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Estimation of leaf water content in sunflower under drought conditions by means of spectral reflectance

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ABSTRACT

The assessment of plant water status under drought conditions is essential for the understanding of the adaptation physiological mechanisms of plants, because water stress is one of the most common limitations of crop growth and yield. This study was carried out to estimate the leaf water content in sunflower plants based on spectral reflectance in the near-infrared region. Two varieties of sunflower plants were cultivated during two autumn seasons inside a non-acclimatized greenhouse until the start of the flowering stage, and later were maintained in a growth chamber with the purpose of submitting the plants to a slow and progressive dehydration rate during 12 consecutive days. Measurements of spectral reflectance and leaf mass (fresh and dry) were accomplished along the water stress period. Increases on the spectral reflectance values were observed as the leaf water loss was intensified. The coefficients of determination (R^2) of models between leaf water content (LWC) and water index (WI) were 0.948 and 0.956 for Sunbright and Sunbright Supreme varieties, respectively, while the R^2 between LWC and wavelength where the first-order derivative of the spectral reflectance is minimum (λd_{min}) were 0.979 and 0.988 for Sunbright and Sunbright Supreme varieties, respectively. Based on the results, the radiometric indicators (WI and λd_{min}) might be useful tools in the development of automatic systems, resulting in a non-destructive, simple, easy, and instantaneous method for monitoring water status in sunflower plants.

1. Introduction

The growing consumption of non-renewable energy and its inherent impacts on climate change have led to policies favoring the use of renewable energy (Rodrigues et al., 2010). Among crops considered for biofuel production, sunflower (*Helianthus annuus* L.) is one of the most widely cultivated in the world to obtain biodiesel.

Agricultural water resources are becoming more limited in many parts of the world due to a combination of natural and anthropogenic factors. As a result, the paradigm of full irrigation and maximized production per unit area of land is gradually being replaced with deficit irrigation and achieving maximum production per unit of applied water (Taghvaeian et al., 2014).

Water is a fundamental chemical constituent of plants, and its abundance in leaves is closely tied to leaf vigor, phylogenic traits such as leaf structure and shape, and photosynthetic efficiency (Cheng et al., 2011). The assessment of plant water status under drought conditions is essential for the understanding of the adaptation physiological mechanisms of plants, because water stress is one of the most common limitations of crop growth and yield.

The most reliable and accurate method to obtain plant water status is by determining mass differences between wet and dry leaves in laboratory. This method is destructive, time consuming, labor demanding, and also subject to measurement and sampling errors, especially when data are extrapolated to the whole plant and canopies (Ordoñez et al., 2010). Despite this traditional method, the near-infrared reflectance spectroscopy offers a non-destructive and instantaneous way of assessing the plant water status based on light absorbance by water contained in leaf tissue at certain wavelengths of the near-infrared region.

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Reliable detection of water stress in plants by near-infrared spectroscopy is dependent upon knowledge of wavelengths most sensitive to leaf water content. In this direction, Peñuelas et al. (2004) showed that water index (WI), defined as the ratio between the reflectance at a reference wavelength (900 nm) and the reflectance at 970 nm (one of the light absorption bands by water), was correlated with leaf water content in several species of trees, shrubs and grasses. The water index was used to estimate water vapor and carbon dioxide fluxes in chaparral vegetation (Claudio et al., 2006), as well as to irrigation scheduling and to monitor vineyard performance (Serrano et al., 2010). Even though there are strong water absorption wavebands in the near- and middle-infrared regions (above 1000 nm), the cost of spectrometers for measuring these wavelengths is high when compared to spectrometers that measure spectral reflectance in lower wavelengths, mainly due to their type of detector technology.

Other radiometric indicators, such as normalized difference vegetation index (NDVI) (González-Fernández et al., 2015), simple ratio water index (SRWI) (Zarco-Tejada et al., 2003), floating position water band (FWBI) (Strachan et al., 2002), and wavelength in the near-infrared region where the first-order derivative of the spectral reflectance is minimum (λd_{min}) (Peñuelas et al., 1993) are also used for estimation of leaf water content in many agricultural crops.

Despite of past researches predicting leaf water content of different species by spectrometry (Claudio et al., 2006; Serrano et al., 2010; González-Fernández et al., 2015; Zarco-Tejada et al., 2003; Strachan et al., 2002; Peñuelas et al., 1993, 1997; Sims and Gamon, 2003; Zhang et al., 2012; Rallo et al., 2014), there are no applications for the sunflower crop under moderate to severe water stress conditions. In this context, this work was carried out to estimate the leaf water content in sunflower plants under progressive dehydration based on spectral reflectance in the near-infrared region.

2. Material and methods

2.1. Greenhouse cultivation

Sunflower plants (*Helianthus annuus* L.) of varieties Sunbright and Sunbright Supreme (Sakata Ornamentals, Morgan Hill, California, USA) were cultivated in a non-acclimatized greenhouse constructed with a steel arch frame and covered with a transparent polyethylene film (150 μm). This greenhouse is located at the experimental area of the Agrarian Sciences Department of the Federal University of São João del-Rei, Sete Lagoas, Minas Gerais, Brazil, with the following geographical coordinates: 19° 28′ 34″ S latitude, 44° 11′ 44″ W longitude, and 796 m elevation. According to Köppen's climate classification for Brazil (Alvares et al., 2013), the local climate is humid subtropical with dry winter and hot summer (Cwa).

The hybrids Sunbright and Sunbright Supreme are excellent as cut flowers, presenting bright golden petals and pollenless. Both varieties are very uniform and vigorous in growth. Sunbright Supreme offers earlier flowering (more precocious), shorter and stronger flower stems, and flowers with more rounded petals when compared to Sunbright.

Sunflower seeds were germinated in substrate consisting of a mixture pine bark, vermiculite and peat (4:3:3 v v^{-1}), which had mean density of 0.42 g cm^{-3}, contained in plastic pots with capacity of 905 cm^3. The pots were disposed inside of the greenhouse with density of 0.25 plant m^{-2}.

The irrigation water was automatically supplied to plants by a time-controlled drip system connected to a 37.3 W pump and a 220 L water reservoir. The sunflower plants were fertigated with a nutrient solution manually prepared from granulated fertilizer (15 N-5 P-15 K-5 Ca-2 Mg, Peters Excel, Scotts, Marysville, Ohio, USA). The nutrient solution concentration was maintained in 1 dS m^{-1} and monitored by an electrode connected to an electronic circuit developed by Steidle Neto et al. (2005) for measuring the electrical conductivity of aqueous solutions.

Four applications of the growth regulator (Pachlobutrazol 100 CE, Wiser, São Paulo, São Paulo, Brazil) were performed on substrate, aiming at avoiding to tutor the plants growing and to facilitate the plant management at laboratory. The application doses were of 4.5 mg of the active ingredient per plant, at weekly intervals starting from the second week after seeding. This growing control technique resulted in sunflower plants with average height of around 0.4 m.

At the start of the sunflower flowering stage, approximately 60 days after seeding, a last irrigation event was accomplished at the early morning with the purpose of maximizing the substrate moisture. From this day, no fertigation events were applied.

The total number of sunflower plants cultivated in greenhouse was greater than the required for the spectral reflectance measurements, since it was considered the selection of the healthy, vigorous and well-formed plants. For accounting variables that can affect plant growth and development, the sunflower cultivation was carried out during the autumn seasons of 2014 (experiment 1) and 2015 (experiment 2).

2.2. Growth chamber and measurements

Plants were submitted to a slow and progressive dehydration rate during 12 consecutive days in a reach-in grown chamber equipped with actuators (heating, cooling, fogging, and lighting systems) and sensors (photometric, pyranometer, air temperature and relative humidity). The chamber was programmed to control internal conditions for 1600 fc (17.2 klux) illumination, 12 h photoperiod, 30 to 25 °C (day-night) temperature, and 40% humidity. There were used 36 plants for each experiment and variety.

Spectral reflectance and leaf mass (fresh and dry) measurements were made in three sunflower plants, randomly selected from day to day. The reflectance measurements were accomplished in three leaves of each plant. Three separate measurements on adaxial surface were performed in each leaf, avoiding its veins and boundaries. Thus, there were made 648 measurements per experiment (12 days × 3 plants × 3 leaves × 3 points per leaf × 2 varieties). Average spectral reflectance curves were generated per plant to analyze these measurements.

The measure equipment of the spectral reflectance was a miniature spectrometer (JAZ-EL350, Ocean Optics, Dunedin, Florida, USA) coupled to a tungsten-halogen light source and preconfigured to acquire and store in memory card reflectance data (500–1000 nm wavelength range), with a spectral resolution of 1.3 nm. A clip probe (SpectroClip-R, Ocean Optics, Dunedin, Florida, USA) was used to collect reflected light from the sunflower leaves. This probe contains an integrating sphere that captures diffuse reflected light more efficiently than lens-based collection optics. The active illuminated leaf area in the clip probe was 5 mm diameter. Two premium fibers (600 μm diameter) interconnected the spectrometer and the light source to the clip probe. A diffuse reflectance standard with Spectralon™ was used as a reference to measure spectral reflectance.

Daily, the warm-up time of the light source was waited and the reference standard measurement was made before of the spectral reflectance measurements in sunflower leaves with the purpose of calibration.

According to Claudio et al. (2006), the ideal wavelengths for predicting leaf water content are those with weak absorption,