nm is characteristic wavelength at which peak values of the first derivative (FD) exist, according to Inoue et al. (1993).

Effective reflectance ratio for estimate leaf moisture condition

Reflectance ratio is an index frequently used to estimate leaf water status. For example, Aoki et al. (1988) proposed an estimation formula of LWC for the woody plant by relating LWC to R_{1650}/R_{1430}. Inoue et al. (1993) presented an estimation formula of LWC for the herbaceous plants by relating it to R_{1200}/R_{1430} and R_{800}/R_{1650}. Here, our examinations focused on relationships between some reflectance ratios (R_λ/R_{1430}, R_λ/R_{1650}, R_λ/R_{1850}, R_λ/R_{1920} and R_λ/R_{1950}) and the water status parameters of the leaves (LWC, SWC or RWC, and LMP or RMP). According to the results, we found that there are different relationships between the reflectance ratios and each of the water status parameters. The functions for expressing those relationships are given in Table 4. For the LWC, SWC and RWC, they have identical function forms for the relationships between the reflectance ratio and each of them. The same trend is for LMP and RMP. In addition, Table 4 indicates that there exist different function forms for the relationships of some reflectance ratios and leaf water status across the whole wavelength band.

Figure 5 demonstrates changes of the R^2 values of the regressions between LWC of soybean and the reflectance ratios (R_λ/R_{1430}, R_λ/R_{1650}, R_λ/R_{1850}, R_λ/R_{1920} and R_λ/R_{1950}). Evidently, the effective wavelengths are different with reflectance ratios. For this, a possible explanation is that R_λ/R_{1430}, R_λ/R_{1650} and R_λ/R_{1850} are more effective than R_λ/R_{1920} and R_λ/R_{1950} in LWC estimation.

For the relationships between LWC, RWC or RMP of the four plant species, and R_λ/R_{1430}, R_λ/R_{1650} or R_λ/R_{1850}, changes of the R^2 values for equations in Table 4 at each wavelength are shown in Figure 6.

According to Figure 6, the most effective empirical formulas (Equations 12.1 to 14.3) for estimating LWC, RWC and RMP were selected and are shown in Table 5. The most effective reflectance ratios for estimating LWC are R_{1483}/R_{1650}, R_{1100}/R_{1430} and R_{1121}/R_{1430}; for estimating RWC are R_{1430}/R_{1650}, R_{1430}/R_{1850} and R_{1483}/R_{1650}; and for estimating RMP are R_{2200}/R_{1430}, R_{1430}/R_{1650} and R_{1483}/R_{1430}. The values of RMSE for LWC calculated with the formulas in Table 5 are smaller than 19.2 g m^{-2}, for RWC smaller than 12.9%, and for RMP smaller than 6.9%. The values of RRMSE for LWC, RWC and RMP were smaller than 16.3%, 20.7% and 8.0%, respectively. It is clear that all formu-

Table 5. Empirical equations for estimating the leaf water status parameters using reflectance ratios based on the data set of all of the four plant species

Leaf water content	Empirical equation	Equation number	R^2	RMSE ($g\ m^{-2}$ or%)	RRMSE (%)
LWC ($gH_2O\ m^{-2}$)	$LWC = 2717.6 \exp(-5.3929\ R_{1483}/R_{1650})$	(12.1)	0.910	17.7	15.0
	$LWC = 68.72\ R_{1100}/R_{1430} - 6.7227$	(12.2)	0.902	19.1	16.3
	$LWC = 69.77\ R_{1121}/R_{1430} - 6.8636$	(12.3)	0.901	19.2	16.3
RWC (%)	$RWC = 612.2 \exp(-4.3521\ R_{1430}/R_{1650})$	(13.1)	0.909	11.5	18.5
	$RWC = 2229.2 \exp(-5.4911\ R_{1430}/R_{1850})$	(13.2)	0.907	12.7	20.4
	$RWC = 1052.1 \exp(-4.8368\ R_{1483}/R_{1650})$	(13.3)	0.884	12.9	20.7
RMP (%)	$RMP = 159.9\ Ln(R_{2200}/R_{1430}) + 74.01$	(14.1)	0.897	6.2	7.5
	$RMP = -126.8\ R_{1430}/R_{1650} + 153.25$	(14.2)	0.885	6.6	7.1
	$RMP = 405.4\ Ln(R_{1483}/R_{1430}) + 40.76$	(14.3)	0.870	6.9	8.0

The abbreviations are identical to those in Table 3. The free degree is 166 for all regression formulas.

las are of higher estimation precision for LWC, RWC and RMP. In addition, it can be found that the R^2 values of RMP are rather lower than those of LWC and RWC, but the RRMSE value of RWC is the biggest and that of RMP the smallest.

Also, if comparing the R^2 values in Table 3 and 5, we can find that using reflectance ratios could improve the R^2 values and make the RMSE and RRMSE values of the empirical formulas smaller than those using reflectances. Therefore, to estimate water status of living leaves, reflectance ratios should be more useful indices.

Verification of the empirical formulas

To verify the empirical formulas mentioned above, measured data for the leaves of soybean and maize grown in pots under water and nutrient stresses were used. Figure 7 shows volumetric water content of soil in the pots and water potentials of the leaves of soybean and maize when reflectance ratios were measured. The values are the averages of the values measured in the morning and afternoon. Soil water content for the water treatment W1 is 0.5 $m^3\ m^{-3}$, equivalent to field capacity. Soil water content for the water treatment W6 is 0.2 $m^3\ m^{-3}$, approximating to plant wilting point. For the same level of soil water stress, soybean and maize had different leaf water potentials. That leaf water potentials decreased with aggravation of soil water stress clearly indicates influences of soil water on leaf water status. The leaf water potential of soybean under water treatment W1 was from −0.4 to −0.6 MPa, and that under water treatment W6 was from −1.7 to −1.9 MPa. For maize, the leaf water potential was from −0.3 to −0.5 MPa in the case of water treatment W1, and from −0.9 to −1.3 MPa in the case of water treatment W6.

On the other hand, LWC of the soybean plants grown in the pots ranged from 11 to 187 $g\ m^{-2}$, with an average of 30.9±32.1 $g\ m^{-2}$. RWC varied from 6.8 to 94.4%, with an average of 65.4±13.7%, and RMP from 23.2 to 97.7%, with an average of 87.6±9.7%. For maize, LWC ranged from 11 to 196 $g\ m^{-2}$, with an average of 137.6±31.8 $g\ m^{-2}$; RWC ranged from 34.9 to 98.4%, with an average of 59.8±11.5%; and RMP ranged from 73.9 to 100%, with an average of 90.7±4.4%.

The mean values of photosynthesis rate, transpiration rate and stomatal conductance of soybean and maize plants grown in the pots during the period of investigation are shown in Figure 8. From Figure 8, it can be seen that the values of these physiological indices increased with amount of fertilizer applied to the pots. However, significant influence arising from fertilizer application was not found in soybean. On the other hand, water stress impacts physiological properties of both soybean and maize significantly. The values of these physiological indices decreased with increasing of soil water stress, especially for the plants grown under fertilizer treatments of medium (F2) and high (F3) levels of amount.

For the living soybean and maize leaves under water and nutrient stresses, the leaf moisture content decreased with soil drying and simultaneously induced enhancement of the spectral reflectance over the whole

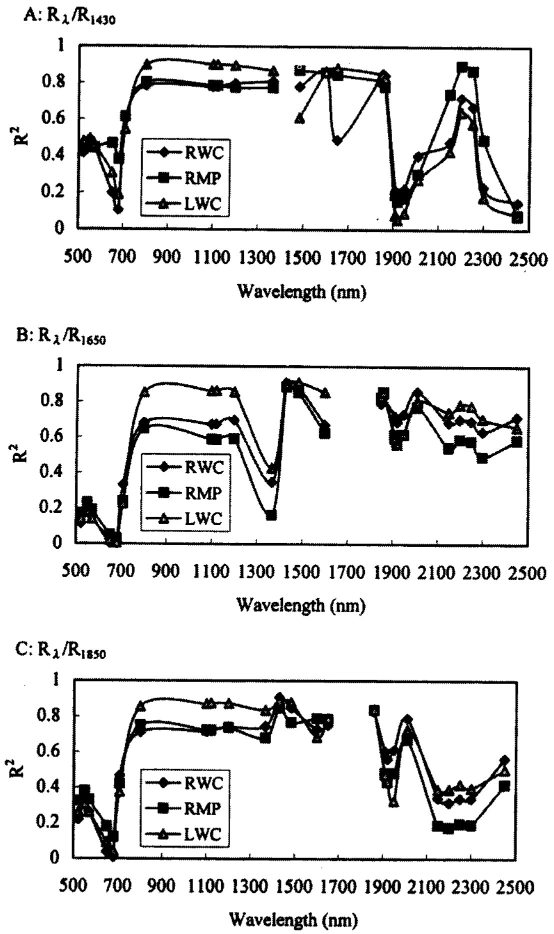


Figure 6. Coefficient of determination (R^2) of the regression formulas in Table 4 expressing for the relationships between reflectance ratios (R_λ/R_{1430}, R_λ/R_{1650}, R_λ/R_{1850}) and leaf water content per unit leaf area (LWC), relative specific leaf water content (RWC) as well as relative leaf moisture percentage on fresh weight basis (RMP). The diagram is based on the data set of the four plant species. The degree of freedom is 166.

band. This is consistent with the results (Figure 2) measured for the air-drying leaves in laboratory. Using the observation data, we examined validation of the empirical formulas listed in Tables 3 and 5.

For LWC, RWC and RMP of soybean, in the case of using the empirical formulas (Equations 9.1 to 11.3) involving the reflectance, the R^2 values of the regression formulae, Y_{mea}=bY_{est}, are higher than 0.56, 0.53 and 0.59, the RMSE values are lower than 15.9 g m^{-2}, 8.67% and 4.98%, and the RRMSE values are lower than 12.2%, 13.1% and 5.7%, respectively. In comparison, in the case of using the empirical formulas (Equations 12.1 to 14.3) involving the reflectance ratios, the R^2 values are higher than 0.89, 0.71 and 0.37, the RMSE values are lower than 14.4 g m^{-2}, 12.1% and 7.7%, the RRMSE values are lower than 10.9%, 18.4%, 8.8% for LWC, RWC and RMP, respectively. It can, therefore, be assumed that those empirical formulas are suitable for estimating the moisture conditions of the soybean leaves with acceptable precision (Figure 9). However, more satisfactory results in estimating the LWC, RWC and RMP were not achieved for the leaves of maize under water and nutrient stresses. The RMSE value for LWC is 46–49 g m^{-2}, for RWC is 17–29%, and for RMP is 6–10%.

For the higher estimation precision in the case of soybean, and the lower precision in the case of maize, the reasons are assumed to be the following two points. First, maize leaf has large leaf veins, which could induce measurement errors of the spectral reflectance and the leaf moisture conditions, when the reflectance spectra were measured outdoors. Second, the formulas were developed without considering effects of the fertilizer on reflectance spectra of the leaves. Figure 8 shows that there is no significant difference in the physiological indices between fertilizer treatments in the case of soybean. However, there exists a significant fertilizer effect in the case of maize. This fertilizer effect also might impact the spectral characteristics of maize leaves. Regarding this, further examinations are needed.

Conclusions

In this study, we analyzed the relationships between water status parameters of plant leaves and characteristic spectral reflectance as well as reflectance ratios, based on the air-drying experimental results of the four plant species. Effective reflectances and reflectance ratios were identified for estimating LWC, SWC, LMP, RWC and RMP. Using these spectral indices, we specified the modeling approaches to estimating LWC, RWC and RMP, and eventually presented the universal models for estimating the leaf moisture conditions of both the herbaceous and woody plants. Furthermore, the formulas were tested on the experimental data of maize and soybean grown under water and nutritional stresses in the field. For soybean, satisfactory results were received. For maize, however, the estimation precision was lower. This might be due to morphological feature of maize leaf and fertilizer effects. Further examinations are necessary.

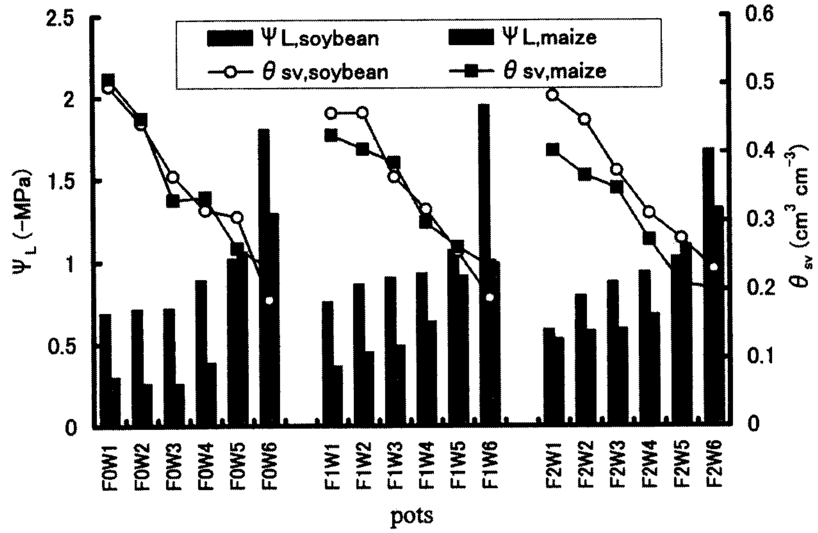
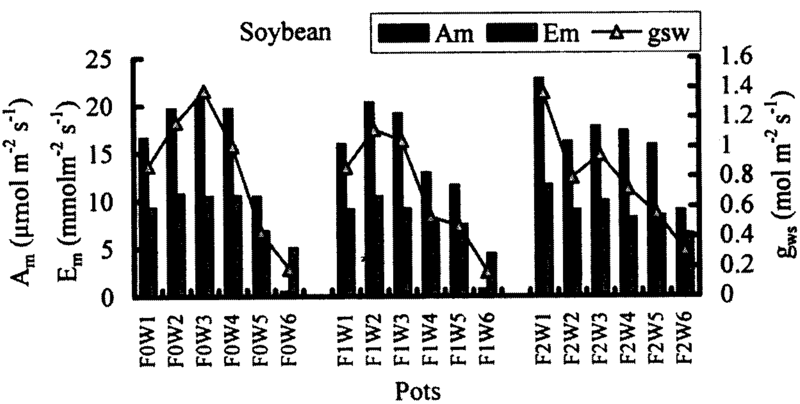


Figure 7. Changes of volumetric water content, θ_{sv}, of soil in the pots and leaf water potential, Ψ_L, of maize and soybean plants grown in the pots under water and nutritional stresses.



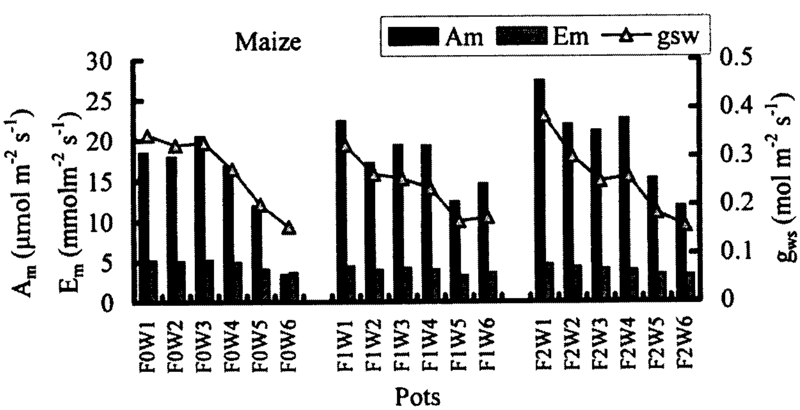


Figure 8. Changes of net assimilation rate, A_m, transpiration rate, E_m, and stomatal conductance, g_{ws}, of soybean (A) and maize (B) plants grown in the pots under water and nutritional stresses.

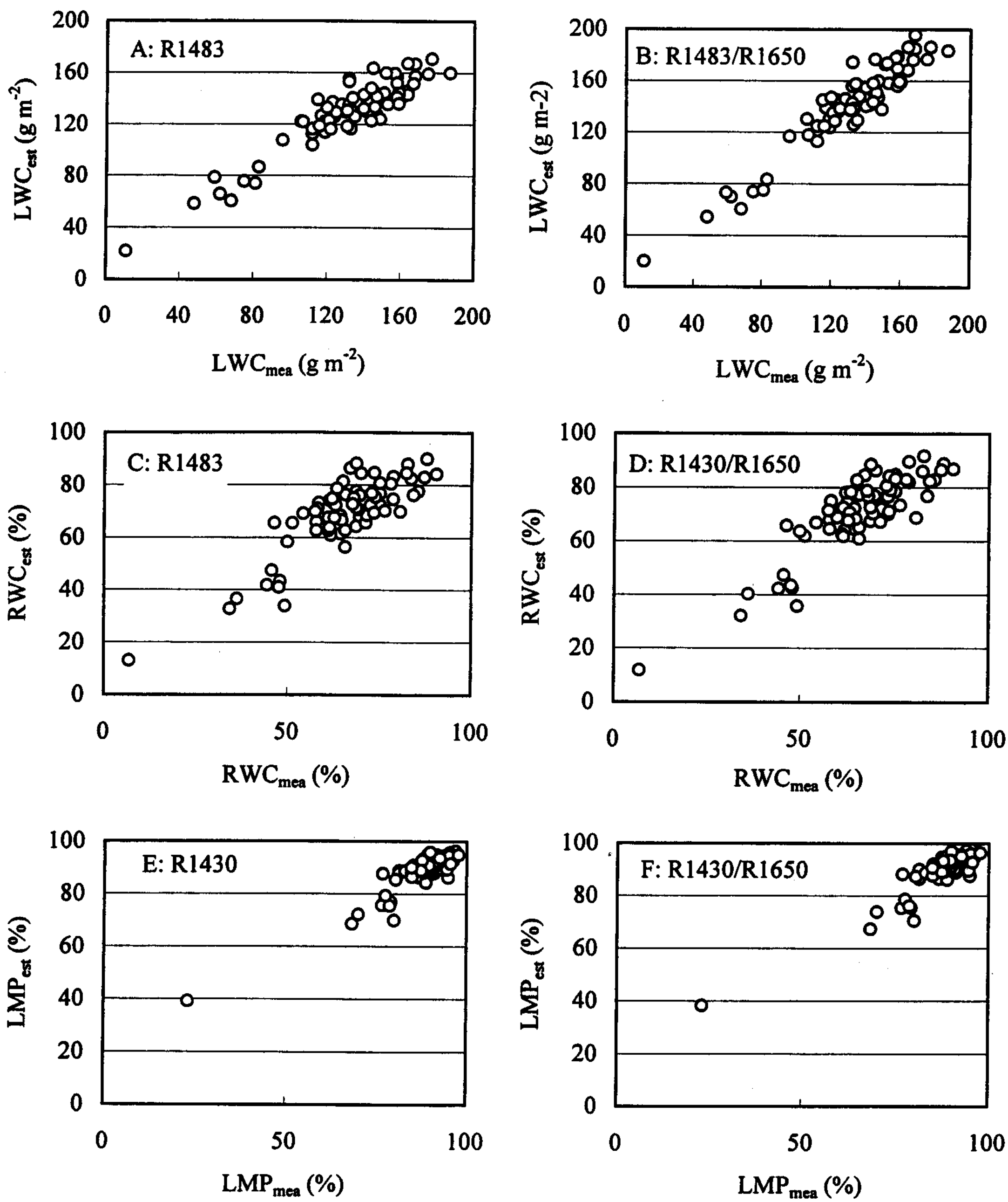


Figure 9. Relationships between the measured and the estimated values of leaf water status parameters, leaf water content per unit leaf area (LWC), specific leaf water content (SWC), leaf moisture percentage on fresh weight (LMP), of soybean under stresses of water and nutrition. (A) Equation 9.1 with reflectance E_{1483}; (B) Equation 12.1 with reflectance ratio R_{1483}/R_{1650}; (C) Equation 10.2 with R_{1483}; (D) Equation 13.1 with R_{143}/R_{1650}; (E) Equation 11.1 with R_{1430} and (F) Equation 14.2 with R_{1430}/R_{1650}. The subscripts 'est' and 'mea' denote the estimated and measured values, respectively.

Acknowledgements

We thank the support of the research project of "The Hundreds-Talents Program" of The Chinese Academy of Sciences. Also, thanks are due to Dr J. Zhuang for the valuable comments on the manuscript, and K. Oda, D. Satou and T. Urushisaki for their assistance in the measurements.

References

Adams M L, Norvell W A, Peverly J H and Philpot W D 1993 Fluorescence and reflectance characteristics of manganese deficient soybean leaves: Effects of leaf age and choice of leaflet. Plant Soil 155/156, 235–238

Aoki M, Yabuki K and Totsuka T 1988 Effective spectral characteristics of leaf for the remote sensing of leaf water content. J. Agric. Meteorol. 44, 111–117.

Aoki M, Yabuki K and Totsuka T and Nishida M 1986 Remote sensing of chlorophyll content of leaf: Effective spectral reflection characteristics of leaf for the evaluation of chlorophyll content in leaves of dicotyledons. Environ. Control in Biol. 24, 21–26.

Al-Abbas A H, Barr R, Hall J R, Crane F L and Baumgardner M F 1974 Spectra of normal and nutrient-deficient maize leaves. Agron. J. 66, 16–20.

Blackmer T M, Schepers J S and Varvel G E 1994 Light reflectance compared with other nitrogen stress measurements in corn leaves. Agron. J. 86, 934–938.

Blackmer T M, Schepers J S, Varvel G E and Walter-Shea E A 1996 Nitrogen deficiency detection using reflected short-wave radiation from irrigated corn canopies. Agron. J. 88, 1–5.

Carlson R E and Yarger D N 1971 An evaluation of two methods for obtaining leaf transitivity from leaf reflectance measurements. Agron. J. 63, 78–81 .

Carlson R E, Yarger D N and Shaw R H 1971 Factors affecting the spectral properties of leaves with special emphasis on leaf water status. Agron. J. 63, 486–489.

Gitelson A A and Merzlyak M N 1996 Signature analysis of leaf reflectance spectra: Algorithm development for remote sensing of chlorophyll. J. Plant Physiol. 148, 494–500.

Chappelle E W, Kim M S and McMurtrey J E 1992 Ratio analysis of reflectance spectra (RARS): An algorithm for the estimation of the concentrations of chlorophyll *a*, and chlorophyll *b*, and cartooned in soybean leaves. Remote Sens. Environ. 39, 239–247.

Datt B 1999 A new reflectance index for remote sensing of chlorophyll content in higher plants: Tests using *Eucalyptus* leaves. J. Plant Physiol. 154, 30–36.

Gausman H W, Allen W A, Myers V I and Cardnas R 1969 Reflectance and internal structure of cotton leaves. Agron. J. 61, 374–376.

Gausman H W, Allen W A, Cardenas R and Richardson A J 1971a Effects of leaf nodal position on absorption and scattering coefficients and infinite reflectance of cotton leaves, *Gossypium birsutum* L.. Agron. J. 63, 87–91.

Gausman H W, Allen W A, Escobar D E, Richardson A J and Cardenas R 1971b Age effects of cotton leaves on light reflectance, transmittance, and absorption and on water content and thickness. Agron. J. 63, 465–469.

Gupta R K and Woolley J T 1971 Spectral properties of soybean leaves. Agron. J. 63, 123–126.

Hunt E R Jr, Rock B N and Nobel R S 1987 Measurement of leaf relative water content by infrared reflectance. Remote Sens. Environ. 22, 429–435.

Inoue Y, Morinaga S and Shibayama M 1993 Non-destructive estimation of water status of intact crop leaves based on spectral reflectance measurements. Jpn. J. Crop Sci. 62, 462–469.

Maas S J and Dunlap J R 1989 Reflectance and transmittance, and absorption of light by normal, etiolated and albino corn leaves. Agron. J. 81, 105–110.

Milton N M, Ager C M, Eiswerth B A and Power M S 1989 Arsenic-and selenium-induced changes in spectral reflectance and morphology of soybean plants. Remote Sens. Environ. 30, 263–269.

Milton N M, Eiswerth B A and Ager C M 1991 Effect of phosphorus deficiency on spectral reflectance and morphology of soybean plants. Remote Sens. Environ. 36, 121–127.

Masoni A, Ercoli L and Mariotti M 1996 Spectral properties of leaves deficient in iron, sulfur. Magnesium and manganese. Agron. J. 81, 937–943.

Schepers J S, Blackmer T M, Wilhelm W W and Resende M 1996 Transmittance and reflectance measurements of corn leaves from plants with different nitrogen and water supply. J. Plant Physiol. 148, 523–529.

Thomas J R, Namken J N, Oerther G F and Brown R G 1971 Estimating leaf water content by reflectance measurements. Agron. J. 63, 845–847.

Thomas J R and Gausman H W 1977 Leaf reflectance vs. leaf chlorophyll and carotenoid concentrations for eight crops. Agron. J. 69, 799–802.

Tucker C J 1980 Remote sensing of leaf water content in near infrared. Remote Sens. Environ. 10, 23–32.

Woolly J T 1971 Reflectance and transmittance of light by leaves. Plant Physiol. 47, 656–662.