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# Chlorophyll fluorescence upper-to-lower-leaf ratio for determination of irrigation time for *Pentas lanceolata*

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## Abstract

The objective of this study was to use nondestructive measurements as the precise irrigation indices for potted star cluster (*Pentas lanceolata*). Drought stress was imposed on plants for 0, 3, 5, 7, 12, and 16 d by withholding water. Measurements were conducted on the third leaf counted from the apex (upper leaves) and on the third leaf from the bottom (lower leaves). Within the range of soil water content (SWC) from 10 to 45%, leaf water potential (WP), SWC, and soil matric potential (SMP), chlorophyll fluorescence, photochemical reflectance index (PRI), adjusted normalized difference vegetation index (aNDVI), and the reflectance (R) at 1950 nm ($R_{1950}$) were measured. The plants reached the temporary wilting point at –3.87 MPa of leaf WP; the maximal fluorescence yield of the light-adapted state ($F_m'$) ratio of upper-to-lower leaves was 1.7. When the $F_m'$ ratio was 1.3, it corresponded to lower-leaf WP $< -2.27$ MPa, SWC $< 21\%$, SMP $< -20$ kPa, PRI $< 0.0443$, aNDVI $< 0.0301$, and $R_{1950} > 8.904$; it was the time to irrigate. In conclusion, the $F_m'$ ratio of upper-to-lower leaves was shown to be a nondestructive predictor of leaf WP and can be used to estimate irrigation timing.

*Additional key words*: nondestructive technique; reflectance spectroscopy; rewatering; water status; water stress.

## Introduction

Many types of physiological stresses occur when plants encounter a water deficiency. Symptoms of drought injury include chlorophyll (Chl) breakdown, protein degradation, membrane permeability decrease, peroxidation, slower leaf expansion, petiole epinasty, and stomatal closure (Taiz and Zeiger 2006). Stomatal closure causes a decline in intercelluar $CO_2$ concentration and a concomitant decline in photosynthesis due to diminished availability of $CO_2$ for carbon fixation (Gogorcena *et al.* 1995, Lilley and Ludlow 1996). Water stress also reduces the capacity of photosynthetic systems to utilize incident photons and leads to photoinhibition (Lu and Zhang 1999, Skotnica *et al.* 2000). Photoinhibition of photosynthesis is characterized by a reduction in the quantum yield of photochemistry and a decrease in Chl fluorescence (He *et al.* 1995). Photoinhibition entails not only the inhibition of PSII but also increases thermal deexcitation of excited Chl (Demmig-Adams *et al.* 1996).

Chl fluorescence measurement, a noninvasive technique, has been widely used in a range of photosynthetic organisms and tissues to study functional changes in the photosynthetic apparatus under different environmental conditions (Richardson *et al.* 2001, Weng *et al.* 2006). These measurements are commonly used to study the responses of plants to water deficits. Prior studies have demonstrated this in chickpea (Rahbarian *et al.* 2011), spruce (Pukacki and Kamińska-Rożek 2005), ramie (Huang *et al.* 2013), wheat (Paknejad *et al.* 2007), and strawberry (Razavi *et al.* 2008) in controlled environments and in the field under varying degrees of water stress. However, no effort has been devoted to determine irrigation timing in response to water stress by studying Chl fluorescence in upper and lower leaves. Reflectance spectroscopy is another underexploited, noninvasive technique that can be used in physiological studies because of its simplicity, rapidity, and nondestructive nature (Levizou

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*Abbreviations*: aNDVI – adjusted normalized difference vegetation index; Chl – chlorophyll; $F_0$ – minimal fluorescence yield of the dark-adapted state; $F_m'$ – maximal fluorescence yield of the light-adapted state; $F_s$ – fluorescence yield at the steady-state; $F_v/F_m$ –maximal quantum yield of PSII photochemistry; PRI – photochemical reflectance index; R – reflectance; SMP – matric potential; SWC – soil water content; WP – water potential; $Y_{II}$ – maximum effective quantum yield.

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*et al.* 2005). Various reflectance spectra from leaves have been employed for calculating vegetation indices used to monitor plant growth. Reflectance spectra are altered, when stress occurs; it can be used for calculation of different vegetation indices, such as photochemical reflectance (PRI), adjusted normalized difference vegetation index (aNDVI), and $R_{1950}$. PRI, the reflectance at 531 nm relative to reflectance at a reference wavelength of 570 nm, has been linked to photosynthetic light-use efficiency (Peñuelas *et al.* 1995, Demmig-Adams and Adams 1996). aNDVI has potential applicability for water content estimation due to water absorption band centered at 1,240 nm (Gao 1996). $R_{1950}$ is a water absorption band; it shows promising for water content estimation (Sims and Gamon 2003). Many theoretical models based on leaf reflectance were developed to predict leaf Chl content, water content, and other variables associated with vegetative structure (Inoue *et al.* 1993, Jacquemoud *et al.* 1996, Dawson *et al.* 1998).

Irrigation indicators provide guidance on the best timing to irrigate crops in order to prevent or mitigate water stress. Their effectiveness, however, is often influenced by soil texture and plant morphology (Sadras and Milroy 1996). SWC is a common indicator, and irrigation is normally applied when the SWC is < 60–80% of field capacity. Common plant morphological indicators for irrigation include stem/leaf wilting, growth rate decreases, and leaf darkening or reddening. Irrigation of plants according to their water status could minimize irrigation water waste. Conventionally, the plant water status is measured by either direct visual observation or destructive testing. Visual observations can easily cause experimental errors, whereas destructive measurements damage plants and make further experiments impossible. Reflectance indices might be useful for measuring leaf WP when developing indices for nondestructive estimation. Hence, we attempted to determine whether spectral reflectance indices of star cluster (*Pentas lanceolata*) could be used as sensitive metrics for developing algorithms for estimating leaf WP corresponding to leaf Chl fluorescence. The objective of this study was to employ nondestructive measurements to create an irrigation index for *P. lanceolata* and develop a precise irrigation strategy. Chl fluorescence measurements were taken on drought-treated plants to determine the upper-to-lower-leaf $F_m$' ratio, while the corresponding reflectance spectrum, SWC, and SMP measurements were recorded in order to establish irrigation indicators for *P. lanceolata*. To our knowledge, this is the first study using these nondestructive techniques to determine irrigation timing for a single species.

## Materials and methods

**Plants, cultural practices, experimental design, and treatments**: Star cluster (*Pentas lanceolata*) is one of the few ornamental plants that grow well under high temperature and humidity of Taiwan summers, and is commonly used in flower beds. This plant has a rapid phenotypic response to water stress (*i.e.*, drought or flooding), thus it was used as a model for developing nondestructive monitoring and optimal water-management techniques for other plants. For our experiments, star cluster was purchased from a local commercial garden in Taipei, Taiwan. Plants with 30–35 cm in height were transplanted into 5-inch plastic pots five weeks after purchase. Pots containing a commercial potting mix of peat moss and perlite (4:1, v/v) were placed in a controlled-environment greenhouse under a 12-h photoperiod with an irradiance of 400 μmol(photon) $m^{-2}$ $s^{-1}$ at 28/22°C day/night temperature and relative humidity (RH) of 70%. They were evenly spaced to encourage similar growth rates and sizes. Plants were watered three times a week, and an optimal amount of a compound fertilizer solution (20 N-8.7 P-16.6 K water-soluble fertilizer at 200 mg $L^{-1}$) was applied once a week. Plants were maintained for 30 d under above conditions before a water stress initiation. Plants of a uniform size were then subjected to drought treatment in which the SWC of each pot was kept at 50% of field capacity. Drought stress was imposed on plants for 0, 3, 5, 7, 12, and 16 d by withholding water. Each drought period represented a treatment, and effects of drought were analyzed at the end of each drought period. Meanwhile, another set of plants subjected to constant drought were monitored continuously with the Chl fluorescence meter, and watering was resumed when signs of wilting began to appear. Six replicates undergoing six drought duration treatments were randomly placed in the greenhouse. The experiment was performed twice independently in a randomized design for the growth environment, sampling day, and physiological analyzes. Following each drought treatment, leaves from each plant were measured for Chl fluorescence, water potential, and reflectance spectrum from 12:00 to 14:00 h.

**Soil water content (SWC) and soil matric potential (SMP)**: The SWC of each pot was measured with a moisture meter (*Wet HH2*, *Delta-T*, Cambridge, UK). In addition, soil water tension, known as SMP, was also detected by a sensor recording *MPS-1* device (*Decagon*, WA, USA). All tested plants were subjected to drought treatment by ceasing irrigation until leaf wilting was observed, after which normal irrigation was resumed.

**Chl fluorescence** parameters for the tested plants were quantified with a portable modulated fluorometer (*Monitoring-PAM Multi-Channel Chl Fluorometer*, *Heinz Walz GmbH*, *Inc*., Effeltrich, Germany). All measurements were performed on mature, healthy, fully expanded third leaves counted from the apex (upper leaves) and bottom (lower leaves) of six plants under continuous drought. Plants were dark-adapted for 40 min prior to measurements. The minimal fluorescence yield of the dark-adapted state ($F_0$) and maximal fluorescence yield of the dark-adapted state ($F_m$) were determined with modulated irradiation of a weak blue LED beam (measuring light) and saturating pulse, respectively. Samples were then irradiated continuously with the natural light. Measurements of the maximal fluorescence yield of the light-adapted state ($F_m'$) and the steady-state fluorescence yield ($F_s$) values were performed every 30 min automatically by saturation pulse through *MONI-Head*. The intensity of the measuring ligh was 0.9 (at $F_0$ and $F_t$) and 9 μmol(photon) $m^{-2}$ $s^{-1}$ (at $F_m$), and the intensity of the saturating pulses was 6,000 μmol(photon) $m^{-2}$ $s^{-1}$. The ratio of $F_v/F_m$ was used as maximal quantum yield of PSII photochemistry (Porcar-Castell *et al.* 2008), where $F_v$ was calculated as $F_m - F_0$. Subsequently, maximum effective quantum yield ($Y_{II}$) was estimated as $\Delta F/F_m'$ ($\Delta F = F_m' - F_s$). Measurements were recorded by the *WinControl-3* software (*Heinz Walz*, Effeltrich, Germany). Chl fluorescence components were used to measure different functional levels of photosynthesis and recorded consecutively for 16 d.

**Leaf water potential (WP)**: The water potential of the upper and lower leaves was measured with a *Dewpoint Potential Meter WP4* (*Decagon*, WA, USA). After the device temperature equaled the sample temperature, the switch was adjusted to READ mode to balance the vapor pressure between the sample and atmosphere so that air potential and WP were equal. After the dew point of air and the sample temperature were determined, the WP of each leaf sample was obtained through calculations and ranged from 0 to –40 ± 0.1 MPa.

**Reflectance (R) spectrum**: Spectroscopy was used to evaluate the water status of the tested plants. Spectral reflectance was measured on the lower leaves at wavelengths of 305–2150 nm ($R_{305}$–$R_{2150}$) at 1-nm intervals using a spectrophotometer *Handy Spec Field 2.2 Tec5* (*Tec5*, Oberursel, Germany). Three indices were calculated from the reflectance spectrum: (*1*) photochemical reflectance index (PRI), calculated as $(R_{531} - R_{570})/(R_{531} + R_{570})$ (Stylinski *et al.* 2002); (*2*) adjusted normalized difference vegetation index (aNDVI), calculated as $(R_{860} - R_{1240})/(R_{860} + R_{1240})$ (Gao 1996); and (*3*) reflectance value at 1950 nm ($R_{1950}$), one of the water absorbance peaks (Sims and Gamon 2003). To investigate spectral bands, where these indices were sensitive to leaf WP, the coefficients $R^2$ between reflectance indices and the estimates of leaf WP were examined. Model development data between the reflectance spectrum and leaf WP were tested as nonlinear regressions.

**Statistical analysis**: Measurements of leaf WP were analyzed by a completely randomized analysis of variance (*ANOVA*) that compared the positions of leaves and time periods under water stress. For significant values, means were separated by the least significant difference (LSD) test at $p \leq 0.05$ using *Costat 6.29* (*CoHort Software*, Berkeley, CA, USA). Model data sets were based on 36 leaves from each time period, and leaf WP was calculated using reflectance data from the model's validation data sets. Predicted lower-leaf WPs were compared to the spectral reflectance indices that were physically measured, and predicted irrigation times were calculated. Relationships between lower-leaf WP and spectral reflectance indices were examined using nonlinear regression models. All graphs were created with *Sigmaplot 10.0* (*Systat Software*, CA, USA).

## Results

**Leaf WP under continuous drought treatment**: WP changed in the upper and lower leaves of star cluster over six different durations of drought treatment (0, 3, 5, 7, 12, and 16 d) (Fig. 1). The trends and rates of increases in WP under drought stress were similar in both two leaf positions. For example, WP of both the upper and lower leaves was reduced as the duration of drought treatment proceeded, although the lower-leaf WP was reduced to a larger degree compared to the upper leaves. WP in the lower leaves after 12 and 16 d of the treatment (–18.95 and –22.60 MPa) was significantly lower than that after 0 to 7 d (from –1.87 to –3.87 MPa), indicating that the long-term drought could induce a decline in the leaf water content that subsequently affected leaf WP. Moreover, the upper leaves after 0 to 5 d of the treatment exhibited significantly higher WP (a less negative value) than that after 7 to 16 d. Thus, the WP of both two leaf positions responded differently to the drought stress duration.

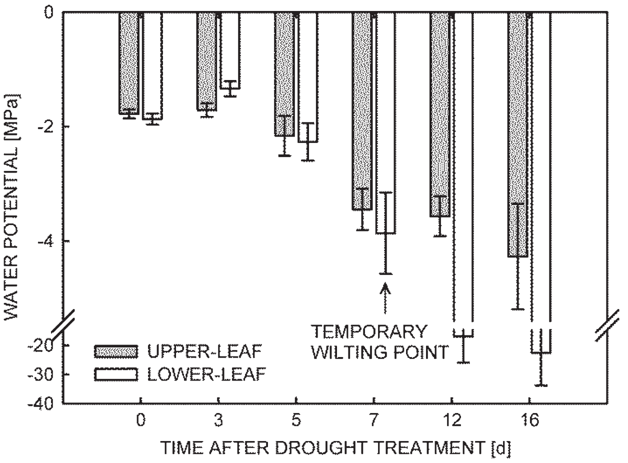

Fig. 1. Leaf water potential of upper and lower leaves of star cluster exposed to 0–16 d of drought treatments. *Vertical bars* represent standard errors.

A temporary wilting point in the lower leaves became observable after 7 d of drought; the upper and lower leaves both wilted after 12 and 16 d.

**Effects of SWC and SMP on the temporary wilting point**: Continuous monitoring of SWC and SMP corresponding to the suggested irrigation time and temporary wilting point of star cluster is shown in Fig. 2. Under continuous drought treatment, SWC declined from 43 to 27%, but SMP remained at –10 kPa until SWC dropped below 27%. When the lower leaves exhibited the temporary wilting point, the WP in the lower leaves was –3.87 MPa after 7 d of drought treatment (Fig. 1), and corresponding SWC and SMP were found to be 14% and –40 kPa, respectively (Fig. 2).

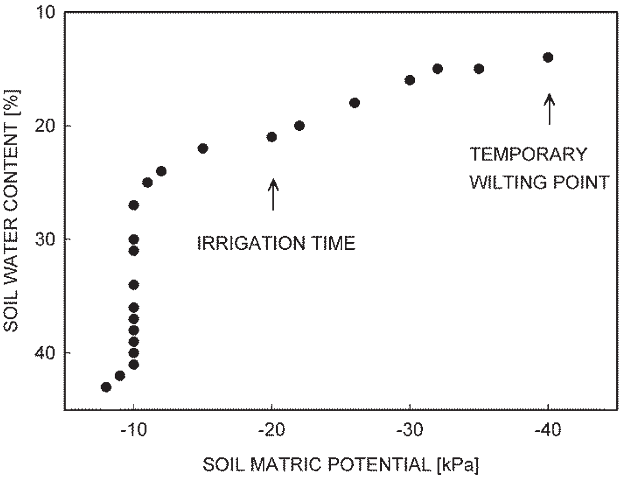

Fig. 2. Soil water content and soil matric potential at the temporary wilting point and when star cluster irrigation is initiated.

**Effects of water stress on Chl fluorescence**: Optimal irrigation timing using leaf WP was determined by observing leaf appearance and Chl fluorescence parameters. In this study, a monitoring PAM device was used to measure the Chl fluorescence ratio of the upper-to-lower leaves, providing useful information on water deficiency. Fig. 3*A* showed that the $F_m'$ variation patterns in the upper and lower leaves caused by drought stress were different. A greater decline in $F_m'$ was observed in the lower leaves. The $F_m'$ ratio of the upper-to-lower leaves corresponding to the temporary wilting point was 1.7 (Fig. 3*B*). After this point, the $F_m'$ ratio showed a tendency to decline from 1.7 to 1.4, but it did not return to initial values (1.2 to 1.3) and the corresponding WP of the lower leaves, SWC, and SMP after 5 d of drought were –2.27 MPa (Fig. 1), 21%, and –20 kPa (Fig. 2), respectively.

**Relationships between leaf WP and reflectance spectrum indices**: The calibration curves of the lower-leaf WP and corresponding spectral reflectance indices could reveal information on the water content of the lower-leaf and serve as a reference for the optimal timing of watering. PRI and aNDVI both showed a positive correlation with lower-leaf WP (Fig. 4*A*,*B*). The suggested irrigation time for star cluster came when the lower-leaf WP reached –2.27 MPa, PRI < 0.0443, or aNDVI < 0.0301. $R_{1950}$ showed a negative correlation with lower-leaf WP, and the suggested irrigation time occurred when the lower-leaf WP reached –2.27 MPa or when $R_{1950}$ > 8.9040 (Fig. 4*C*). Furthermore, relationships between reflectance in the visible spectrum and leaf WP were nonlinear. Regression analysis showed that the PRI, aNDVI, and $R_{1950}$ were significantly and positively correlated with leaf WP at $R^2$=0.86, 0.93, and 0.95, respectively. The distinctive spectral reflectance between vegetation indices and leaf WP clearly showed that drought-induced substantial physiological changes were observable spectrally. Therefore, these vegetation indices can be used as more precise metrics for developing leaf WP estimation algorithms and irrigation times.

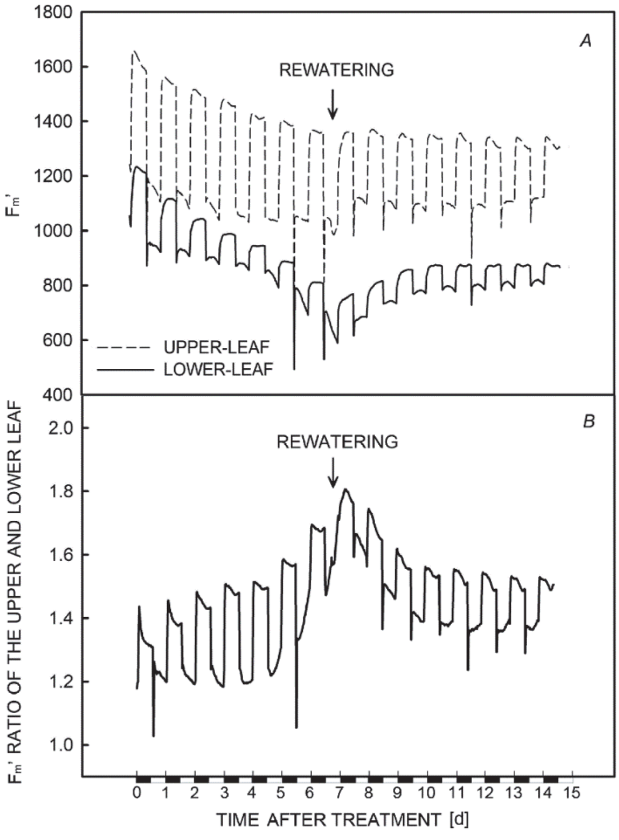

Fig. 3. The light-adapted maximum fluorescence ($F_m'$) changes (*A*) and its ratio (*B*) of upper-to-lower leaves in star cluster following drought and rewatering treatments. The arrow indicates rewatering time. *Black* and *white* on the *x*-axis represents nighttime and daytime, respectively.

## Discussion

Leaf WP progressively decreased with increasing drought time, indicating that the water relationships of all tested plants were affected during water stress. The reason for the decreased WP is that a water deficit induces stomatal closure and consequently reduces leaf WP. Moreover, when plants were subjected to drought treatment over 7 d, all lower leaves had significantly reduced WP values compared to the upper leaves. The reasons for the faster decline in the lower-leaf WP could be that water movement was fast when drought occurred and water in the lower leaves was preferentially transported to the upper leaves to support physiological activities. Consequently, the lower leaves were sacrificed for the whole plant to survive the drought. We found that the lower leaves of all tested plants looked epinastic and senescent after 5 d of drought. Under control conditions (0 d of drought), the plants were able to retain the higher water content and higher WP compared to those under water stress. The bulk flow of water in the well irrigated plants reached a maximum at noon and retained higher WP values (–1.8 MPa). However, when the plants suffered from drought, bulk flow would alternatively reach a maximum in the morning followed by a more drastic decline at noon. Hence, the plants with differing water status have the largest WP differences at noon, while WP values are closest to each other at dawn and twilight (Remorini and Massai 2003). All measurements in the study were carried out at noon, when leaf WP reached its lowest value. Determining leaf WP is one of the most common methods to measure the water status of a plant (Williams *et al.* 2012, Deb *et al.* 2012).

SMP was used to examine the degree of drought in soil. SMP declines as vapor pressure decreases, and is an index of water status for regularly irrigated crops (Thompson *et al.* 2007). When the lower leaves of star cluster reached the temporary wilting point, leaf WP and SMP were –3.87 MPa and –40 kPa, respectively. The $F_m'$ ratio of the upper-to-lower leaves was 1.7 at this point. As a result, the recommended leaf WP to start irrigation was –2.27 MPa and the SMP was –20 kPa. Augé *et al.* (2003) measured lethal leaf WP values for 30 herbaceous and woody plants and found that the permanent wilting points ranged from –2.01 MPa (*Geranium*) to –6.67 MPa (goldencup St. John's wort). Compared to the above data, star cluster (–3.87 MPa) is relatively drought-tolerant herbaceous plant species, even approaching some woody species. In addition, many researchers have determined SMP for different crops, such as sweet corn (–30 kPa) (Rivera-Hernández *et al.* 2010), cucumber (–15 to –30 kPa) (Suojala-Ahlfors and Salo 2005), crisphead and romaine lettuce (–50 kPa) (Gallardo *et al.* 1996), tomato (–50 kPa) (Wang *et al.* 2007a), and potato (–25 to –30 kPa) (Wang *et al.* 2007b). The SMP (–20 to –40 kPa) for star cluster in our study fits within the reference ranges.

$F_m$ and $F_m'$ values for the upper and lower leaves declined after a period of drought stress, during which $F_m'$ at daytime declined more dramatically than $F_m$ at night. Furthermore, the $F_m'$ of the lower leaves declined to a larger degree than that of the upper leaves, which may be due to greater photoinhibition during the day when plants were suffering from drought stress (Shirke and Pathre 2003). Drought increased the likelihood of photoinhibition, characterized by a decline in Chl fluorescence of the upper and lower leaves; photoinhibition generally occurred more rapidly in the lower leaves than in the upper leaves. Another reason for lower-leaf $F_m'$ declining more under stress during daytime than that at night was that dark adaptation reduced the differences between night and day. Water stress led to an obvious decrease in the indicated Chl fluorescence parameters and caused chronic photoinhibition. In general, when the plants were drought-stressed for 7 d, they gradually lost PSII functioning and $F_m'$ declined with duration of the drought treatment. When the $F_m'$ ratio started changing on day 7, water stress exhibited a turning point for rewatering. Leaf position differences in water transport characteristics may be involved in imparting drought tolerance. Although the visual appearance of the plants remained unchanged at this point, physiological changes had begun. At the critical turning point, the $F_m'$ ratio of the upper-to-lower leaves, leaf WP, SWC, and SMP were 1.3–2.27 MPa, 21%, and –20 kPa, respectively. The severer drought, the larger the $F_m'$ ratio of the upper-to-lower leaves. When the temporary wilting point of star cluster became evident, leaf WP, SWC, SMP, and $F_m'$ ratio values were –3.87 MPa, 14%, –40 kPa, and 1.7, respectively. If a single leaf is used for Chl fluorescence tests, the results is ambiguous due to lack of reference; alternatively, if both upper and lower leaves are used for determining Chl fluorescence parameters, such as the $F_m'$ ratio, readings might be more accurate. The long-term goal of our work is to help breed drought-tolerant plants by using Chl fluorescence and reflectance spectra as selection indices. $F_m'$, $F_m$, and $F_0$ from a dark-adapted sample can be obtained instantly. It means that a large number of individual plants may be screened per day, providing ample opportunity to discover plants that manifest irrigation requirement indicators and those that exhibit greater tolerance to drought stress.

Chl fluorescence measurements are often applied to gain a stress tolerance index for plants. The function of thylakoid membranes influences PSII directly or indirectly because it sensitively responds to various types of stress. Consequently, a lower Chl fluorescence value can often be found in a drought-stressed plant. (Hirotsu *et al.* 2005, Špunda *et al.* 2005, D'Ambrosio *et al.* 2006). Chl fluorescence measurements can be used not only to detect response mechanisms to stress, but also to allow quantitative analysis and comparison of these responses (Souza *et al.* 2004). When a plant is subjected to water stress, PSII can be damaged by photoinhibition resulting from stomatal

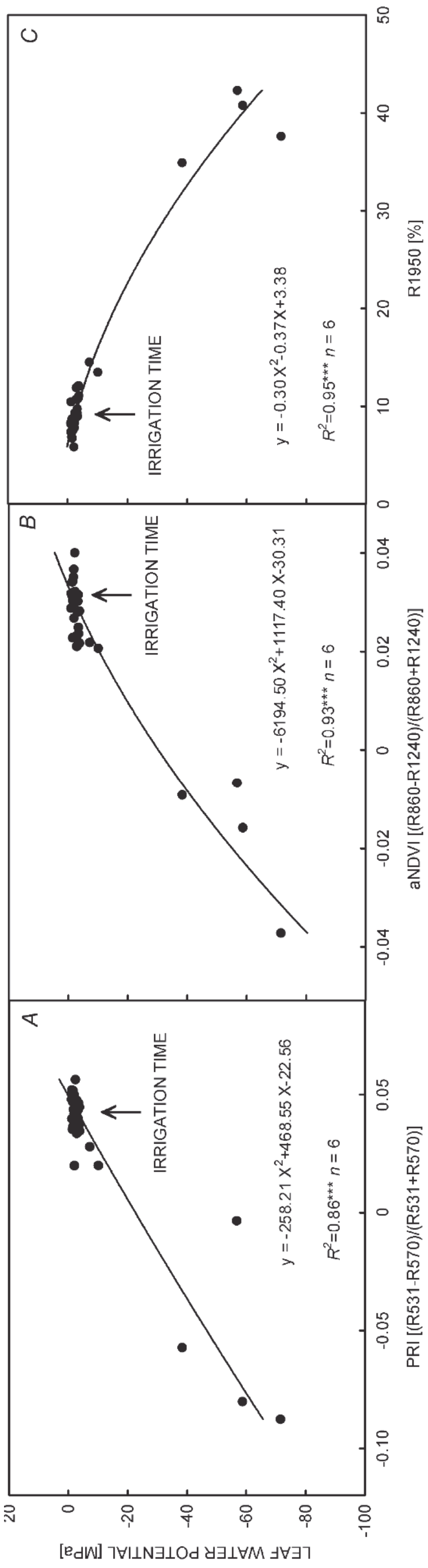

Fig. 4. Relationship between leaf water potential and photochemical reflectance index (*A*), adjusted normalized difference vegetation index (*B*), and $R_{1950}$ nm (*C*) of star cluster. The arrow indicates the irrigation time. Filled circles represent 36 leaves from each time period used; leaf water potential was calculated using reflectance data from the model's validation data sets. The coefficient $R^2$ between leaf water potential and reflectance spectrum indices was calculated.

closure. Therefore, declining Chl fluorescence values suggests that PSII is impaired or damaged (Gulías *et al.* 2002, Colom and Vazzana 2003). In our study, $F_0$ increased but $F_m$ and $F_v/F_m$ decreased when plants encountered water stress (data not shown). Water stress can induce chronic photoinhibition that deactivates the light reaction in the PSII center and damages the D1 protein (He *et al.* 1995). Water stress destablizes the PSII, resulting in a separation between LHCII and PSII reaction centers that raise $F_0$. This destablization causes a decline in the activity of the water-splitting enzyme complex in PSII which induces deactivation of the protein in the PSII reaction center that eventually lowers $F_m$ and cyclic electron transport, while raises nonphotochemical quenching (NPQ) (Aro *et al.* 1993). Such reductions lead to photoinhibition and reduce the ability to photosynthesize. The growth environment can lead to an adjustment in the ratio between photochemistry and NPQ, *i.e.*, a stress may shut down the PSII reaction center and cause 95.0–97.0% of energy to be released as heat and 2.5–5.0% as fluorescence (Kitao *et al.* 2006). The proportions for each of quenching routes can be evaluated by the Chl fluorescence index. Leaves with higher photosynthetic rates show also larger proportions of photochemical quenching (Maxwell and Johnson 2000). The electron transfer rate rises as light intensity increases until light intensity reaches the photoinhibition point, after which the efficiency of PSII declines when light intensity further increases (Jia and Li 2002).

PRI is significantly correlated to the quantum yield of electron transfer in PSII and is indicative of xanthophyll cycle-mediated thermal energy dissipation. Indices using spectral bands (570 and 531 nm) were suggested for the estimation of the photosynthetic rate (Zou *et al.* 2011, Dillen *et al.* 2012, Huang *et al.* 2012). aNDVI is used to assess a Chl content (Whitehead *et al.* 2005) and as an index for the water content. The water contents of peanut and wheat are highly and negatively correlated with reflectance at 1450 nm (Peñuelas and Inoue 1999). The effect of aNDVI on leaf WP differed with drought duration. Because leaves undergoing a short period of drought were 'greener' than those undergoing the long-drought period, aNDVI was typically lower for the latter than in the former ones. The lower aNDVI of the long-drought-stressed leaves compared to short-drought-stressed leaves indicated that the long-drought-stressed leaves had lower Chl concentrations, consistent with visual observations (not shown). Sims and Gamon (2003) illustrated the relationship between the water index and reflectance at 970–1450 nm, where a heat lamp was used on bean leaves to produce water stress and generate a water gradient. They found that the lower water content produces the higher reflectance. These vegetation indices are highly correlated with the relative water content and leaf WP; for instance, irrigation should be performed either when the crop water stress index is > 0.4 or when the leaf WP drops below –1.2 to –1.4 MPa (Pierce *et al.* 1990, Riggs and Running 1991).

Thompson *et al.* (2007) reported that the leaf WP of sorghum dramatically fell from –0.18 to –1.6 MPa within 15 d after irrigation was ceased, and recovery took four weeks. Additionally, SWC was used to set leaf WP (< –0.4 MPa) as the irrigation indicator for chili pepper was at –58 kPa, melon at –35 kPa, winter tomato at –59 kPa, and tomato at –39 kPa.

PRI is highly correlated to Chl fluorescence (Fiella *et al.* 1996). Under water stress, PRI, Chl fluorescence, and photochemical reaction declined (Peñuelas *et al.* 1995). Both PRI and aNDVI were highly correlated with the water potential of the lower-leaves, which reached its lowest level at noon. Consistent and high correlations ($R^2$=0.86–0.95) were observed between the vegetation indices and leaf WP, demonstrating the applicability of vegetation indices for measuring leaf WP. The $F_m'$ ratio of the upper-to-lower leaves was predicted using reflectance data from validated data sets, and then compared to actually measured $F_m'$ values. It is expected that the new index can be comprehensively used to estimate the plant water content. When the $F_m'$ ratio of the upper-to-lower leaves was 1.3, which corresponded to lower-leaf WP < –2.27 MPa, SWC < 21%, SMP < –20 kPa, PRI < 0.0443, aNDVI < 0.0301, and $R_{1950}$ > 8.9040, it was time to irrigate. With validation of the indices, the $F_m'$ ratio of the upper-to-lower leaves was shown to be a nondestructive predictor of leaf WP and can be used to estimate irrigation timing.

In conclusion, we found $F_m'$ and the water potential significantly higher in the lower leaves than that of the upper leaves at different drought durations. Our newly developed models for evaluating water deficit in plants used nondestructive spectroscopic measurements that are applicable to large-scale water management of ornamental plants, and enable scarce water resources to be conserved.

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