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ESTIMATING WATER STRESSED DWARF GREEN BEAN PIGMENT CONCENTRATION THROUGH HYPERSPECTRAL INDICES

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Abstract

In this study, the relationship between leaf pigment concentration (analyzed in the laboratory) and four spectral indexes (measured in the field) was investigated. For this purpose, field experiments consisting of six different irrigation treatments were conducted with dwarf green beans during 2005 growing season. Based on spectral data, spectral indexes were plotted against pigment concentration. Results showed that under water stress, the chlorophyll and carotene contents of green bean leaves rose. According to linear regression analysis between spectral indexes and pigment contents, the Normalized Difference Pigment Chlorophyll Index (NPCI) and Normalized Difference Vegetation Index (NDVI) had the highest correlations with the chlorophyll (a, b and total), and carotene content of leaves.

Introduction

The sustainable management of irrigation water is fundamental for conserving the natural resources and it requires efficient monitoring and decision support tools. Since agricultural data varies on temporal and spatial bases, it is very difficult and expensive to accurately study agricultural lands through on-site sampling and analysis. Remote sensing techniques can very effectively discriminate between agricultural crops. However, conversion of remotely sensed data to a meaningful form is based on the determination of the relationship between these data and the physiological parameters. Hand held remote sensing devices and satellites measure equivalent data in the same spectral region. Therefore, in order to develop new indicators related to crop properties, various studies have been carried out by means radiometers (Jackson *et al.,* 1980).

Pigments of plants are integrally related to the physiological functions of leaves, which play very important roles in plant growth. Chlorophylls tend to decline more rapidly than carotenoids when plants are under stress or during leaf senescence (Merzlyak *et al*., 1999). Because of the importance of Chlorophyll a (Chl a), Chlorophyll b (Chl b) and carotenes for leaf function, variations in pigment content provide information concerning the physiological state of leaves.

Consequently, assessment of pigment content has become an effective means of monitoring plant growth and estimating potential photosynthetic productivity (Chen *et al*., 2007). Techniques have been developed to estimate the concentrations of individual pigments within leaves using high spectral resolution (hyperspectral) reflectance measurements (Penuelas *et al*., 1995). Blackburn (1998) reported that it is essential to investigate the relative merits of different hyperspectral measurements of pigment concentrations for different species of plants and at a range of scales, from the individual leaf to the whole canopy. In a related study, it was reported that remote estimates of pigment concentrations will provide an improved evaluation of the spatial and temporal dynamics of vegetation stress (Filella *et al.,* 1995).

The aim of this study was to determine relationships between spectral indices and pigment content of water stressed and fully irrigated dwarf green beans. For this purpose, field experiments were conducted with six different irrigation treatments, pigment analyzes were done in the laboratory and spectral measurements were carried out.

Materials and Methods

Field experiments were conducted at the Ankara Soil and Water Resources Research Institute (39” 53’ N and 32” 45’ E, altitude 924 m) with dwarf green beans *(Phaseolus vulgaris humilis)* during growing period of 2005. The dwarf green beans were planted at 0.70 m row spacing on May 12th in 2005. The experimental design was randomized complete block design with three replications. The irrigation treatments were based on US Weather Bureau Class A pan evaporation for dwarf green beans and were B1 (120%), B2 (90%), B3 (60%), B4 (30%), B5 (10%) and B6 (rainfed). Drip irrigation was used and the irrigation interval was one week. Fertilizer was applied on the basis of a soil analysis. Sixty kg ha^{-1} P_2O_5 and 23.4 kg ha^{-1} N were applied pre-sowing and 5.3 kg ha^{-1} N was applied five times via drip irrigation at regular intervals during the growing season. The plants were harvested at one week intervals from mid-June to the end of September.

During the growing period, spectral reflectance, climatic parameters and soil water content were measured. The Chl a, Chl b, total chlorophyll (Chl t) and carotene content of sampled leaves were analyzed. Spectral observations were made with a spectroradiometer (Model LI-1800, Licor, Inc., Lincoln, NE, USA) with a 15^{o} field of view lens. The radiance of the crops in the region ranging from 300 to 1100 nm was detected at 2 nm intervals from 1.5 m above the crop canopy with nadir orientation of the telescope body by means of a tripod. As a result, spectral data from an area nearly 40 cm in diameter were obtained. Irradiance spectra of sunlight were recorded before and after each crop measurement with a spectralon panel (Lapsphere, North Sutton, USA) and their average were used as the reflectance standard. The reflectance of individual wavelength was calculated as the ratio of radiance and irradiance. All spectral data collections were performed at solar zenith angles of 45^{o}–50^{o} on cloudless days and at least two measurements were taken each week.

In this study, the Normalized Difference Vegetation Index (NDVI) (Tucker, 1979), Normalized Difference Pigment Chlorophyll Index (NPCI) (Aparicio *et al*., 1994), Difference Vegetation Index (DVI) (Tucker, 1979) and Modified Chlorophyll Absorption in Reflectance Index (MCARI) (Kim, 1994; Daughtry *et al*., 2000) were used as spectral index based on Equations 1, 2, 3 and 4, respectively.

$$NDVI = \frac{R800 - R680}{R800 + R680} \qquad (1)$$

$$NPCI = \frac{R680 - R430}{R680 + R430} \qquad (2)$$

$$DVI = R800 - R680 \qquad (3)$$

$$MCARI = (R700 - R670) - (0.2(R700 - R550)(\frac{R700}{R670}) \qquad (4)$$

where R800, R700, R680, R670, R550 and R430 are the reflectance values at 800, 680, 670, 550 and 430 nm wavelengths, respectively.

Pigment concentration was determined through laboratory analyses based on the method of Arnon (1949) and Withan and Blayedes (1971). The three replicates for each irrigation treatment were sampled. The samples were bulked and later sub-sampled for testing. The sub-samples were chipped, weighed and then immersed in 40 ml of 80% acetone for 24 h to extract the chlorophylls and carotenoid from the leaves. Absorbance values at 470, 663 and 645 nm were measured by using a spectrophotometer. The content in mg g^{-1} of Chl a, Chlb, Chl t and Carotenes (Cars) in the extract were calculated using the following equations (5 to 8).

Chl a = [(12.7 D663) – (2.69 D645)] V/W/1000 (5)
Chl b = [(22.91 D645) – (4.68 D663)] V/W/1000 (6)
Chl t = [(20.2 D645) + (8.02 D663)] V/W/1000 (7)
Cars = [(1000 D652) - (2.27 Chl a) - (81.4 Chl b)] / (V x 2.27 / W /1000) (8)

where, V is the volume, W is the weight and D663, D645 and D470 are absorbance values measured at 663, 645 and 470 nm wavelengths, respectively.

Linear regression analyses were carried out for pigment contents and spectral indexes recorded the same Day After Planting (DAP). Determination coefficients were evaluated for two significance levels [P<0.01 (**) and P<0.05 (*)].

Results and Discussion

In the current study, laboratory analysis identified pigment contents in the various water treatments between DAP 50–130. Course of Chl a, Chl b, Chl t and carotene concentrations in leaves sampled from all irrigation treatments throughout the study period are given in Fig. 1. In general, pigment contents varied significantly, depending on the irrigation level which affected the physiology of the crop directly. Lower water regimes caused lower pigment concentrations. While Chl a ranged between 0.14 and 0.95 mg g^{-1}, Chl b ranged from 0.08 to 0.82 mg g^{-1}. Minimum and maximum Chl t were 0.22 and 1.77 mg $g^{-1,}$ respectively. In addition, the lowest carotene contents were observed for treatments B4, B5 and B6 at the end of the growing season. The highest values were determined in the middle of the growing period for treatment B3 which received 60% of Class A pan evaporation as irrigation. Similar relationships between irrigation regime and pigment concentration have been determined for peanuts (Arunyanark *et al*., 2008), birinjal plants (Prakash & Ramachandran, 2000), sorghum (Younis *et al*., 2000), wheat (Sarker *et al*., 1999), finger millet (Maqsood & Azam Ali, 2007), canola (Kauser *et al*., 2006), mung bean (Farooq & Bano, 2006) and four other plant species (Aziz, 2007).

Crop spectral data were recorded a total of twenty four times (three times in July, seven times in June, nine times in August and five times in September) for each treatment by means of spectroradiometer. Monthly courses of average NDVI, NPCI, DVI and MCARI throughout the measurement period are given in the Fig. 2. Generally, it shows that variation of these indexes was consistent with the irrigation regime.

NDVI is one of the most prominent spectral indicators for the assessment of water stress on plants.. The effect of water stress on green bean growth was clearly apparant in the NDVI graphic in Fig. 2. NDVI values of lesser and non-water stressed treatments

(B1, B2 and B3) were close to each other and distinguished from the higher water stress treatments (B4, B5 and B6). After DAP 90, the NDVI of B1, B2 and B3 treatments varied slightly, whereas it's value in treatments B4, B5 and B6 was significantly reduced.

DVI is a simple spectral index sensitive to the vegetation occupying the field of view of the sensor. Graphically, variation of DVI values was similar to the NDVI (Fig. 2). However, while the NDVI ranged between from 0.5 – 0.9, the DVI varied from 0.2 to 0.55. The lowest DVI values were measured for the rain fed treatment (B6) and the highest DVI values were observed for the non-water stressed crop (B1).

The NPCI, which was developed especially for the detection of the chlorophyll content of crops, differentiated between the irrigation treatments and varied between approximately 0.18 and 0.41 (Fig. 2). NPCI values decreased from the beginning to middle of the growing period and rose after mid-season. Trends in NPCI values were consistent in that the lowest water level (B6) shows the highest NPCI and the highest water level (B1) has the lowest NPCI (Fig. 2).

For the final index, MCARI values throughout the observation period differed from the other spectral indexes. The range of the MCARI was between 0.02 and 0.08. Rainfed crops showed the lowest MCARI values and the highest values were observed both for B1 and B2 irrigation treatments (Fig. 2). MCARI values for the rainfed treatment decreased from the beginning to the end of the observation period. Although MCARI values of B1, B2, B3, B4 and B5 irrigation treatments decreased from the outset to mid-season, these values increased after mid-season.

The sensitivity of the spectral indexes for estimation of pigment concentration of green beans was evaluated through statistical analysis. Figure 3 summarizes relationships between the four spectral indexes and pigment concentrations. Three of four spectral indexes had significant relationships with pigment concentrations. Only the relationship between MCARI and pigment concentration was non-significant (ns) for dwarf green beans.

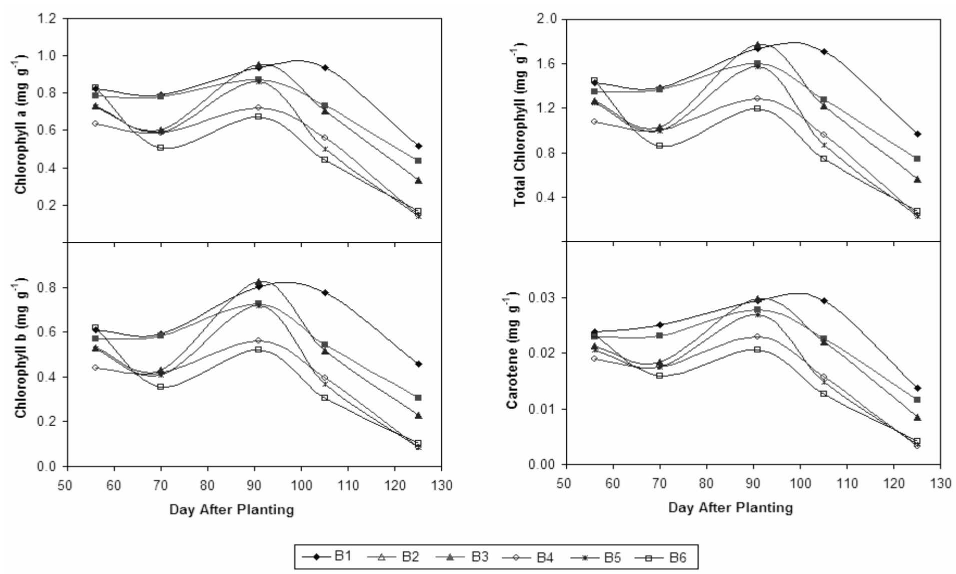

Fig. 1. Course of pigment concentrations throughout the sampling period.

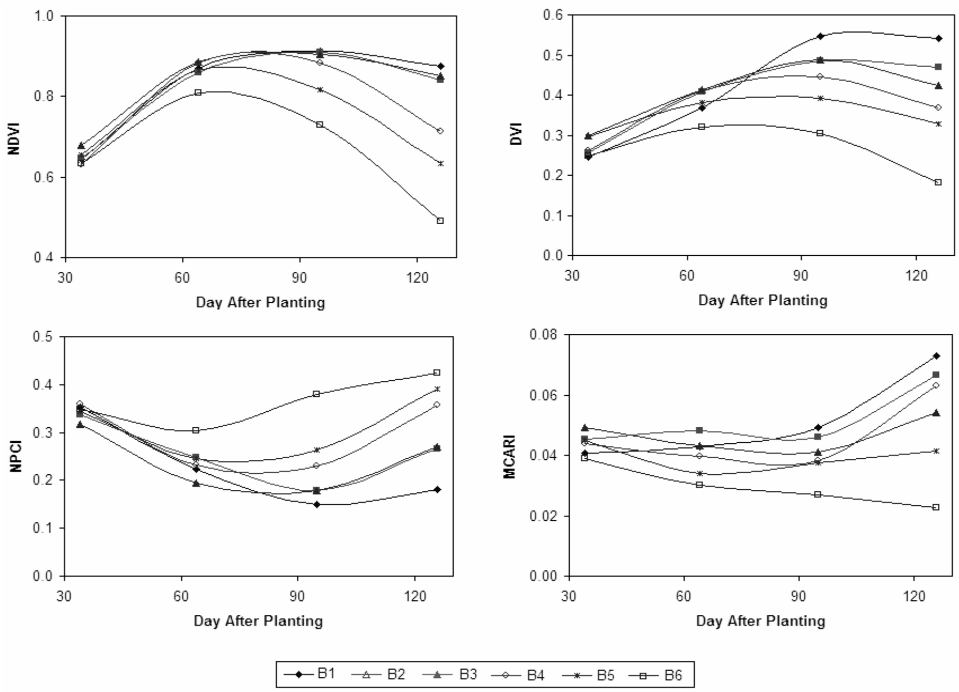

Fig. 2. Variation of spectral indices from beginning to end of measurement period.

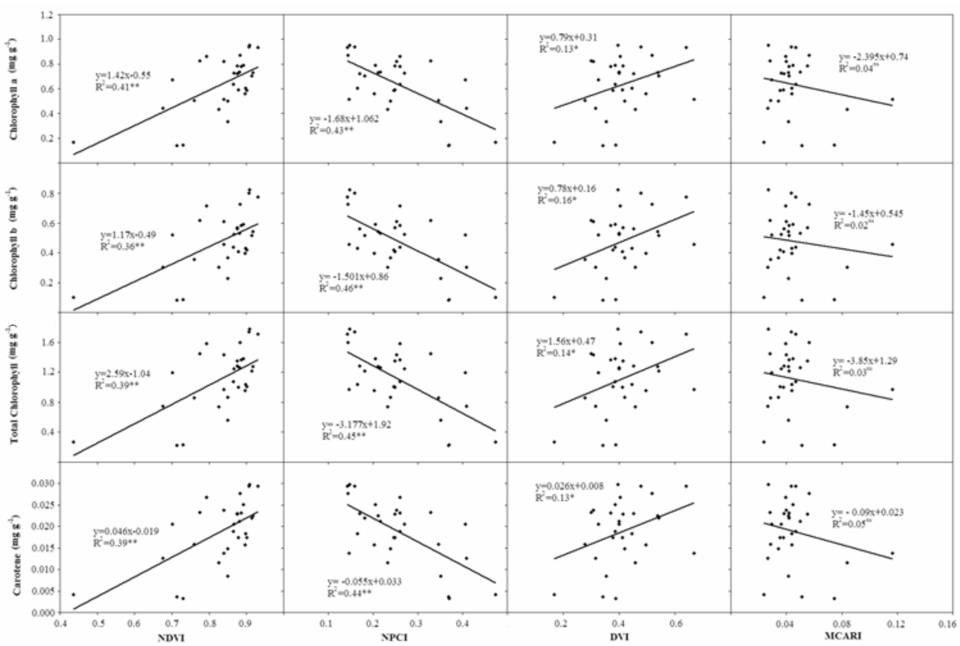

Fig. 3. Comparisons between spectral vegetation indices and pigments.

The highest coefficients of determination (R^2 between 0.43 and 0.46) were calculated using NPCI ($p<0.01$). Maximum coefficient of determination value was obtained for the NPCI-Chl b relationship (R^2=0.46**). Correlations between NDVI and pigment concentrations (R^2 between 0.36** and 0.41**) were higher than that of DVI (R^2 between 0.13* and 0.16*). Both NDVI and NPCI showed linear trends with pigment concentrations. While increasing pigment concentrations produced higher NDVI values, in contrast they were reflected in lower NPCI values. According to these results, spectral data could be used to estimate the Chl a, Chl b, Chl t and carotenoid concentration of green beans. For this purpose, NPCI and NDVI are suggested as spectral indexes.

Previous studies related to estimation of pigment content by means of hyperspectral indexes yielded similar results. Datt (1998) determined a correlation coefficient varying from 0.43 to 0.47 between NDVI and pigments for eucalyptus. Bannari *et al*., (2007) evaluated relationships between wheat crop chlorophyll content and some spectral indices and defined the highest correlation by means of NPCI (R^2:0.84). According to a different study, MCARI was determined to be very sensitive to the chlorophyll content of corn leaves. In addition to this leaf area index (LAI) and background reflectance had a big effect on MCARI (Daughtry *et al*., (2000). Haboudanea *et al*., (2002) determined significant relationships between low pigment concentrations and MCARI.

Conclusions

In this study, four hyperspectral vegetation indexes were investigated to determine their sensitivity in the estimation of Chl a, Chl b, Chl t and carotene concentration. Indexes those are composed of reflectance at 800, 680 and 430 (NDVI [800, 680] and NPCI [680, 430]) proves to have better correlation with pigment contents. The significant relationship between pigment concentrations and NDVI is indirect and appears to be attributable to the degree of water stress. However, NPCI could be more sensitive to the pigment contents of dwarf green bean leaves. The primary results of this study may provide frame of reference for further research to estimate pigment content by means of remote sensing instruments.

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