**Predicting the relative water content of sunflower plant using RGB reflectance**

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**Abstract:** With an increasing request of fresh water resources in arid/semi-arid parts of the world, researchers and practitioners are relying more than ever on remote sensing techniques for monitoring and evaluating crop water status and for estimating crop water use. The goal of the present study was to evaluate relative water content (RWC) to different levels of irrigation and deploy a digital imaging system for high spatial and temporal monitoring using of vegetation indices and investigate their relationship with RWC. According to results, with increase in water stress degree the RWC value decrease. The results of this study show that significant linear relationships between the image parameters and RWC. There was a strong relationship between the normalized difference red blue index (NDRBI) and RWC with correlation coefficient of 0.90\*\*. Overall, the results of this study show the potential of using vegetation indices derived from digital Red, Green and Blue (RGB) images as a low-cost technique for assessing RWC under different levels of irrigation availabilities.

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**Keywords:** RWC, digital RGB imaging, image processing technology, vegetation indices

**1. Introduction**

Sunflower is one of the four major oilseed crops (soybean, peanut, rapeseed and sunflower), grown for edible oil in the world. Irrigation water availability has been identified as one of the major limiting factors of sunflower productivity (O’Shaughnessy et al., 2011; Wang et al., 2012). Irrigation scheduling based upon crop water status (Yazar et al., 1999; Candogan et al., 2013) a conventional method is to measure the relative water content (RWC). So the RWC is considered as a reliable indicator of plant water balance (Rauf. 2008). Similarly, RWC represents an adequate description of the water stress history experienced by plants (Sinclair and Ludlow., 1986, Ritchie et al., 1990, Rauf. 2008, Canvar et al., 2014). Historically, RWC has been used as an index of water stress. However, makes in the RWC measurement impractical for irrigation scheduling over large areas. Another methods, for collecting crop irrigation management information at the field level is the use of remotely sensed images.

With the use of digital image technologies have as tools for monitoring and mapping crop water status, improving water use efficiency and precisely managing irrigation is feasible to obtain the related parameters to water requirements and crop growth modelling (Escarabajal- Henarejos et al., 2015a, b). The exploration of leaf reflectance in the visible region as an indicator of plant water status is justified because plant water status, photosynthesis, and visible leaf reflectance are interrelated (Carter, 1991; Schlemmer et al., 2005; Wheeler, 2006). Moreover, with this technology it is possible to analyze deficiencies in management and to quantify the excess in irrigation (Escarabajal- Henarejos et al., 2015a, b). Many studies used spectral vegetation indices to find a correlation with crop water status (Zakaluk and Sri Ranjan., 2008; Kim et al., 2011; Lorente et al., 2012; Guendouz et al., 2013; Elazab et al., 2015; González-Esquiva et al., 2016). The specific objectives of this study were to (1) develop and deploy a digital imaging system for monitoring sunflower canopy at high spatial; (2) investigate full relationships between RWC values and vegetation indices by automatically evaluating digital images.

**2. Material and Methods**

**2.1. General information of the experiment site**

The field experiment was conducted during the summer of 2015 at the limited irrigation research farm, located near the city of Shoushtar in northern Khozestan, Iran (32◦ 27ʹ N, 48◦ 53ʹ E, elevation 110 m). Local Steppe climate prevails in the experimental area. The average annual rainfall is 371 mm and average annual temperature 25.3 ◦C. Available water in the upper 0.60 m of the soil profile depth is 101 mm. In the root zone, soil water contents at the field capacity and permanent wilting point are 193 and 92 mm, respectively. According to soil analysis, at the time of planting 69 kg N ha−1, 100 kg P ha−1 and 50 kg K ha−1 were applied to avoid nutrient deficiencies on all treatments. 69 kg N ha−1 remaining of fertilizer N at stem elongation stage was added to the ground. Treatments were varying levels of regulated deficit irrigation based on cumulative evaporation from class A evaporation pan consisted of control (T50= irrigation after 50 mm evaporation), mild water stress (T90=irrigation after 90 mm evaporation), moderate water stress (T130=irrigation after 130 mm evaporation) and severe water stress (T170=irrigation after 170 mm evaporation). Treatments were laid out in a randomized block design with 3 replications.

Photographs were taken vertically over the crop at a fixed height of 1m distance from the sunflower canopy using a digital camera (A3300 IS, Canon, Japan). With a resolution of 16 mega pixels was taken to capture color images of sunflower canopy of each plot an interval of 7 days once and stored as 8-bit color images with a resolution of 3456 × 4608 pixels and focal length of 28-140mm. The images were processed with the image processing toolbox using the ENVI® (Environment for Visualizing Images) software (Research System Inc., Boulder, CO, USA) version 4.7. A set of 160 images were processed.

**2.2. Sample collection and plant measurements**

Plant vegetive growth was monitored by means of subsequent destructive samplings manually realized throughout the growing period. For RWC determination leaf disks taken from two to three leaves of similar physiological maturity and weighted (FW), the samples were immediately hydrated to full turgidity for 4h at 25 °C and then turgid leaf disks weight were measured (TW). Samples in oven dried at 80°C for 24 h and weight (DW), were measured per plot and measurements were averaged within each plot, defined by Eq. (1).

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| RWC (%) = $\left[\frac{F\_{W}-D\_{W}}{T\_{W}-D\_{W}}\right]×100$ | (1) |

Where, FW is fresh weight, DW dry weight and TW turgid weight (Ferrat and Loval, 1999).

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| Table 1. Vegetation indices derived from the RGB images. |
| Index | Name | Equation | Citation |
| NDGRI | normalized difference green red index | $$\frac{G-R}{G+R}$$ | Gitelson et al (2002) |
| NDGBI | normalized difference green blue index | $$\frac{G-B}{G+B}$$ |
| NDRBI | normalized difference red blue index | $$\frac{R-B}{R+B}$$ |
| GRS | green red slope transformation | $$\frac{G-R}{GPWL+RPWL}$$ | Zakaluk1 and Sri Ranjan (2008) |
| GBS | green blue slope transformation | $$\frac{G-B}{GPWL+BPWL}$$ |
| RBS | red blue slope transformation | $$\frac{R-B}{RPWL+BPWL}$$ |
| GPWL: peak wavelength (nm) of the green image band, RPWL: peak wavelength (nm) of the red image band and BPWL: peak wavelength (nm) of the blue image band. |

**2.3. Image segmentation and data analysis**

Images were represented in the RGB color model, each pixels in the displayed by an RGB triplet (red, green, blue value). Various approaches to the use of image segmentation to extract gap deficit from digital photograph under different scenarios have been reported (e.g., Booth et al., 2006; Demarez et al., 2008; Graham et al., 2009). Green vegetation had an intensive reflectance peak in the green band, whereas soil did not cause any apparent change in albedo in the visible band. Therefore In this study, the difference between the canopy and non-canopy area became obvious in Greenness value (Liu and Pattey, 2010), as defined in Eq. (2).

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| Greenness = 2G − B − R | (2) |

Where R, G and B represent the severity levels recorded by the red, green and blue channels of the digital camera. Once a threshold was set, pixels with Greenness value higher than the threshold were sorted as the sunflower canopy and the rest as the background (soil or plant residues). The second step, crop water stress indices computation: the digital images were further processed to calculate plant stress indices. Six different spectral indices were evaluated for plant water stress detection (Table 1). They can provide valuable information about water stress using just a few individual wavelengths. Pearson linear correlation and regression analyses were performed to determine the association among the measured physiological parameters and the leaf spectral reflectance.

**3. Results and discussion**

**3.1. Relative water content of sunflower**

It can be clearly seen that plant response, expressed in terms of RWC, was very muchdependent on theapplied irrigation regimes. As depicted in Figure.1 the changes of RWC for different irrigation regimes confirming the results of Canavar (2014). It is known that, treatment T50 had the highest RWC on all days of measurement that the cause of it ability to absorb more water from the soil and compensate transpiration was done from plant leaves (Siddique et al., 2000). Compared with T50, the RWC was significantly lower by 11%, 16% and 21% under T90, T130 and T170, respectively (Table 2).

Figure 1. Relative water content variation during the sunflower growing season in all treatments.

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| Table 2. The effect of irrigation treatments on the relative water content (RWC) and vegetation indices in September and October. |
| Treatment | RWC (%) | NDGRI | NDGBI | NDRBI | GRS | GBS | RBS |
| T50 | 72.84 | 0.0827 | 0.1204 | 0.0383 | -0.0006 | 0.0006 | 0.0001 |
| T90 | 64.52\*\* | 0.0679 | 0.1529\* | 0.0668\*\* | -0.0005\*\* | 0.0010\* | 0.0002\* |
| T130 | 60.95\*\* | 0.0639\* | 0.1619\*\* | 0.0777\*\* | -0.0005\* | 0.0017\*\* | 0.0003\*\* |
| T170 | 57.36\*\* | 0.0519\*\* | 0.1690\*\* | 0.0778\*\* | -0.0006 | 0.0019\*\* | 0.0003\*\* |
| ANOVA |  |  |  |  |  |  |  |
| B | \* | NS | NS | NS | NS | NS | NS |
| IRR | \*\* | \* | \* | \*\* | \* | \* | \*\* |
| B, block, IRR, irrigation treatments (T50- irrigation after 50 mm evaporation, T90- irrigation after 90 mm evaporation, T130- irrigation after 130 mm evaporation, T170- irrigation after 170 mm evaporation).Treatments with RWC and vegetation indices significantly different than T50 treatment are marked by NS,\* and \*\* for not significant, P < 0.05 and P < 0.01, respectively. |
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| Table 3. Pearson correlation coefficients between sunflower water content indices and the image feature parameters extracted from segmented images. |
|  | NDGRI | NDGBI | NDRBI | GRS | GBS | RBS | Number of samples |
| RWC | 0.65 | -0.81\*\* | -0.90\*\* | -0.56 | -0.80\*\* | -0.86\*\* | 160 |
| \*\* P < 0.01. |

**3.4. Relationships between image feature parameters and RWC**

The effect of irrigation treatments was significant for all studied vegetation indices (P < 0.05) except for the NDRBI and RBS (P < 0.01) (Table 2). The image feature parameters, i.e., NDBGI, NDRBI, GBS and RBS extracted from images were significantly correlated with RWC (Table 3). Among them, NDGBI, NDRBI, GRS, GBS and RBS were significantly with RWC negatively correlated, while NDGRI was significantly with RWC positively correlated. NDRBI had the highest correlation coefficient with RWC reaching 0.90\*\* and followed by RBS, NDGBI and GBS, while NDGRI and GRS had lower correlation coefficient compared to the others (Table 3). Elsayed et al. (2011) offered that water deficiency causes alteration in leaf pigment composition, concentration, and cell structure by changing the properties of connections between air spaces and cell walls, cell wall combination and structure or cell size and shape. It is apparent in our study that observed reduction the RWC were as a result of water stress. The image feature parameters, NDRBI and RBS, were well correlated with the RWC. This paper focuses on the analysis of the relationships between the two image feature parameters and sunflower water content indices. Regression analysis showed that NDRBI and RBS had linear relations with the RWC (Figure 2), and the following linear function was identified to best fit the linear relationships:

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| y = a+bx | (3) |

Where y is a dependent variable, representing RWC or CWSI, x is an independent variable, representing NDRBI or RBS. Both a and b were parameters obtained by the least square method.

Regression analysis was performed using the concatenation of the different irrigation treatments, the feature parameters were in good linear relations with RWC. The correlation coefficients between NDRBI with the RWC reached 0.90 and between RBS with the RWC was 0.86 (Fig.2). Carter (1991) found that RWC was sensitive to the red and blue visible wave--lengths centered at 480 and 680 nm for the leaves of six species. Our finding here was similar to those reported by Carter (1991), Schlemmer et al. (2005) and Guendouz et al. (2013), which showed a strong linear relationship between sunflower water status (RWC) with image feature parameters.

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| Figure 2. Relationships of NDRBI (normalized difference red blue index) and RBS (red blue slope) against (a and b) RWC (relative water content), fitted with Eq. (3). |

**4. Conclusions**

This study demonstrated a reliable, fast, and cost-effective approach for estimating the water status of sunflower using digital camera images. As observed from this study, the feature parameters after image segmentation, are closely related to sunflower water status. NDRBI extracted from segmented images are in linear relationship with RWC of sunflower reaching 0.90. Overall, our results suggest that near-ground remote sensing technology using digital camera has the potential to improve the efficiency of water application to agricultural fields for managing irrigation water. The use of digital cameras as a tool for near-ground remote sensing in precision agriculture is a new field of research.

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