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Photochemical reflectance index and solar-induced fluorescence for assessing cotton photosynthesis under water-deficit stress

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Abstract

Rapid and non-destructive assessment of water status is essential to enhance crop performance. This study aimed to evaluate photosynthetic performance and to monitor water status in cotton under field conditions. A two-year experiment was conducted with three irrigation regimes to measure the following parameters: photochemical reflectance index (PRI), structural independent pigment index (SIPI), water index (WI), solar-induced fluorescence (SIF; retrieved from reflectance by using Fraunhofer line-depth method), gas exchange, and chlorophyll fluorescence (CF). The results showed that PRI decreased > 30 % in moderate drought (MD) and >50 % in severe drought (SD), compared with control. PRI was found to be positively correlated with net photosynthetic rate (P_N), stomatal conductance, transpiration rate, actual quantum yield of photosystem II photochemistry (Φ_{PSII}), but a negatively correlated with nonphotochemical quenching (NPQ). Solar-induced fluorescence around 761 nm (SIF_{761}) had significant correlations with P_N, Φ_{PSII}, and NPQ, but not with maximal quantum yield of PS II photochemistry (F_v/F_m). The relationship between PRI and P_N was stronger at the beginning of water stress ($R^2 = 0.86$) than for the all stress stages ($R^2 = 0.54$), indicating that PRI could be more effective for assessing P_N of cotton at early water stress. PRI was better correlated with relative water content and photosynthetic parameters than SIPI and WI and so it could also be a good indicator to evaluate cotton water status.

Additional key words: chlorophyll *a* fluorescence, drought, *Gossypium hirsutum*, net photosynthetic rate, stomatal conductance, transpiration rate.

Introduction

Water deficit is a major abiotic factor limiting plant growth and crop productivity around the world and it is considered a severe threat for sustainable crop production under climate change (Le Houerou 1996). Water stress can induce morphological, physiological, and biochemical changes in plants. Rapid, accurate, and non-destructive monitoring of water relations and photosynthetic status is essential to determining crop productivity and improve water use efficiency. Optical remote sensing techniques, especially hyperspectral remote sensing can provide an efficient and quantitative approach for stress detection in plant canopies. Major progress has been made with remotely sensed vegetation indices to assess physiological traits associated with plant water status (Peñuelas and Filella 1998, Sun *et al.* 2008, Mobasheri and Fatemi 2013). Nevertheless, most of traditional vegetation indices have no direct link to photosynthetic functioning and maybe low sensitive to track changes of water status. The most promising remote sensing techniques are measurement of the photochemical reflectance index (PRI, Gamon *et al.*

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Abbreviations: CF - chlorophyll fluorescence; F_v/F_m - maximal quantum yield of PS II photochemistry; g_s - stomatal conductance; MD - moderate drought; NPQ - nonphotochemical quenching; PRI - photochemical reflectance index; P_N - net photosynthetic rate; RWC - relative water content; SD - severe drought; SIF - solar-induced fluorescence; SIF_{761} - solar-induced fluorescence around 761 nm; SIPI - structural independent pigment index; T_r - transpiration rate; WI - water index; Φ_{PSII} - effective quantum yield of PS II photochemistry.

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1992), and of solar-induced fluorescence (SIF) in the oxygen absorption bands (Plascyk and Gabriel 1975, Carter *et al.* 1996, Zarco-Tejada *et al.* 2001, Meroni *et al.* 2009).

PRI was originally derived as a measure of xanthophyll cycle activity and determined using proximal remote sensing of leaves on a diurnal time scale. This index is sensitive to the de-epoxidation of the xanthophyll cycle pigments and can be used to detect dynamic variations of nonphotochemical quenching of chlorophyll fluorescence (Gamon *et al.* 1992, 1997). When radiation exceeds the amount that can be used for photosynthesis (for example under stress conditions), excess energy must be dissipated to avoid photoinhibition and photooxidation. One of the mechanisms for dissipation of the excess energy is fluorescence emitted by chlorophyll *a* of photosystem II. The other mechanism is heat dissipation, linked to inter-conversion of the xanthophyll cycle pigments, and inducing changes in the leaf reflectance at 531 nm which are detected by PRI. Thus PRI provides a new remote sensing method to estimate photosynthetic efficiency (Peñuelas *et al.* 1997, Rascher and Pieruschka 2008). Most studies suggested that drought resulted in declined PRI (Winkel *et al.* 2002, Van Gaalen *et al.* 2007, Sarlikioti *et al.* 2010, Sun *et al.* 2014). At the leaf level, PRI is positively related to variable to maximum fluorescence ratio (F_v/F_m), actual photochemical efficiency of PS II ($\triangle F/F_m'$), and photosynthetic radiation-use efficiency, but negatively related to nonphotochemical quenching (NPQ). Dobrowski *et al.* (2005) found that heat and water-stress induce changes in steady-state chlorophyll fluorescence (CF) measured with the PRI, which is a more effective real time indicator of photosynthetic function than indices based upon leaf water content and pigment content. Suárez *et al.* (2008) demonstrated that the airborne-level PRI index is sensitive to the de-epoxidation of the xanthophyll cycle pigments caused by water stress. However, the relationship between PRI and photosynthetic parameters has been found to vary quite widely in different studies. Sun *et al.* (2014) reported that PRI is very sensitive to pigment content and net photosynthetic rate (P_N) in olive trees during water-stress and recovery. PRI correlates more closely with carotenoid content than with structural independent pigment index (SIPI). Gamon *et al.* (2002) argued that a consistent relation between PRI and radiation-use efficiency at the canopy level is not always found, and PRI does not work well under severe drought. Naumann *et al.* (2010) reported that PRI is not effective in tracking changes in $\triangle F/F_m'$ during times of stress in *Elaeagnus umbellata*, supporting the view that xanthophyll cycle dissipation is not the photoprotective mechanism for some species. Wong and Gamon (2015) reported that three distinct processes affect PRI in evergreen conifers: a short-term, facultative response related to the operation of the xanthophyll cycle; a long-term, constitutive response related to seasonally changing carotenoid:chlorophyll ratio; and reversible response to cold.

Recently, CF kinetics technique has found wide applications in many fields, such as plant physiology, plant nutrition, and plant ecology. Many parameters could be derived under dark or under irradiance from different sources (Maxwell and Johnson 2000). The CF induction kinetics technique is a useful tool to explain photosynthetic mechanisms. Nevertheless, scientists have been looking for new approaches to record fluorescence remotely and rapidly. Many studies suggested that SIF is currently the most promising approach towards space platform (Plascyk and Gabriel 1975, Carter *et al.* 1996). Although SIF signal is rather weak, its detection is possible using vegetation indices and solar Fraunhofer line discrimination (FLD) methods from plant apparent reflectance (Plascyk and Gabriel 1975, Moya *et al.* 2004, Liu e*t al.* 2005). Two broad oxygen absorption bands of terrestrial atmosphere at 688 nm (O_2-B) and 760 nm (O_2-A) are usually used to estimate SIF. SIF has been retrieved and tested at the ground and airborne scales, showing a promising potential to evaluate plant photosynthetic activity. At the leaf level, Amoros-Lopez *et al.* (2008) reported that the SIF emissions at 687 and 760 nm measured by *FieldSpec FR* spectroradiometer can discriminate between different health conditions of the plants. An increased magnitude of SIF (both at 760 nm and 687 nm) in herbicide-treated bean leaf was found by Meroni and Colombo (2006), while a decreased SIF at 760 nm under water stress in maize was measured by Ni *et al.* (2015). Therefore, there are still uncertainties regarding the inter-play between SIF and photosynthesis under different conditions. At the canopy level, Pérez-Priego *et al.* (2005) showed that the CF in-filling in the O_2-A band at 760 nm was sensitive to diurnal variations of fluorescence and water stress in orchard trees. Also, some works demonstrate that SIF could be retrieved from airborne narrow-band data (Zarco-Tejada *et al.* 2009, Damm *et al.* 2014) and it is feasible to detect water stress in trees (Zarco-Tejada *et al.* 2009) and maize (Panigada *et al.* 2014).

The effects of water stress on cotton depend on the severity and duration of stress, the growth stage at which stress is imposed, and the genotype of the plant (Zhang *et al.* 2014). At the flowering stage (in late June to early July) cotton is very sensitive to drought (Loka *et al.* 2011) and accurate detection of water stress to achieve an optimum water supply for productivity and to maximize the water-use efficiency has an increasing importance. Although the characterization on PRI and solar-induced fluorescence has been reported in various plant species, the performance of them in detecting drought effects on plant activity is still debated. Also relatively few studies have been conducted on the relationship between PRI and photosynthetic parameters. The objectives of this paper were to evaluate behavior of P_N, PRI, and SIF under different water treatments in the field condition, and to investigate the applicability of PRI and SIF in assessing early stage of water stress in cotton.

Material and methods

Plants and treatments: The experiment of this study was conducted in 2011 and 2012 at the experimental station of Hebei Agricultural University, located in Baoding city (38° 49' 17.4" N, 115° 26' 18.8" E). In this area, the average temperature and accumulative precipitation from April to September during the cotton growth period were 21.7 °C and 405.6 mm in 2011, and 22.2 °C and 511.6 mm in 2012, respectively. Three water regimes were imposed under rain-sheltered control conditions: severe drought (SD, soil relative water content of 35 - 45 %), moderate drought (MD, 45 - 55 %), and control (CK, 55 - 70 %). Seeds of cotton (*Gossypium hirsutum* L.; transgenic cultivar Yinrui361 selected by Institute of Cotton Research, Dezhou, in 2007, and derived from transgenic cultivar sGK321 harboring Bt/CpTI gene) were sown in pots filled with mixtures of soil and compound fertilizer (N: P_2O_5: K_2O = 16:8:16; m/m/m), one plant per each pot, on April 28 2011 and April 19 2012. There were 50 plants in each treatment. All seedlings were well irrigated before water treatments, which started in mid-June (bud stage). Irrigation timing and amount were controlled based on measurement of soil water content using a time domain reflectometry (*TZS-2X*, *Zhejiang Top Instrument*, Hangzhou, Zhejiang, China). For each treatment, irrigation was timely applied when the soil relative water content RWC reached the lower limit, by which to adjust the soil water content to the upper limit of the treatment.

Measurements were carried out 3 - 5 d after irrigation under clear sky conditions between 09:00 - 12:00. Photosynthetic photon flux densities (PPFD) ranged between 800 and 1500 μmol m^{-2} s^{-1}, air temperatures between 33 and 39 °C, and water vapor pressure between 1 and 4 kPa. Each year we measured three time points: July 9 (flowering stage), July 26 (boll-filling stage) and August 4 (boll-filling stage) in 2011, July 3 (flowering stage), July 16 (boll-filling stage) and August 6 (boll-filling stage) in 2012. In order to avoid shadows caused by the shelter frames, pots were moved outside 1.5 h before measurement to allow plants to adapt to consistent high irradiance. The pots were arranged to ensure that the fourth fully expanded leaves from the top were oriented to the same direction.

Determination of leaf relative water content (RWC): The fourth fully expanded leaf from the top was removed, weighed quickly and immediately immersed into double distilled water to saturate with water during 24 h and then the leaves were oven dried to a constant mass at 80 °C. Leaf RWC was calculated according to the following formula: RWC [%] = [(fresh mass - dry mass)/(water saturated mass - dry mass)] × 100.

Measurement of leaf reflectance: Leaf reflectance was measured using a portable *GER 1500* spectroradiometer (*Spectra Vista Corporation*, Poughskeepsie, NY, USA) with a spectral range of 350 - 1050 nm. The spectroradiometer has 512 channels with 1.5 nm bandwidth and 3 nm spectral resolution at 700 nm. A 25 × 25 cm^2 $BaSO_4$ calibration panel was used to measure the solar irradiance. The fourth fully expanded leaf from the top was put horizontally above a pure black pad to avoid the influence of background. A 23° field-of-view fiber optic was held at a distance 8 cm above the leaf. The scanned area of a leaf was approximately 8 cm^2. Three spectra were collected and averaged for each leaf to acquire a representative value. Each treatment was replicated 5 times and different parameters were calculated:

$\mathrm{PRI} = (R_{531} - R_{570}) / (R_{531} + R_{570})$

$\mathrm{SIPI} = (R_{800} / R_{445}) / (R_{800} - R_{680})$

$\mathrm{WI} = R_{970} / R_{900}$

where terms with different R subscripts indicate reflectance at the corresponding wavelengths.

SIF at O_2-A band was retrieved from leaf irradiance and solar irradiance based on the Fraunhofer line-depth method by using three bands (3 FLD) (Maier *et al.* 2003, Ni *et al.* 2015).

$$\mathrm{SIF} = \frac{L(\lambda_{in}) - \dfrac{E(\lambda_{in}) \times (w_{left} \times L(\lambda_{left}) + w_{right} \times L(\lambda_{right})}{w_{left} \times E(\lambda_{left}) + w_{right} \times E(\lambda_{right})}}{1 - \dfrac{E(\lambda_{in})}{w_{left} \times E(\lambda_{left}) + w_{right} \times E(\lambda_{right})}}$$

$$w_{left} = \frac{\lambda_{in} - \lambda_{left}}{\lambda_{right} - \lambda_{left}} \qquad w_{right} = \frac{\lambda_{right} - \lambda_{in}}{\lambda_{right} - \lambda_{left}}$$

where $L(\lambda_{left})$ and $L(\lambda_{right})$ represent the target radiances in the left and right shoulders of the Fraunhofer well, respectively. In addition, $E(\lambda_{left})$ and $E(\lambda_{right})$ represent the solar irradiance in the left and right shoulder of the Fraunhofer well, respectively, and w_{left} and w_{right} are the weights of two bands outside the Fraunhofer line (for more information about FLD principle, see Fig. 5 from Meroni *et al.* 2009). In this paper, λ_{right} was 772.4 nm, λ_{in} was 761.6 nm, and λ_{left} was 753.8 nm. Due to the spectral resolution (3 nm) of *GER 1500* spectroradiometer, in this experiment only the O_2-A absorption band was used to calculate SIF_{761}.

Gas exchange parameters: Immediately following the reflectance measurement, gas exchange parameters [P_N, stomatal conductance (g_s), transpiration rate (T_r)] were measured in the same leaves using a portable photosynthesis system *LI-6400* (*Li-Cor*, Lincoln, NE, USA).

Chlorophyll fluorescence parameters: Chlorophyll fluorescence measurements were carried out using a portable fluorometer *FMS-2* (*Hansatech*, Kings Lynn, UK) on the same leaf as the reflectance measurement. First steady-state fluorescence (F_s) was obtained under natural irradiance, then a saturation pulse (8 000 μmol m^{-2} s^{-1}, 0.8 s) was used to acquire maximum yield of chlorophyll fluorescence in light (F_m'), which was followed by dark adaption for 20 min to acquire the maximal fluorescence (F_m) and minimal fluorescence (F_0). Maximum quantum yield of PS II photochemistry ($F_v/F_m = (F_m - F_0)/F_m$), effective quantum yield of PS II photochemistry ($\Phi_{PSII} = (F_m' - F_s)/F_m'$), and nonphotochemical quenching ($NPQ = F_m/F_m'-1$) were calculated.

Statistical analysis: Statistical analysis was performed using *SPSS* software *v. 22.0* (*IBM*, Armonk, NY, USA). Data was analyzed by one-way *ANOVA*, followed by Duncan's multiple comparison test. The values are reported as means with their standard errors. Differences were considered significant at $P < 0.05$. Column and scatter plots were produced by *Origin 8* (*OriginLab*, Northampton, MA, USA).

Results

Leaf relative water content significantly decreased with the increased water stress. Average values of leaf RWC in CK, MD, SD were 80.2, 72.1, and 64.6 % at three time points in 2011 and 81.9, 71.8, and 65.1 % at three time points in 2012 (Fig. 1). Average RWC in MD and SD decreased by 10.1 and 19.5 % in 2011, 12.3 and 20.5 % in 2012, respectively, compared with the control. At each measuring time, RWC showed significant differences among the water stress treatments, which were consistent across the two years. Likewise, P_N also decreased with the increase in water stress (Fig. 2). The differences in P_N among different treatments were larger in 2012 than those in 2011. There were significant declines in PRI of cotton leaves at each measuring time in both years in response to water stress (Fig. 3*A*,*D*). On average, the PRI in MD and SD decreased by 34.0 and 70.9 % in 2011, by 35.8 and 58.1 % in 2012, compared with control. The largest differences among treatments were found in 9 July, 2011 and 3 July, 2012 (cotton in flowering stage and the initial stage of drought stress). Compared with control, the PRI

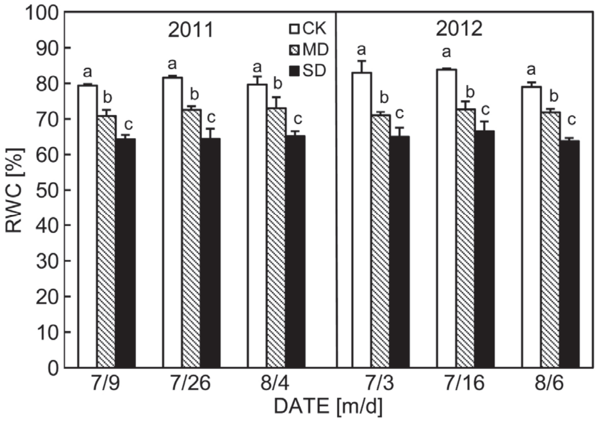

Fig. 1. Cotton leaf relative water content (RWC) under three water regimes at different time points in years 2011 and 2012. CK - control, MD - moderate drought, SD - severe drought. Means ± SEs, $n = 3$, different letters indicate significant differences at $P < 0.05$.

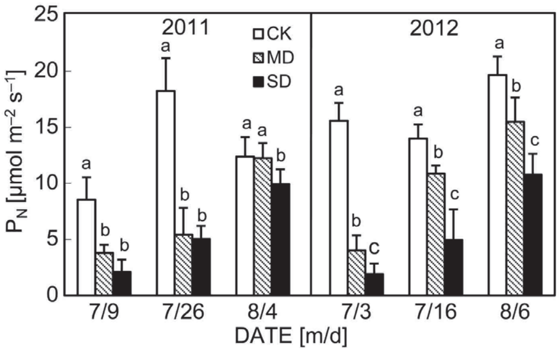

Fig. 2. Net photosynthetic rate under three water regimes at different time points in years 2011 and 2012. CK - control, MD - moderate drought, SD - severe drought. Means ± SEs, $n = 5$, different letters indicate significant differences at $P < 0.05$.

in MD and SD decreased by 47.3 and 90.7 % in 9 July, 2011 and by 58.0 and 80.2 % in 3 July, 2012. In the last two time points (cotton in boll-filling stage), the differences in PRI among three treatments were less obvious than in the initial stage.

As concern SIPI, under SD it was higher than in CK (Fig. 3 *B*,*E*) but only in July 9 in 2011 and July 3 in 2012 significant differences were found among CK, MD, and SD. An increasing trend was also found in WI along with the increased water stress (Fig. 3*C*,*F*). Under SD, WIs were significantly higher than in CK for all measuring time points.

SIF was retrieved from field hyperspectral radiance around 761 nm (O_2-A band) based on Fraunhofer line discrimination principle by using three bands (3 FLD). SIF_{761} decreased with the severity of drought stress (Fig. 4). On the average, the SIF_{761} in MD and SD decreased by 15.6 and 34.1 % in 2011, by 40.0 and 52.3 % in 2012, compared with control.

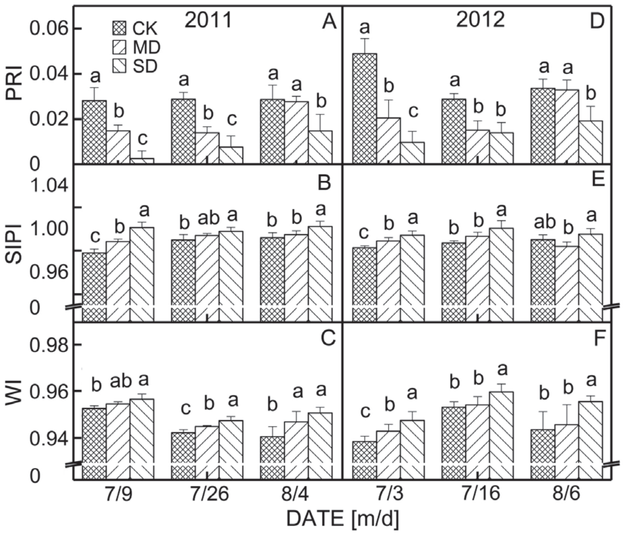

Fig. 3. Photochemical reflectance index (PRI) (*A* and *D*), structural independent pigment index (SIPI) (*B* and *E*), and water index (WI) (*C* and *F*) under three water regimes at different measuring time points in 2011 (*A*, *B*, and *C*) and 2012 (*D*, *E*, and *F*). CK - control, MD - moderate drought, SD - severe drought. Means ± SEs, $n = 5$, different letters indicate significant differences at $P < 0.05$.

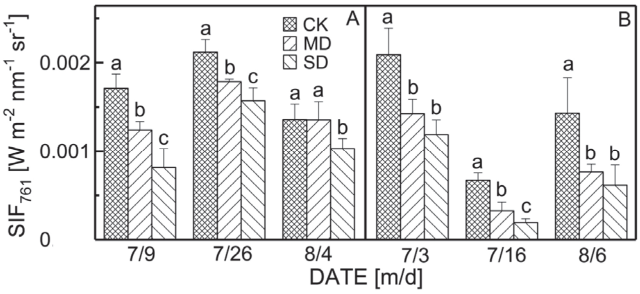

Fig. 4. Solar-induced fluorescence around 761 nm (SIF_{761}) under three water regimes at different measuring time points in 2011 (*A*) and 2012 (*B*). CK - control, MD - moderate drought, SD - severe drought. Means ± SEs, $n = 5$, different letters indicate significant differences at $P < 0.05$ (sr - steradian)

Correlation analysis was performed to determine the relationships between spectral indices, solar-induced fluorescence, gas exchange and chlorophyll fluorescence parameters under three water regimes at different time points for 60 cotton leaves. Pearson's correlation-based relationships among all parameters are presented in Table 1 Suppl. PRI was found to be positively correlated with P_N, g_s, T_r ($R^2 > 0.7$, $P < 0.01$), and Φ_{PSII} ($R^2 = 0.51$, $P < 0.01$), but negatively with NPQ ($R^2 = -0.510$, $P < 0.01$). Water index was found to be negatively correlated with P_N, g_s, T_r, F_v/F_m, and Φ_{PSII} ($P < 0.01$). A negative correlation between SIPI and P_N, g_s, T_r, Φ_{PSII} was observed, but all correlation coefficients were lower than those concerning PRI. Comparing the correlation coefficients between P_N and three spectral indices, we found that the value for PRI was the largest (0.762). Also SIF_{761} was positively related to P_N, g_s, and T_r ($P < 0.01$), but the correlation coefficients were lower. In addition, we found that SIF_{761} were significantly correlated with Φ_{PSII} and NPQ ($P < 0.01$). No significant correlation was found between SIF_{761} and F_v/F_m.

For the PRI - P_N relationship, we found that when data from all 3 measuring time points were pooled ($n = 45$) to make a linear fit, the correlation coefficient was 0.58 in 2011 and 0.49 in 2012 (Fig. 5); it was 0.54 for two years combined. For data from the first measurement only ($n = 15$), the correlation coefficient increased to 0.87 in 2011 and 0.85 in 2012 (Fig. 5), and that for the two years combined was 0.86. Therefore, the result indicated that PRI could be more effective for assessing P_N the beginning of water stress in cotton. SIPI was negatively and significantly related to PRI (Table 1 Suppl.).

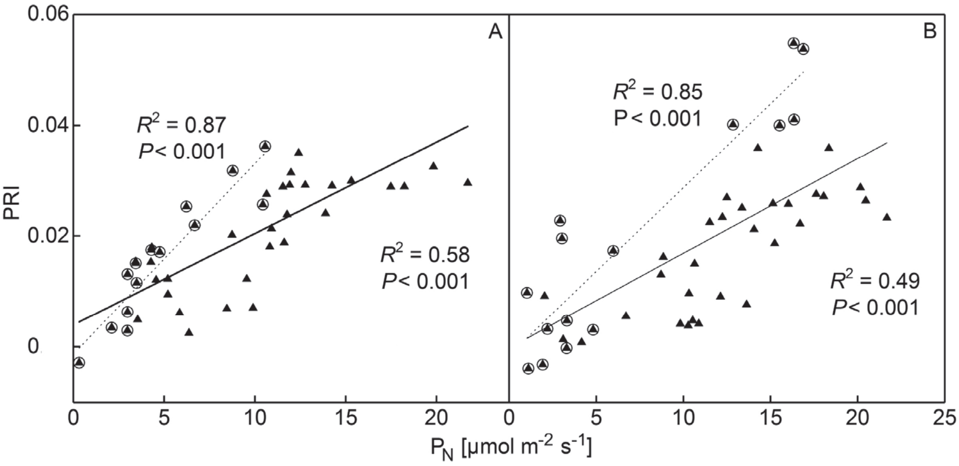

Fig. 5. Relationships between photochemical reflectance index (PRI) and net photosynthetic rate (P_N) in 2011 (*A*) and 2012 (*B*) The *straight line* shows the trend of data of 3 measuring time points ($n = 45$, *solid triangles*) and the *dotted line* shows the trend of data of the first measuring time ($n = 15$, *circles*). For each fitting correlation coefficients (R^2) and P values are reported.

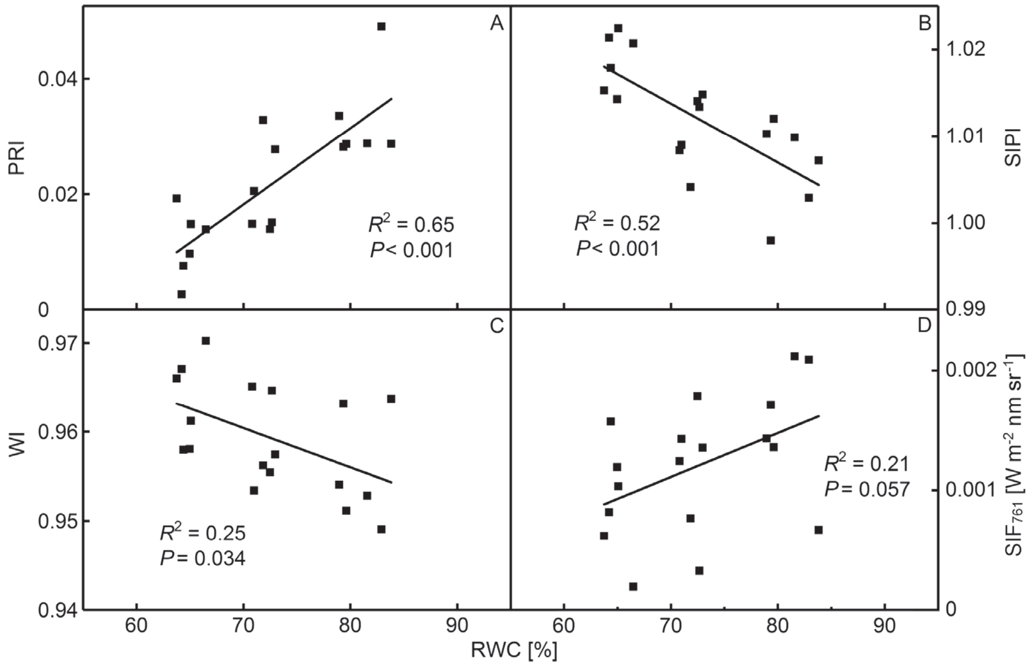

Fig. 6. Relationships between leaf relative water content (RWC) and photochemical reflectance index (PRI) (*A*), structural independent pigment index (SIPI) (*B*), water index (WI) (*C*), and solar-induced fluorescence around 761 nm (SIF_{761}) (*D*) ($n = 18$). The data was analyzed on the basis of mean values of 3 treatments from 3 measuring time points in 2011 (July 9, July 26, and August 4) and 2012 (July 3, July 16, and August 6).

Scatterplots and linear regressions between RWC and PRI, SIPI, WI, and SIF_{761} are shown in Fig. 6. Each data point of RWC represents an average of three leaf samples; others were averaged from five samples. RWC had a stronger linear relation with PRI than with SIPI or WI (Fig. 6*A*,*B*,*C*), indicating that PRI is more sensitive to water stress in cotton than SIPI and WI. The weak linear relation across treatments between SIF_{761} and RWC (Fig. 6*D*) suggests that SIF may not be a good parameter for water stress detection at the seasonal scale.

Discussion

We quantified leaf photochemical reflectance index, structural independent pigment index, water index, and solar-induced fluorescence under three water regimes and established the relationships with gas exchange and chlorophyll fluorescence parameters. These helped to evaluate the applicability of PRI and SIF for assessing cotton photosynthetic performance and water status.

Although leaf level measurements are less practical from a management perspective, they provide insights about changes in leaf spectral properties accompanied by changes in plant water status. Water stress in plant affects many physiological processes such as net photosynthetic rate, stomatal conductance as well as the photochemical efficiency of chloroplasts. As PRI is sensitive to changes in xanthophyll cycle pigments which occur during stress (Sarlikioti *et al.* 2010), it could be used to monitor changes in the photosynthetic parameters. Our results demonstrated that PRI was well related to P_N, g_s, and T_r under water stress (Table 1 Suppl.). Water stress may also result in changes in radiation energy use efficiency, which can be reflected by chlorophyll fluorescence parameters (Krause and Weis 1991). Previous studies have reported that PRI shows linear relationships with Φ_{PSII} and NPQ; however, this relationship is dependent on plant species (Naumann *et al.* 2010, Sun *et al.* 2014). The results of no significant correlation between PRI and F_v/F_m in our study are consistent with the findings of Winkel *et al.* (2002), but contradicted the results of Gamon *et al.* (1997) and Peñuelas *et al.* (1995b). In addition, some studies reported that the relationships between PRI and photosynthetic efficiency vary quite widely and they are affected by species, growth stages, and solar radiation (Nakaji *et al.* 2006, Rahimzadeh-Bajgiran *et al.* 2012). This was also seen in our study. The relationship between PRI and P_N at the flowering stage was much stronger than that for the whole stages (Fig. 5), indicating that the changes of leaf age, irradiance, pigmentation, *etc*., might result in weak relationship between these two parameters.

We also compared the responses of different reflectance indices to water stress. Whereas PRI, SIPI, and WI all tended to change in response of three water regimes (Figs. 3 and 6), only PRI emerged as a reliable indicator of water stress in cotton. The correlation coefficients between PRI and gas exchange parameters and chlorophyll fluorescence parameters were higher than those considering WI and SIPI (Table 1 Suppl.), which demonstrated that PRI was more suitable than WI and SIPI for monitoring photosynthesis similarly as reported Eitel *et al.* (2006) and Marino *et al.* (2014). Similar relationship between PRI and RWC has been found by Sarlikioti *et al.* (2010) when irradiance is above 700 $\mu mol\ m^{-2}\ s^{-1}$.

The increased interest in remote sensing of solar-induced fluorescence stems from the fact that CF is highly related to photosynthesis and that it has been a powerful tool to stress analysis (Zarco-Tejada *et al.* 2001). Although a large body of literature focused on signal retrieval techniques, few researches applied them for water stress detection (Pérez-Priego *et al.* 2005, Meroni *et al.* 2009, Panigada *et al.* 2014). The results presented in this study showed that solar-induce fluorescence (SIF_{761}) decreased under drought stress (Fig. 4). These results are in agreement with Liu *et al.* (2013) and Ni *et al.* (2015) who reported fluorescence at 760 nm decreased under herbicide treatment or water stress, but are not with results of Meroni and Colombo (2006). Also, SIF_{761} showed significant positive correlations with P_N, g_s, T_r, and Φ_{PSII} and negative correlation with NPQ (Table 1 Suppl.), which demonstrated that SIF was related to changes in plant photosynthetic activities. Water stress resulted in a decline in SIF_{761} partly due to an increase in heat dissipation. Our results showed that there was no significant relationship between SIF_{761} and RWC when data from two years were used. Leaf physiology, experimental design, and environment (especially irradiance) may account for these conflicting results.

Conclusions

The present study showed that in cotton leaves under water stress, PRI correlated more closely with photosynthetic and chlorophyll fluorescence parameters (except F_v/F_m) than spectral indices SIPI and WI. Further, PRI was more sensitive to water status than SIPI and WI, and could be used as a reliable indicator especially at early drought stage. The SIF_{761} was also significantly related to gas exchange parameters P_N, g_s and T_r, and chlorophyll fluorescence parameters Φ_{PSII} and NPQ, but not to F_v/F_m. However, SIF was less sensitive to water stress in cotton than PRI at the seasonal scale.

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