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Crop water status assessment in controlled environment using crop reflectance and temperature measurements

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Abstract Crop water status is an important parameter for plant growth and yield performance in greenhouses. Thus, early detection of water stress is essential for efficient crop management. The dynamic response of plants to changes of their environment is called 'speaking plant' and multisensory platforms for remote sensing measurements offer the possibility to monitor in real-time the crop health status without affecting the crop and environmental conditions. Therefore, aim of this work was to use crop reflectance and temperature measurements acquired remotely for crop water status assessment. Two different irrigation treatments were imposed in tomato plants grown in slabs filed with perlite, namely tomato plants under no irrigation for a certain period; and well-watered plants. The plants were grown in a controlled growth chamber and measurements were carried out during August and September of 2014. Crop reflectance measurements were carried out by two types of sensors: (i) a multispectral camera measuring the radiation reflected in three spectral bands centred between 590–680, 690–830 and 830–1000 nm regions, and (ii) a spectroradiometer measuring the leaf reflected radiation from 350 to 2500 nm. Based on the above measurements several crop indices were calculated. The results showed that crop reflectance increased due to water deficit with the detected reflectance increase being significant about 8 h following irrigation withholding. The results of a first derivative analysis on the reflectance data showed that the spectral regions centred at 490–510, 530–560, 660–670 and 730–760 nm could be used for crop status monitoring. In addition, the results of the present study point out that sphotochemical reflectance index, modified red simple ratio index and modified ratio normalized difference vegetation index could be used as an indicator of plant water stress, since their values were correlated well with the substrate water content and the crop water stress index; the last being extensively used for

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crop water status assessment in greenhouses and open field. Thus, it could be concluded that reflectance and crop temperature measurements might be combined to provide alarm signals when crop water status reaches critical levels for optimal plant growth.

Keywords Remote sensing · Speaking plant approach · Multispectral camera · Crop water stress index

Introduction

Plant stress caused by biotic or abiotic factors that adversely affect plant growth significantly reduces productivity. For a certain crop, water stress may be the result of a single or a combination of abiotic factors such as the aerial microclimate (e.g. air temperature, relative humidity, solar radiation intensity, air velocity) and root zone (e.g. available water and electrical conductivity). When a plant becomes stressed, stress is expressed in many types of symptoms. Water stress, for example, closes stomata and impedes photosynthesis (Chaves et al. 2002; Sarlikioti et al. 2010) and transpiration, resulting in changes in leaf colour and temperature (Jackson et al. 1981; Katsoulas et al. 2009). Crop reflectance (Knipling 1970), chlorophyll fluorescence (Norikane and Kurata 2001) and thermal radiation emittance (Jones and Schofield 2008) are also affected. Other symptoms of water stress include morphological changes such as leaf curling or wilting due to loss of cell turgidity. This dynamic response of plants to changes of their environment is called 'speaking plant' (Takakura 1974).

Early detection of plant stress is very critical especially in intensive production systems in order to minimize both acute and chronic loss of productivity. Thus, plant based sensing could be valuable to better understand the interactions between plants and their microclimate (Kacira et al. 2005).

Methods such as substrate water content for soilless crops or soil water tension, leaf water potential and sap flow, among others, have been widely used to assess plant water status. Though, soil or substrate water content indicates the availability of water in the root zone, this is not always directly correlated with plant water status. In addition, although leaf water potential and sap flow measurements do provide direct information about plant water status, they require plant contact or destructive sampling, something difficult to realize in commercial scale. Non-contact and non-destructive sensing techniques can continuously monitor plants and enable automated sensing capabilities (Ling et al. 1996). That is why several studies have attempted to detect and quantify water stress using thermal emittance and reflectance measurements obtained by remote sensing (e.g. Sclemmer et al. 2005; Zakaluk and Sri Ranjan 2007; Jones and Schofield 2008).

Although remote sensing has been successfully used for years in open fields and relevant calculation models have been developed [e.g. Jacquemoud et al. (2009) for crop reflectance or Chavez et al. (2010) for crop temperature measurements], it has not been extensively tested for crops grown under controlled environmental conditions such as greenhouses or growth chambers. Open field methods cannot directly be applied in greenhouses due to difficulties arising mainly from reflections and shadows casted by the greenhouse frame and equipment. Such problems could be addressed by combining data from two or more spectral bands to form reflectance indices (Kacira et al. 2005; Sarlikioti et al. 2010; Tsirogiannis et al. 2013). According to Zakaluk and Sri Ranjan (2008), the most common forms of reflectance indices are: (a) reflectance ratios corresponding to the ratio of two spectral bands, which are referred to as simple ratio (SR) reflectance indices and (b) normalized reflectance indices (NRI), which are defined as ratios of the difference in reflectance between two spectral bands to the sum of the reflectance at the same bands.

In addition, the crop water stress index (CWSI), which is based on temperature measurements, proved to be one very successful tool for crop water stress detection under open field (Alchanatis et al. 2010; Meron et al. 2010; González-Dugo et al. 2013; Cohen et al. 2015) and greenhouse conditions (Katsoulas et al. 2001; Kacira et al. 2002; Katsoulas et al. 2002).

For commercial production settings, it is more advantageous to develop a real-time plant canopy health, growth and quality monitoring system with multi-sensor platforms (Story and Kacira 2015). This can be achieved by a sensing system equipped with a multi-sensor platform ultimately using plants as 'sensors' to communicate their true status and needs. Such systems can be potentially used not only for crop water status assessment but also to detect crop deviations from normal development, e.g. due to crop stress (i.e., nutrient deficiencies).

Therefore, the objective of the present work was to explore the use of multispectral and hyperspectral canopy reflectance and crop temperature measurements, carried out remotely, under controlled environmental conditions to assess crop water status. Further aim is to develop a computer vision plant monitoring system for real-time crop diagnostics. For this purpose the measurements obtained in well-watered plants are compared to those obtained from plants where irrigation was stopped for some days.

Materials and methods

Growth chamber facilities and plant material

The experiments were carried out during August and September of 2014 in a controlled growth chamber, located at Velestino, Central Greece. The geometrical characteristics of the growth chamber were as follows: height = 3.2 m, width = 4 m, length = 7 m, ground area = 28 m^2. Air temperature, relative humidity, light intensity and CO_2 concentration were automatically controlled using a climate control computer (Argos Electronics, Greece). Air temperature and humidity were controlled by means of a heat exchanger while CO_2 concentration was controlled by adding pure CO_2. The light intensity was controlled by means of 24 high pressure sodium lamps, 600 W each (MASTER GreenPower 600 W EL 400 V Mogul 1SL, Philips), operated in four groups with 6 lamps per group. The groups where turned on progressively during the morning and switched off progressively in the evening to simulate the daily evolution of solar radiation at greenhouse conditions. The average light intensity obtained at the level of the plants when all 24 lamps were used was 240 W m^{-2}.

The tomato plants (*Solanum lycopersicum* cv. *Elpida*, Spyrou SA, Athens, Greece) were grown in slabs filled with perlite (ISOCON PerloflorHydro 1). Two units comprising two crop lines each (six slabs per line, three plants per slab) were used. The mean volumetric water content of perlite at field capacity was 53–55%. The measurements started thirty days after transplanting, when the plants had about 10 leaves each, were about 1 m high and had a leaf area index of about 0.8. The nutrient solution was supplied to the crop via a drip system and controlled by a commercial time irrigation controller (eight irrigation events per day at 03:30, 07:00, 10:00, 12:00, 14:00, 16:00, 18:00, 19:30 local time), with set points for electrical conductivity at 2.4 dS m^{-1} and pH at 5.6.

In order to study the effects of irrigation water deficit on crop reflectance characteristics, two different irrigation treatments were applied for 9 days (starting 30 days after transplanting): (a) a water deficit treatment, by withholding the nutrient solution supply through removal of drippers in five perlite slabs and (b) a control treatment where the plant water needs were completely covered and a drainage rate of about 35% was obtained. During the first three days of the measurements both treatments were irrigated according to the time schedule irrigation program set by the irrigation computer (Days 1–3). Then, in treatment (a) the drippers were removed from the slabs for five days (Days 4–8) and placed back to the slab on Day 9 of the experiment. Each experiment lasted 9 days and was repeated three times, imposing different slabs in water deficit each 9 days period. The data selected for analysis in this work correspond to one 9 days period.

Measurements

Air temperature (*T*, in °C) and relative humidity (*RH*, in %) were measured using two temperature–humidity sensors (model HD9008TR, Delta Ohm, Italy) calibrated before the experimental period, placed 2 m above the ground level. Using *T* and *RH* values, air vapour pressure deficit values were calculated. Light intensity ($R_{g,i}$, in W m^{-2}) inside the growth chamber was recorded using a solar pyranometer (model SKS 1110, Skye instruments, Powys, UK) located 2 m above ground level. Leaf temperature (T_l, in °C) was measured using two infrared thermographs (type OS5551A, Omega Engineering Inc., Manchester, USA) installed at a distance of 0.9 m from the crop, at a constant angle of 45° from the vertical axis of the target plants (control and water deficit treatment). Thus, the surface area sensed was about 5000 mm^2. The infrared thermographs were calibrated according to international standards (American National Standard 1998; O'Shaughnessy et al. 2011) prior to their installation, by measuring the temperature of a black target (flat piece of black metal) that emitted the highest amount of energy than any other surface at the same temperature. Substrate volumetric water content (θ, %) was estimated using capacitance sensors (model WCM, Grodan Inc., The Netherlands). Four water content sensors were placed horizontally in the middle (height and width) of the hydroponic slabs (two sensors per treatment). The above data were automatically recorded in a data logger system (Zeno 3200, Coastal Environmental Systems Inc., Seattle). Measurements took place every 30 s and 10 min average values were recorded.

Two spectra sensors were used in this work:

(1) The radiation reflected by the plants was monitored every day between 8:00 and 9:00 for periods of 9 consecutive days, using a portable spectroradiometer (model ASD FieldSpec Pro, Analytical Spectral Devices, Boulder, CO, USA), measuring the radiation reflected in the range between 350 and 2500 nm (with sampling interval of 1.4 nm and 2; and spectral resolution of 3 and 10 nm for the regions between 350–1000 and 1000–2500 nm, respectively). In order to measure leaf reflectance, a contact reflectance probe connected to the spectroradiometer was used, providing a 25° field of view fiber optic of 100 W halogen reflectorised lamp with fibre-optic input socket and color temperature at least 3000° K. Leaf reflectance measurements were taken in contact with the leaf. Reflectance measurements of 20 leaves per treatment were taken randomly within the canopy (20 leaves within 5

slabs per treatment). A white spectralon panel was used to calibrate the sensor every 20 min.

(2) During Days 3–6 and 9 of the experiment, the radiation reflected by the plants under water deficit treatment was also monitored every hour (from 08:00 to 19:00) using a multispectral camera (custom model, spatial resolution 1280 × 1024 pixels, Quest Innovation, OH, USA) measuring in three spectral bands centered between 590 and 680 nm [$R_{(590–680)}$], 690–830 nm [$R_{(690–830)}$] and 830–1000 nm [$R_{(830–1000)}$] regions. The camera included an F-type lens and an 8 bit CMOS-type sensor and was controlled through a PC by means of a special software (Frame Link Express Application, Imperx, USA). For optimal radiometric calibration of the imaging system, a calibration protocol was followed in two steps. The first step was performed in the laboratory, using known light sources. The responsiveness of the multispectral camera to three different spectral bands under different radiation intensities was recorded and analysed in order to choose the appropriate camera settings (frame rate and exposure time) and decrease the signal-to-noise ratio. During this step, the image noise characteristics are determined and the initial gain and offset are adjusted. Furthermore, the response of the instrument was adapted to a polynomial to obtain the instrument response function and evaluate the nonlinear characteristics. The second step of the calibration was performed in the controlled growth chamber. During the second step, the appropriate camera's settings were further improved according the light conditions, the growth chamber structure, the surrounding surfaces (background, ground) and the density and architecture of the canopy. Katsoulas et al. (2016) also described the calibration methodology for a similar imaging system used to formulate appropriate reflectance indices for plant water stress detection. The camera was located stable 1 m from the vertical axis of the canopy level to sample the canopy area of young, fully developed leaves between the 3rd and 6th branch of three plants. At the time of measurements a black surface was used as background, in order to block out any reflection from growth chamber surfaces, ensuring a constant field of view without shadows. A sample of 10 images per treatment per hour between 08:00 and 19:00 was taken and the mean reflectance values of the region of interest (fully developed leaves between the 3rd and 6th branch) were calculated. Before that image segmentation was performed to segment leaves out from the background using isodata and *k*-mean statistical methods. After segmentation, a mask was created on the area covered by the young and fully developed leaves. The mask was applied to all other bands of the multispectral image to extract the mean intensity value of the relevant leaf area. MATLAB (by MathWorks®) and ENVI (by Exelis®) software packages were used for image analysis and plant reflectance acquisition. To further overcome the reflection differences due to shadows and reflections and measure true percent reflectance, a white low-density polyethylene sheet of known reflectance (W) was positioned 1 m in front of the sensor and its measured reflectance served as a normalizing reference for all readings. Before each calibration (steps one and two referred above) or measuring process, dark images were acquired as well by covering the lens with a cap. Thus, once a day, before each diurnal cycle of measurements on plants, a measurement on the white and dark references were taken. This frequency of white and dark reference was considered acceptable since the light conditions into the growth chamber during the period of measurements were stable. Plant reflectance (r) was calculated by the ratio of the difference between actual image reflectance (R, i.e. the intensity of reflected light signal in a

certain bandwidth) and dark reference (D) to the difference of white and dark references ($r = (R-D)/(W-D)$).

Calculations

Based on the available reflectance measurements the following indices were calculated and evaluated to explore their relationship with water stress:

– AB, Average bands, by estimating the mean reflectance between 490–510, 530–560, 660–670, 730–760, 1400–1500 and 1600–1700 nm. The above spectrum areas were estimated following a first derivative analysis as proposed by Köksal et al. (2010).
– Three bands centred between 590–680 nm ($R_{(590-680)}$), 690–830 nm ($R_{(690-830)}$) and 830–1000 nm ($R_{(830-1000)}$) regions.
– Two Simple Ratio indices, defined as (Peňuelas and Inoue 1999; Jones et al. 2004):
–

$$\mathrm{WI\ (Water\ Index)} = \mathrm{R970/R900},$$

–

$$\mathrm{VOGREI\ 1(Vogelmann\ Red\ Edge\ Index)} = \mathrm{R740/R720}$$

– Thirteen normalized reflectance indices defined as (Tsirogiannis et al. 2013; Sarlikioti et al. 2010; Shimada et al. 2012; Kim et al. 2010; Köksal et al. 2010; Amatya et al. 2012):

$$\mathrm{PRI\ (Photochemical\ Reflectance\ Index)} = (\mathrm{R531} - \mathrm{R570})/(\mathrm{R531} + \mathrm{R570})$$

$$\mathrm{sPRI} = (\mathrm{R560} - \mathrm{R510})/(\mathrm{R560} + \mathrm{R510})$$

$$\mathrm{NDVI}_{(490-620)}\mathrm{(Normalized\ Difference\ Vegetation\ Index)} \\ = (\mathrm{R490} - \mathrm{R620})/(\mathrm{R490} + \mathrm{R620})$$

$$\mathrm{NDVI}_{(800-680)} = (\mathrm{R800} - \mathrm{R680})/(\mathrm{R800} + \mathrm{R680})$$

$$\mathrm{NDVI}_{(710-810)} = (\mathrm{R710} - \mathrm{R810})/(\mathrm{R710} + \mathrm{R810})$$

$$\mathrm{NDVI}_{(790-670)} = (\mathrm{R790} - \mathrm{R670})/(\mathrm{R790} + \mathrm{R670})$$

$$\mathrm{NDVI}_{(790-550)} = (\mathrm{R790} - \mathrm{R550})/(\mathrm{R790} + \mathrm{R550})$$

$$\mathrm{rNDVI\ (red\ Normalized\ Vegetation\ Index)} = (\mathrm{R750} - \mathrm{R705})/(\mathrm{R750} + \mathrm{R705})$$

$$\mathrm{VOGREI\ 3} = (\mathrm{R734} - \mathrm{R747})/(\mathrm{R715} + \mathrm{R720})$$

$$\mathrm{sNDVI1} = (\mathrm{R810}-\mathrm{R710})/(\mathrm{R810} + \mathrm{R710})$$

$$\mathrm{sNDVI2} = (\mathrm{R810}-\mathrm{R560})/(\mathrm{R810} + \mathrm{R560})$$

$$\text{mrSRI (modified red Simple Ratio Index)} = (\text{R750} - \text{R445})/(\text{R705} - \text{R445})$$

and

$$\text{mrNDVI} = (\text{R750} - \text{R705})/(\text{R750} + \text{R705} - 2 * \text{R445}).$$

Based on the available temperature measurements the following indices were calculated and evaluated to explore their relationship with water stress:

– Crop water stress index *CWSI* defined as (Baille et al. 2001):

$$CWSI = \frac{T_c - T_m}{T_M - T_m}$$

where T_c is the canopy temperature (°C), T_M (°C) is the higher limit of canopy temperature which was assumed to be achieved with minimum canopy conductance and T_m (°C) is the lower limit of canopy temperature which was assumed to be achieved with maximum canopy conductance estimated as described in Katsoulas et al. (2002).

The temperature stress index TSD (Clawson et al. 1989) calculating the temperature difference between the canopy temperature and the lower limit of canopy temperature (T_c–T_m). TSD is separated to: (a) TSD_{meas} index in which the canopy temperature (T_c) is measured by a temperature sensor while the lower limit of canopy temperature (T_m) is calculated as referred by Katsoulas et al. (2002) considering a maximum canopy conductance and (b) TSD_{field} in which the canopy temperature (Tc) and the lower limit of canopy temperature (T_m) are measured using remote temperature sensors.

Statistical analysis

Comparison of means was performed by applying one-way ANOVA at a confidence level of 95% ($p \leq 0.05$) using SPSS (Statistical Package for the Social Sciences, IBM, USA). Additionally, linear regression was performed between the reflectance indices studied and some abiotic parameters. The mean value along with the measurement variability (standard error of the mean, ±SE or standard deviation, ±SD) of the parameters measured are presented.

Results

The mean value of air temperature inside the growth chamber during the period of measurements was 26 and 19 °C, for daytime and night-time periods, respectively, with small variations around these values. In addition, the mean values of air relative humidity during the same time period were 85 and 65%, respectively. In the following sections the results obtained during a sequence of 9 consecutive days are presented. The data obtained during the other two sequences of days showed a similar trend to those presented herein.

While in the case of the control treatment the volumetric water content (θ) (mean θ data calculated by the measurements recorded from 7:00 to 9:00 each morning during the days of the experiment (n = 12/day/treatment)) in the slabs was kept at field capacity levels (about 53% ± 0.02, Fig. 1), the θ values observed in the water deficit treatment decreased progressively after drippers withdrawing from the slab, to reach the level of 34% (±SD > 0.17; ±SE > 0.07) 5 days later (Day 8). Then, it increased again during the last day of measurements as soon as the drippers where placed back to the slabs. The difference

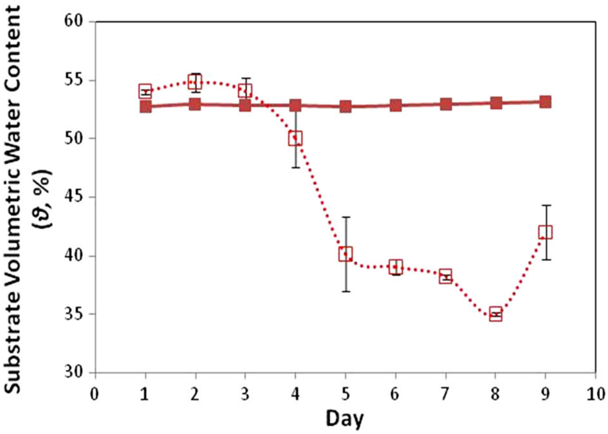


Fig. 1 Diurnal evolution of volumetric water content values measured in the perlite slabs in the two treatments (n = 12/day/treatment). *Continuous line*: control treatment; *dotted line*: water deficit treatment. The *error bars* represent the standard deviation (±SD) of the means (one-way ANOVA, $p < 0.05$)

in θ values between control and treated plants was statistically significant ($p < 0.05$) as soon as the θ values observed in water deficit treatment were reduced below 50%.

Diurnal crop reflectance

Diurnal crop reflectance measured by means of the multispectral camera in the spectral bands centred between $R_{(590–680)}$, $R_{(690–830)}$ and $R_{(830–1000)}$ regions is presented in Fig. 2, during a day with normal irrigation used as reference (Day 3) and during the second (Day 4) and the third day (Day 5) after irrigation withholding. It can be seen that during the reference day, the plant reflectance values varied slightly during the day with no statistically significant differences ($p > 0.05$) along the day. However, the reflectance values measured at $R_{(690–830)}$ increased more than 5% ($p < 0.05$) during the first day of irrigation withholding after midday (Day 4, 16:00). The data remained high the following day (Day 5). The mean reflectance values observed at $R_{(830–1000)}$ spectrum area progressively increase ($p < 0.05$) during the second day of irrigation withholding after midday (Day 5, 16:00). This augmentation overcame to 20% during late in the afternoon (Day 5, 18:00).

The daily mean values of crop reflectance measured from 8:00 to 9:00 by means of the multispectral camera from plants imposed to water deficit are presented in Fig. 3, for the different days studied before (Day 3) and after irrigation withholding. In addition, the mean reflectance values calculated by the mean reflectance measured in the early morning by the spectroradiometer (to the same spectral regions with those of the multispectral camera i.e. 590–680, 690–830 and 830–1000 nm) from well-watered plants during the same time period are also shown. It can be seen that during the day that both treatments were well watered (Day 3) both systems measured similar levels of reflectance (no significant difference between the data observed in control and water deficit treatment was observed). However, during the first day of irrigation withholding the plants under water deficit tended to have higher reflectance values at $R_{(690–830)}$ and $R_{(830–1000)}$ than the well-watered plants ($p < 0.05$), while the values measured at $R_{(590–680)}$ remained constant during the period of measurements for both treatments.

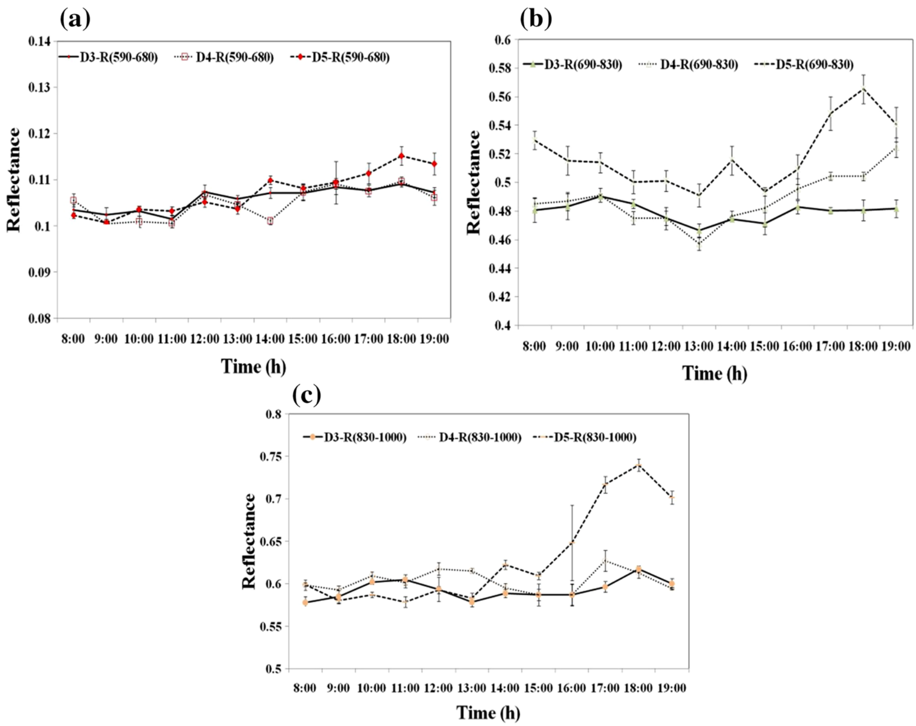


Fig. 2 Daily evolution of plant reflectance measured in the spectral bands centred at $R_{(590–680)}$, $R_{(690–830)}$, $R_{(830–1000)}$ spectrum regions by means of the multispectral camera, 1 day before (Day 3) and 2 days (Days 4–5) after irrigation withholding. **a** $R_{(590–680)}$; **b** $R_{(690–830)}$; **c** $R_{(830–1000)}$; *solid line* day 3-Irrigation; *dotted line* day 4-irrigation withholding; *dash line* day 5-irrigation withholding. The *error bars* represent the standard deviation (±SD) of the means

The mean value of the leaf reflectance measured by means of the spectroradiometer in the spectral region from 350 to 2500 nm are presented in Fig. 4, for the different days studied before and after irrigation withholding. It can be seen that the reflectance values measured were similar for both cases before irrigation withholding ($p > 0.05$) but as soon as irrigation stopped (Day 3) the reflectance values of plants under water deficit started to differentiate from those of the control plants ($p < 0.05$)(Fig. 4d).

According to Köksal et al. (2010), based on a first derivative analysis, certain parts of the spectrum can be selected for further study. Applying this analysis, the following spectral regions were discriminated: 490–510 nm (B_{peak}), 530–560 nm (G_{peak}), 660–670 nm (R_{peak}), 730–760 nm ($rNIR_{peak}$), 1400–1500 nm ($mNIR_{peak}$) and 1600–1700 nm ($fNIR_{peak}$). Subsequent the above discrimination, the grey areas shown in Fig. 4a–i indicate those from the above spectral regions where statistically significant differences ($p < 0.05$) were observed between the reflectance measured in the reference and treated plants during the 9 consecutive days.

Three days after initiating irrigation withholding (Day 6), the reflectance differences between the reference and treated plants were significantly increased ($p < 0.05$) in the entire range of the spectral regions studied (B_{peak}: 3.8%, G_{peak}: 3.7%, R_{peak}: 9.8%, $rNIR_{peak}$: 3.7%, mNIR: 9% and $fNIR_{peak}$: 5.8%). Restarting irrigation during Day 9 resulted in a decrease of the difference between the reflectance values measured in the two

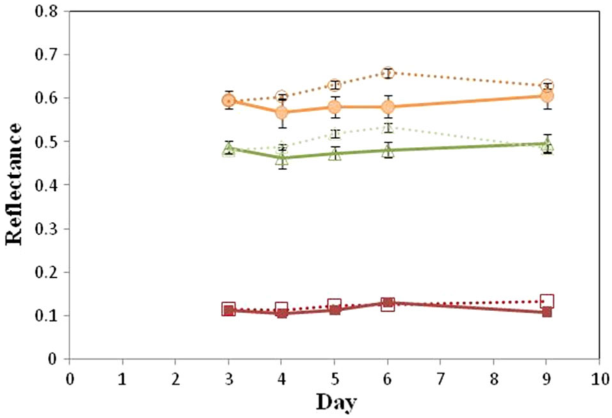


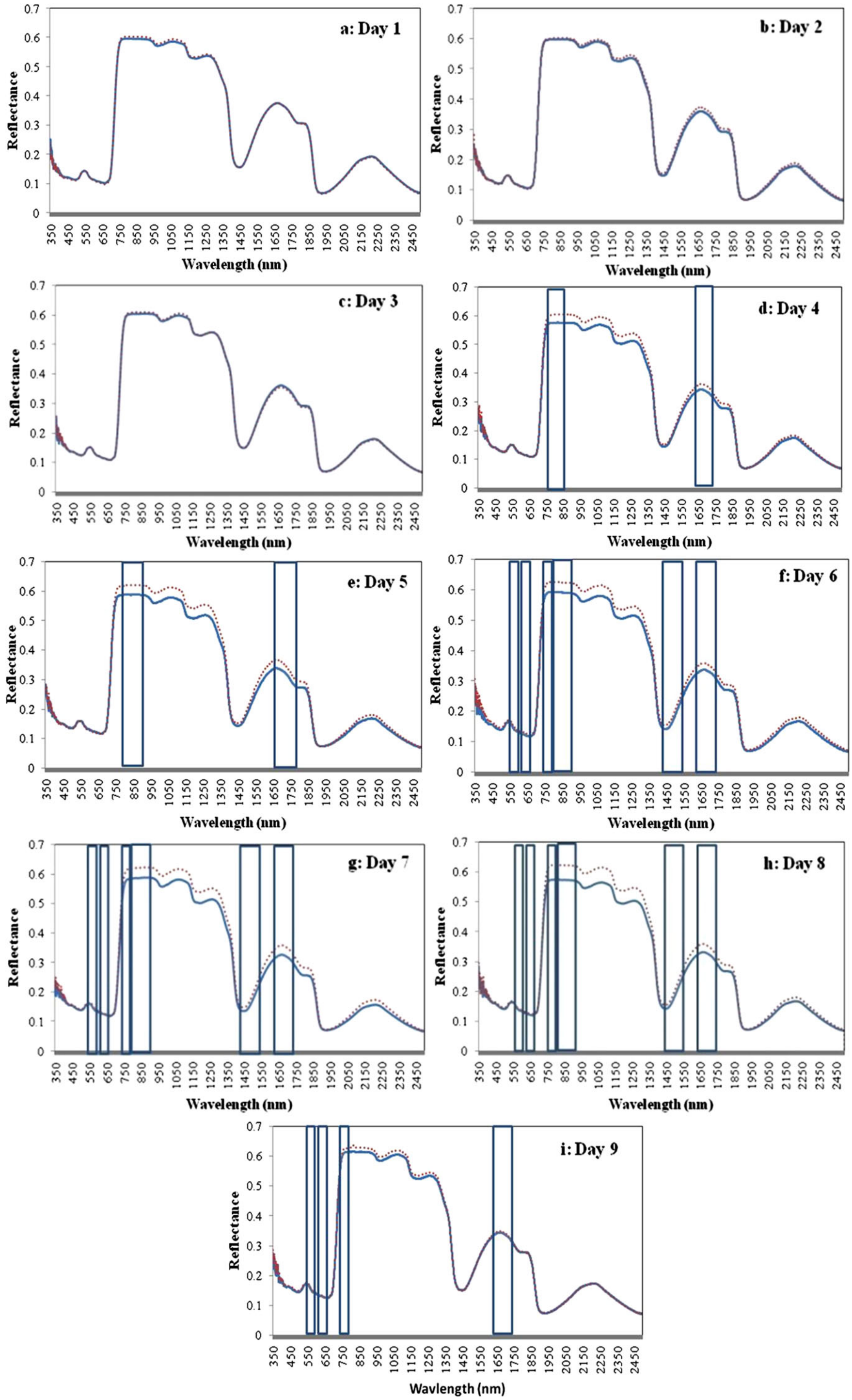
Fig. 3 Daily evolution of reflectance of plants under water deficit measured by means of the multispectral camera (n = 20, data recorded from 8:00 to 9:00) and well-watered plants measured by means of the spectroradiometer in the spectral bands centred at $R_{(590–680)}$, $R_{(690–830)}$, $R_{(830–1000)}$ spectrum regions (n = 20, data recorded from 8:00 to 9:00) (one-way ANOVA, $p < 0.05$). Day 3: all plants were well-watered; days 4–8 irrigation withholding; day 9: irrigation restart. *Squares* $R_{(590–680)}$; *triangles* $R_{(690–830)}$; *circles* $R_{(830–1000)}$; *continuous line* control treatment; *dotted line* water deficit treatment. The *error bars* represent the standard deviation (±SD) of the means (one-way ANOVA, $p < 0.05$)

treatments showing that plants started recovering. However, from the above mentioned spectrum regions, only the reflectance measured in the 730–760 nm (rNIRpeak), 1400–1500 nm (mNIRpeak) spectrum reached similar values between the two treatments 3 days after irrigation restart, while statistically significant differences ($p < 0.05$) were still observed in the rest of the regions studied (data not shown).

Reflectance indices

The measured values of leaf reflectance obtained by means of the spectroradiometer were used for the calculation of the indices presented in Sect. 2.3. The indices values observed were similar even 2 days after irrigation withholding initiation (Fig. 5). However, statistically significant differences were observed between the two treatments ($p < 0.05$) almost 48 h after irrigation withholding for mrSRI and mrNDVI and 72 h later for $NDVI_{(790–670)}$ and sPRI. In the case of $NDVI_{(790–670)}$ and sPRI, the reflectance index values estimated for the plants under stress were about 1.5 and 25%, respectively, lower than those estimated for the reference plants, while in the case of mrSRI and mrNDVI the reflectance index values estimated for the plants under stress were about 70 and 4%, respectively, higher than those estimated for the reference plants.

It was shown that as θ decreased, $NDVI_{(790–670)}$ and sPRI also decreased, while mrSRI and mrNDVI increased. Nonetheless, for the same value of θ observed in the two irrigation treatments, different values of $NDVI_{(790–670)}$, sPRI, mrSRI and mrNDVI (measured in the perlite slabs between 07:00 and 09:00) were observed. Thus, in order to eliminate the effect of reflectance evolution during time, the indices values difference between the treated and control plants [Δindex(Treatment–Control)] were correlated to the volumetric water content differences between the two treatments during the same time period [$\Delta\theta$(Treatment–Control)]. When plotting Δindex vs. $\Delta\theta$ values, a strong linear relationship was found (Fig. 6) of the type:


a: Day 1
b: Day 2
c: Day 3
d: Day 4
e: Day 5
f: Day 6
g: Day 7
h: Day 8
i: Day 9
Reflectance
Wavelength (nm)
Wavlength (nm)

◀**Fig. 4** Evolution of leaf reflectance of control and treated plants (n = 20/day/treatment), during the period of measurements (**a**–**c** days 1–3, well-watered plants, **d**–**h** days 4–8 irrigation withholding, **i** day 9 irrigation restart). *Continuous line* control treatment; *dotted line* water deficit treatment (one-way ANOVA, $p < 0.05$)

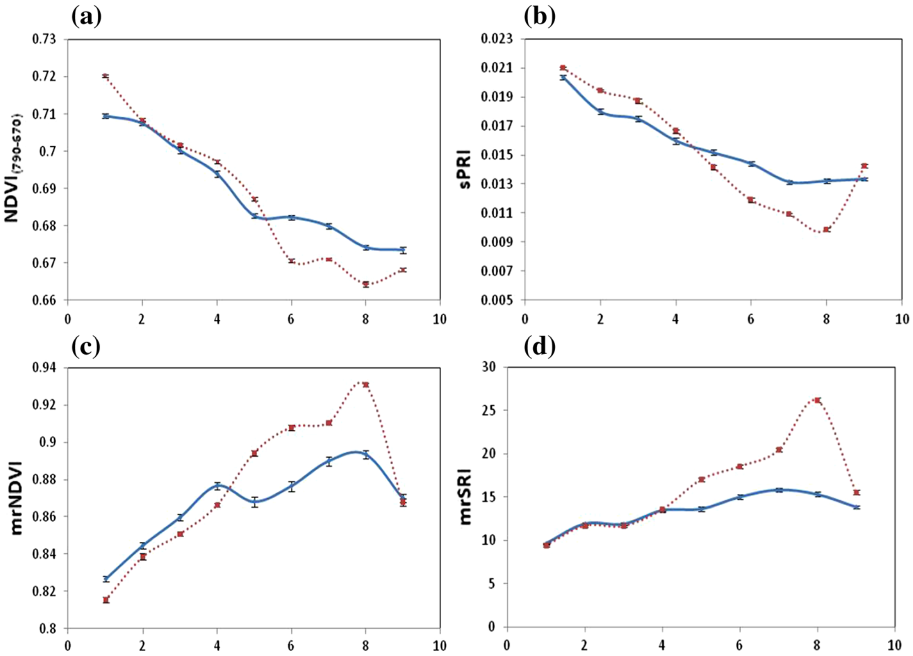


Fig. 5 Evolution of **a** $NDVI_{(790–670)}$, **b** sPRI, **c** mrNDVI and **d** mrSRI values (n = 20/day/treatment) of well-watered plants and plants under water deficit during the period of measurements (days: 1–3 well-watered plants, days 4–8: irrigation withholding in treated plants, day 9: irrigation restart). *Continuous line* control treatment; *dotted line* water deficit treatment. The *error bars* represent the standard errors of the means (±SE)

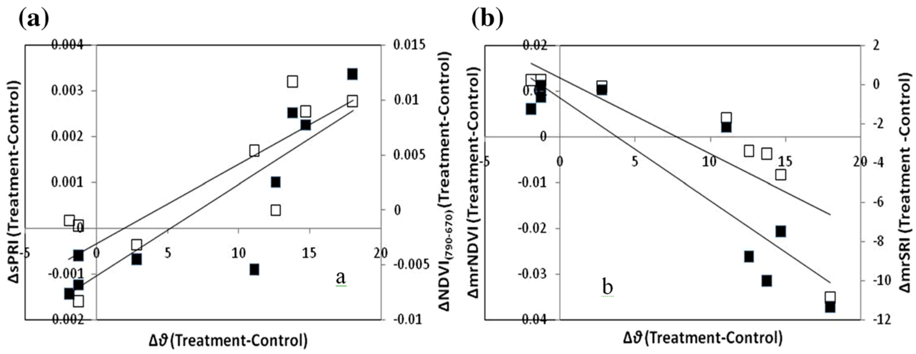


Fig. 6 Relationship between the differences observed in indices values (**a** $NDVI_{(790–670)}$ and sPRI, and **b** mrSRI and mrNDVI) between the two treatments with respective differences in substrate volumetric water content (θ, %) values observed. The *straight lines* represent the best fit regression lines between the Δ indices and Δθ values. *Empty symbols*: **a** $\Delta NDVI_{(790–670)}$ and **b** ΔmrSRI; *solid symbols* **a** ΔsPRI and **b** ΔmrNDVI

$$\Delta NDVI_{(790-670)} = 0.0007\Delta\theta - 0.003, \quad \text{with } R^2 = 0.72$$

$$\Delta sPRI = 0.0002\Delta\theta - 0.001, \quad \text{with } R^2 = 0.76$$

$$\Delta mrNDVI = -0.002\Delta\theta + 0.008, \quad \text{with } R^2 = 0.80$$

$$\Delta mrSRI = -0.389\Delta\theta + 0.367, \quad \text{with } R^2 = 0.72$$

The determination coefficient (R^2) of the mentioned regression was found to be higher than 0.70 ($p < 0.05$) for $NDVI_{(790–670)}$, sPRI, mrNDVI and mrSRI (Fig. 6). As for the rest of the indices presented in Sect. 2.3, the R^2 values were lower than 0.60, were excluded from further study, since the aim of the work is to identify indices that correlate well with water availability in the root.

Crop temperature and thermal indices evolution

The average daily value of crop temperature for the well-watered plants varied from 22 to 24 °C during the experiment period, while three days after irrigation withholding the respective values for the plants under irrigation deficit were more than 1 °C higher than the reference plants, as substrate water content decreased from 54 to 39%.

Figure 7 shows that both TSD_{meas} and TSD_{field} thermal indices and CWSI had a good correlation ($p < 0.05$) with substrate volumetric water content (R^2 higher than 0.7). The average daily value of TSD_{meas} and TSD_{field} three days after irrigation withholding increased from −0.7 to 2.6 and 0.3 to 3.3 °C, respectively.

The average daily value of CWSI for the well-watered plants varied from 0.43 to 0.48 (SE = ±0.029), while the respective values for the water stressed plants increased from 0.42 to 0.71 (SE = ±0.021) three days after irrigation withholding (69% increase, $p < 0.05$).

A good relationship was obtained between TSD_{meas}, TSD_{field} and CWSI with θ:

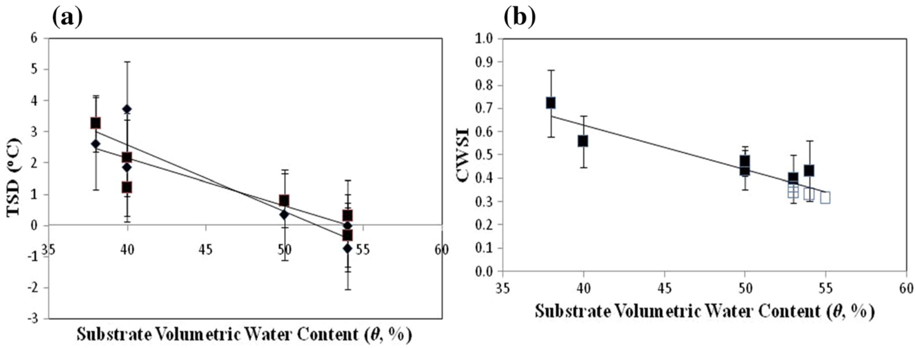


Fig. 7 Relationship between mean daily values of **a** TSD_{field} and TSD_{meas} and **b** CWSI and substrate volumetric water content (θ, %) in well-watered plants and plants under water deficit (n = 12, data recorded from 7:00 to 9:00). *Square* TSD_{field}; *rhomb* TSD_{meas}; *solid squares* CWSI of water deficit treatment; *empty squares* CWSI of control treatment. The *straight lines* represent the best fit regression lines (Linear regression, $p < 0.05$) and the *error bars* represent the standard deviation (±SD) of the means

$$\mathrm{TSD_{meas}} = -0.15\theta + 8.34(\pm 2.1), \quad \text{with } \mathrm{R}^2 = 0.79\ (p < 0.05),$$

$$\mathrm{TSD_{field}} = -0.21\theta + 11.06\,(\pm 1.8), \quad \text{with} \quad \mathrm{R}^2 = 0.85\ (p < 0.05),$$

$$\mathrm{CWSI} = 0.02\theta + 1.40\ (\pm 0.15), \quad \text{with} \quad \mathrm{R}^2 = 0.87\,(p < 0.05).$$

Discussion

Reflectance evolution

In hydroponic crops, it is not possible to obtain water deficit (when withholding irrigation completely) without affecting also the availability of nutrients in the root zone. Thus, the effect on spectral reflectance observed in the water stressed plants may be the combination of both water and nutrients stress. Nevertheless, considering that each water deficit treatment lasted only 6 days and taking into account that for very mobile nutrients such as nitrogen and potassium, deficiency symptoms develop predominantly in the older and mature leaves (Taiz et al. 2015) and that the measurements in the present study were made in young and fully developed leaves, it could be considered that the symptoms detected were mainly due to water stress. Yet, this was also the methodology followed in similar preview studies (e.g. Sarlikioti et al. 2010).

The reflected radiation of the tomato leaves followed the typical reflectance signature of a common healthy green leaf (Jacquemoud and Ustin 2008).Water stress is generally linked to reflectance increase particularly in the near infrared region due to radiation scattering by air content risen in sponge cavities (less water content, Jones et al. 2004; Sclemmer et al. 2005; Vigneau et al. 2011; Amatya et al. 2012). The reflectance increase in blue and red spectrum regions could be explained by the effects of plant water stress on leaf pigments and chlorophyll concentration reduction (Peňuelas et al. 1997; Ray et al. 2006; Kim et al. 2010; Kruse 2004; Sclemmer et al. 2005; Jain et al. 2007).

Indeed, the results of the present study showed that the reflectance of the treated canopy in the complete spectrum area measured was affected by the irrigation treatment. The mean reflectance measured between 730–760 and 1600–1700 nm was correlated to the plant water deficit from the first day of irrigation withholding (Fig. 4a–i). In contrast to near infrared region, in the middle infrared region, more absorption and less reflectance and transmittance is observed in green leaves due to the fact that water absorbs more radiation in that spectrum. Thus, this region contains more information about sponge parenchyma that includes water, cellulose, nitrogen, lignin and starch. Therefore, several authors (e.g. Hunt and Rock 1989; Bowman et al. 1989; Verdebout et al. 1994; Cordon and Lagorio 2007; Jacquemoud and Ustin 2008) have already concluded that the use of middle infrared reflectance is insufficient to estimate the leaf water status, due to the fact that reflectance changes within a biologically meaningful range are too insignificant and the light signal at that spectrum is too low (high light signal noise). Thus, it is not easy to be measured reflectance data in greenhouse conditions based on remote sensing techniques (such as multispectral and hyperspectral machine visions). Even the most advanced hyperspectral sensors present instability in measurement up to 1000 nm over time, due to the intense effect of solar radiation in the target area (Tuominen and Lipping 2011). Usually, reflectance spectrum areas up to 1000 nm are measured through satellites, portable spectroradiometer based on contact reflectance probe or laboratories protocols, something difficult to realise in greenhouse commercial scale. Thus, the most suitable indices in greenhouse conditions include spectrum areas below to 1000 nm.

Reflectance and thermal indices evolution

The values of mrSRI and mrNDVI were higher in the treated plants, which is in agreement with the results presented by Amatya et al. (2012), who found similar increase under waters stress conditions.

Previous research has indicated that the PRI is a sensitive indicator of water stress (Suárez et al. 2009; Sarlikioti et al. 2010), however this was not confirmed in the present study. This could be due to the low level of lighting occurring during the period of measurements (240 W m^{-2}) since according to Sarlikioti et al. (2010) PRI could be sensitive to water stress for radiation levels higher than about 350 W m^{-2}.

The values of TSD_{field} had good correlation with substrate volumetric water content. However, the existence of well irrigated plants as a reference point during the measurements is of high importance for the method. On the other hand, TSD_{mea} could detect plant water stress without recording the temperature of well-watered plants. Simultaneously, the CWSI was sensitive to plant water stress from the first day of irrigation withholding, as the index variation rate was increased more than 25%, without the need of measuring the temperature of well-watered plants. Finally, a good correlation (Fig. 8) was also observed between the differences in CWSI observed between the two treatments and the sPRI, mrSRI, mrNDVI indices values observed between the two treatments, which indicates that those indices could also be used for crop water status assessment:

$$\Delta sPRI = 0.01\Delta CWSI - 0.001, \quad \text{with } R^2 = 0.87$$

$$\Delta mrSRI = -11.82\Delta CWSI + 0.263, \quad \text{with } R^2 = 0.85$$

$$\Delta mrNDVI = -0.13\Delta CWSI + 0.014, \quad \text{with } R^2 = 0.80$$

Previous research studies have supported the idea that the $NDVI_{(800-680)}$ is a sensitive indicator when it comes to water stress detection (Jones et al. 2007; Liu et al. 2004) but this was not confirmed in the present study and others as well. For instance, Jones et al. (2004) claim that the index provides a medium estimate of plant water content, while it is a better

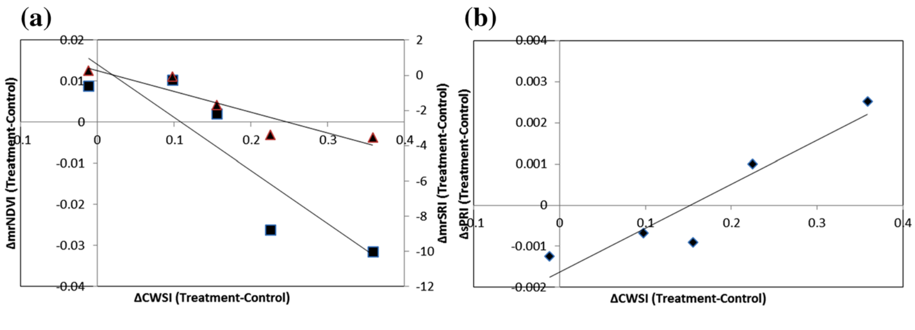


Fig. 8 Relationship between the differences observed in indices values (**a** mrNDVi and mrSRI and **b** sPRI) between treated and control plants with the respective differences observed in the CWSI values of the two treatments (n = 12, Days 3, 4, 5, 6 and 9 of the experiment). *Squares* mrNDVI; *triangles* mrSRI; *rhomp* sPRI. The *straight lines* represent the best fit regression lines (Linear regression, $p < 0.05$) and the *error bars* represent the standard deviation (±SD) of the means

indicator of nitrogen content and biomass. Kim et al. (2010) and Genc et al. (2011) showed that $NDVI_{(800–680)}$ has good correlation to water treatment patterns only when water field capacity is less than 60%, where the canopy coverage is quite low, while Köksal (2011) states that the index is highly correlated with yield increase. In this experiment, the 5-day time of plant irrigation withholding is not long enough to affect plant leaf area and/or cause yield reduction. WI (water index) was another index that did not correlate significantly with substrate volumetric water content in this study. Peňuelas and Inoue (1999) presented results in which WI decreased during the first water losses in monocotyledonous plants (wheat), while in the case of dicotyledonous plants (peanut), with double leaf water concentration due to leaf structure capacity, WI started to decrease when leaf water concentration reached 60%. Finally, VOGREI is commonly used to correlate the reflectance radiation at 720 and 740 nm with total chlorophyll content at the leaf level (Vogelmann et al. 1993). Amatya et al. (2012) found good correlation between VOGREI and soil water content two months after treatment initiation among different water levels (10, 15, 20 and 25% of field capacity).

Concluding remarks

The results of the present study show that NDVI, sPRI, mrSRI and mrNDVI could be used as an indicator of plant water stress in greenhouses, up to a certain limit. sPRI, mrSRI and mrNDVI differences between water stressed and well-watered plants were correlated well with the respective differences in volumetric water content and CWSI values of the two treatments. TSD_{meas} is more convenient index for canopy water stress deficit without the need of measuring the temperature of well-watered plants. Reflectance and temperature measurements in greenhouses could be integrated over time in order to trigger irrigation events. Nevertheless, it has to be noted that the results presented correspond to a nine days period and the correlations observed are relevant to the conditions of the measurements and the specific crop studied.

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References

Alchanatis, V., Cohen, Y., Cohen, S., Moller, M., Sprinstin, M., & Meron, M. (2010). Evaluation of different approaches for estimating and mapping crop water status in cotton with thermal imaging. *Precision Agriculture, 11,* 27–41. doi:10.1007/s11119-009-9111-7.

Amatya, S., Karkee, M., Alva, A. K., Larbi, P., & Adhikari, B. (2012). *Hyperspectral imaging for detecting water stress in potatoes*. Annual international meeting sponsored by ASABE, Paper Number 12-1345197.

American National Standard. (1998). *Standard test methods for measuring and compensating for reflected temperature using infrared imaging radiometers*. American Society for Testing and Materials Licensed, E1862-97.

Baille, A., Kittas, V., & Katsoulas, N. (2001). Influence of whitening on greenhouse microclimate and crop energy. *Agricultural and Forest Meteorology, 107,* 293–306.

Bowman, W. D., Hubick, K. T., Von Caemmerer, S., & Farquhar, G. D. (1989). Short-term changes in leaf carbon isotope discrimination in salt- and water-stressedgrasses. *Plant Physiology, 90,* 162–166.

Chaves, M. M., Pereira, J. S., Maroco, J., Rodrigues, M. L., Ricardo, C. P. P., & Osório, M. L. (2002). How plants cope with water stress in the field: photosynthesis and growth. *Annals of Botany, 89,* 907–916.

Chavez, J. L., Pierce, F. J., Elliott, T. V., & Evans, R. G. (2010). A remote irrigation monitoring and control system for continuous move systems. Part A: Description and development. *Precision Agriculture, 11,* 1–10. doi:10.1007/s11119-009-9109-1.

Clawson, K. L., Jackson, R. D., & Pinter, P. J. (1989). Evaluating plant water stress with canopy temperature differences. *Agronomy Journal, 81,* 858–863.

Cohen, Y., Alchanatis, V., Sela, E., Saranga, Y., Cohen, S., Meron, M., et al. (2015). Crop water status estimation using thermography: Multi-year model development using ground-based thermal images. *Precision Agriculture, 16,* 311–329.

Cordon, G. B., & Lagorio, M. G. (2007). Absorption and scattering coefficients: A biophysical-chemistry experiment using reflectance spectroscopy. *Journal of Chemical Education, 84*(7), 1167–1170.

Genc, L., Demirel, K., Camoglu, G., Asik, S., & Smith, S. (2011). Determination of plant water stress using spectral reflectance measurements in watermelon (*Citrullus vulgaris*). *American-Eurasian Journal Agriculture & Environment Science, 11,* 296–304.

González-Dugo, V., Zarco-Tejada, P., Nicolas, E., Nortes, P. A., Alarcon, J. J., & Intrigliolo, D. S. (2013). Using high resolution UAV thermal imagery to assess the variability in the water status of five fruit tree species within a commercial orchard. *Precision Agriculture, 14,* 660–678. doi:10.1007/s11119-013-9322-9.

Hunt, E. R., & Rock, B. N. (1989). Detection of change in leaf water content using near and middle infrared reflectance. *Remote Sensing of Environment, 30,* 43–54.

Jackson, R. D., Idso, S. B., Reginato, R. J., & Pinter, P. J. (1981). Canopy temperature as a crop water stress indicator. *Water Resources Research, 171,* 133–138.

Jacquemoud, S., & Ustin, S. L. (2008). Modeling leaf optical properties. Photo biologica lsciences online. American Society for Photobiology, from http://photobiology.info/

Jacquemoud, S., Verhoel, W., Baret, F., Bacour, C., Zarco-Tejada, P. J., Asner, G. P., et al. (2009). Prospect + sail models: A review of use for vegetation characterization. *Remote Sensing of Environment, 113,* S56–S66.

Jain, N., Ray, S. S., Singh, J. P., & Panigrahy, S. (2007). Use of hyperspectral data to assess the effects of different nitrogen applications on a potato crop. *Precision Agriculture, 8,* 225–239.

Jones, H. G., & Schofield, P. (2008). Thermal and other remote sensing of plant stress. *General and Applied Plant Physiology, 34,* 19–32.

Jones, C. L., Weckler, P. R., Maness, N. O., Jayasekara, R., Stone, M. L., & Chrz, D. (2007). Remote sensing to estimate chlorophyll concentration in spinach using multi-spectral plant reflectance. *American Society of Agricultural and Biological Engineers, 50*(6), 2267–2273.

Jones, C. L., Weckler, P. R., Maness, N. O., Stone, M. L., & Jayasekara, R. (2004). *Estimating water stress in plant using hyperspectral sensing*. Annual International Meeting Sponsored By ASAE/CSAE, Paper Number 043065.

Kacira, M., Ling, P. P., & Short, T. H. (2002). Establishing crop water stress index (CWSI) threshold values for early, non-contact detection of plant water stress. *Transactions of the ASAE, 45,* 775–780.

Kacira, M., Sase, S., Okushima, L., & Ling, P. P. (2005). Plant response-based sensing for control strategies in sustainable greenhouse production. *Journal Agricultural Meteorology, 61,* 15–22.

Katsoulas, N., Baille, A., & Kittas, C. (2001). Effect of misting on transpiration and conductances of a greenhouse rose canopy. *Agricultural and Forest Meteorology, 106,* 233–247.

Katsoulas, N., Baille, A., & Kittas, C. (2002). Influence of leaf area index on canopy energy partitioning and greenhouses cooling requirements. *Biosystems Engineering, 83*(3), 349–359.

Katsoulas, N., Elvanidi, A., Ferentinos, K.P., Bartzanas, T., & Kittas, C. (2016). Calibration methodology of a hyperspectral imaging system for greenhouse plant water stress estimation. *Acta Horticulturae*, *1142*, 119-126.

Katsoulas, N., Savas, D., Tsirogiannis, I., Merkouris, O., & Kittas, C. (2009). Response of an eggplant crop grown under Mediterranean summer conditions to greenhouse fog cooling. *Scientia Horticulturae, 123,* 90–98.

Kim, Y., Glenn, D. M., Park, J., Ngugi, H. K., & Lehman, B. L. (2010). *Hyperspectral image analysis for plant stress detection* (p. 1009114). Paper Number: Annual International Meeting.

Knipling, E. B. (1970). Physical and physiological basis for the reflectance of visible and near-infrared radiation from vegetation. *Remote Sensing of Environment, 1,* 155–159.

Köksal, E. S. (2011). Hyperspectral reflectance data processing through cluster and principal component analysis for estimating irrigation and yield related indicators. *Agricultural Water Management, 98,* 1317–1328.

Köksal, E. S., Güngör, Y., & Yildirim, Y. E. (2010). Spectral reflectance characteristics of sugar beet under different levels of irrigation water and relationships between growth parameters and spectral indexes. *Irrigation and Drainage, 60,* 187–195.

Kruse, J. K. (2004). *Remote sensing of moisture and nutrient stress*. Dissertation, Ioawa State of University, Ames.

Ling, P. P., Giacomelli, G. A., & Russell, T. P. (1996). Monitoring of plant development in controlled environment with machine vision. *Advances in Space Research, 18*(4–5), 101–112.

Liu, L., Wang, J., Huang, W., Zhao, C., Zhang, B., & Tong, Q. (2004). Estimating winter wheat plant water content using red edge parameters. *International Journal of Remote Sensing, 25*(17), 3331–3342.

Meron, M., Tsipris, J., Orlov, V., Alchanatis, V., & Cohen, Y. (2010). Crop water stress mapping for site-specific irrigation by thermal imagery and artificial reference surfaces. *Precision Agriculture, 11,* 148–162. doi:10.1007/s11119-009-9153-x.

Norikane, J. H., & Kurata, K. (2001). Water stress detection by monitoring fluorescence of plants under ambient light. *Transactions of the ASAE, 44,* 1915–1922.

O'Shaughnessy, S. A., Hebel, M. A., Evett, S. R., & Colaizzi, P. D. (2011). Evaluation of wireless infrared thermometer with a narrow field of view. *Computer and Electronics in Agriculture, 76,* 59–68.

Peňuelas, J., & Inoue, Y. (1999). Reflectance indices indicative of changes in water and pigment contents of peanut and wheat leaves. *Photosynthetica, 36,* 355–360.

Peňuelas, J., Pinol, J., Ogaya, R., & Filella, I. (1997). Estimation of plant water content by the reflectance water index WI(R900/R970). *International Journal of Remote Sensing, 18,* 2869–2875.

Ray, S. S., Das, G., Singh, J. P., & Panigrahy, S. (2006). Evaluation of hyperspectralindices for LAI estimation and discrimination of potato crop under different irrigation treatments. *International Journal of Remote Sensing, 27,* 5373–5387.

Sarlikioti, V., Driever, S. M., & Marcellis, L. F. M. (2010). Photochemical reflectance index as a mean of monitoring early water stress. *Annals of Applied Biology, 157,* 81–89. doi:10.1111/j.1744-7348.2010.00411.x.

Sclemmer, M. R., Francis, D. D., Shanahan, J. F., & Scepers, J. S. (2005). Remotely measuring chlorophyll content in corn leaves with differing nitrogen levels and relative water content. *Agronomy and Horticulture, 97,* 106–112.

Shimada, S., Funatsuka, E., Ooda, M., & Takyu, M. (2012). Developing the monitoring method for plant water stresss. *Journal or Arid Land Studies, 22,* 251–254.

Story, D., & Kacira, M. (2015). Design and implementation of a computer vision-guided greenhouse crop diagnostics system. *Machine Vision and Applications, 26,* 495–506.

Suárez, L., Zarco-Tejada, P. J., Berni, A. J., González-Dugo, V., & Fereres, E. (2009). Modelling PRI for water stress detection using radiative transfer models. *Remote Sensing of Environment, 113,* 730–744.

Taiz, L., Zeiger, E., Møller, I. M., & Murphy, A. (2015). *Plant physiology and development* (6th ed.). Washington: Sinauer Associates.

Takakura, T. (1974). Greenhouse production in engineering aspects. In Y. Hashimoto & W. Day (Eds.), *Mathematical and control applications in agriculture and horticulture*. Oxford: Pergamon Press.

Tsirogiannis, I. L., Katsoulas, N., Savvas, D., Karras, G., & Kittas, C. (2013). Relationship between reflectance and water status in a greenhouse rocket (*Erucasativa mill.*) cultivation. *European Journal of Horticultural Science, 78,* 275–282.

Tuominen, J. & Lipping, T. 2011. *Detection of environmental changes using hyperspectral remote sensing at Olkiluoto repository site*. Working Report 2011-26, Posiva, OY, Finland.

Verdebout, J., Jacquemoud, S., & Schmuck, G. (1994). Optical properties of leaves: Modeling and experimental studies. In J. Hill & J. Megier (Eds.), *A tool for environmental observations* (pp. 169–191). Brussels: Springer.

Vigneau, N., Ecarnotb, M., Rabatela, G., & Roumet, P. (2011). Potential of field hyperspectral imaging as a non destructive method to assess leaf nitrogen content in wheat. *Field Crops Research, 122,* 25–31.

Vogelmann, J. E., Rock, B. N., & Moss, D. M. (1993). Red edge spectral measurements from sugar maple leaves. *International Journal of Remote Sensing, 14*(8), 1563–1575.

Zakaluk, R., & Sri Ranjan, R. (2007). Artificial neural network modelling of leaf water potential for potatoes using RGB digital images: A greenhouse study. *Potato Research, 49,* 255–272.

Zakaluk, R., & Sri Ranjan, R. (2008). Predicting the leaf water potential of potato plants using RGB reflectance. *Canadian Biosystems Engineering Journal, 50,* 7.1–7.12.