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Investigation on the Capability of GreenSeeker Sensor in Predicting Nitrogen Status and Fractional Vegetation Cover of Spinach Crop

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Abstract

Fractional vegetation cover (FVC) and normalized difference vegetation index (NDVI) are the most important indicators of greenness and have a strong correlation with green biomass. The objective of this study was to evaluate a hand-held GreesnSeeker (GS) active remote sensing instrument to estimate NDVI and FVC in the spinach plant. In this study, the color indices of the G-B index and Excess Green (ExG) were used as color vegetation indices to discriminate leaves from soil background. During 28 to 44 days after emergence (DAG), the results showed good correlations between chlorophyll yield and NDVI (R = 0.61 to 0.91), and the correlation between NDVI of GS and biomass was significant. In addition, in this growth stage, the results showed a good coefficient of correlation between NDVI of GS and FVC (R = 0.67 to 0.82). In assessing the nitrogen rate on the NDVI of GS, the results showed significant differences only at the short period of growth stage (28 to 36 DAG). The results revealed that GreenSeeker performed well for estimation both chlorophyll and biomass yield of spinach crop and it could be used as a suitable instrument for estimation of leaf area index in the middle of the plant growth period.

Keywords: Canopy, Chlorophyll, Optical sensor, Segmentation

Introduction

The main challenges for global food security and sustainable development are how to increase food production whereas improving resource use efficiencies and reducing risks of environmental contamination (Guo *et al.*, 2010; Chen *et al.*, 2014). Blanket fertilizer nitrogen (N) recommendations lead to low N use efficiency (NUE) due to field-to-field variability in soil N supply (Cao *et al.*, 2016). Traditionally, pre-plant nitrogen requirements have been estimated by using soil samples and crop yield levels from previous years. The estimated rate is then applied uniformly to the field (Sawyer, 1994). Many farmers often apply fertilizer N in doses higher than the blanket recommendations to ensure that there is no yield loss because of N deficiency (Purba *et al.*, 2015). Using fertilizer over plant demand may result in surface runoff and pollution of streams. Under-application of nitrogen may diminish crop production and result in low economic returns to the producer (Miao *et al.*, 2011).

Advances in technology have led to development of active remote sensing systems that are now available commercially (Inman *et al.*, 2007). In principle, they can be mounted on a VRT fertilizer system that is used to vary the amount of fertilizer for a given area in 'real-time (Williams, 2006).

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Recently, farmers using precision agriculture tools and technologies such as leaf color chart (LCC), chlorophyll meter (SPAD), GreenSeeker, and real-time on-the-go optical sensing measurements (OPM) based variable rate (VRT) fertilizer application. These tools and technologies can reduce under or over-application of N (Boyer *et al.,* 2011; Bagherpour *et al.,* 2017). Raun *et al.* (2002) analyzed optical sensing and VRT for Oklahoma, USA winter wheat production. They showed that the extra income owning to an increase in NUE (N use efficiency) can cover the expected costs of the technology and that OPM-based VRT would be most profitable in areas of high spatial variability.

Leaf color chart has been used successfully to optimize fertilizer N application in wheat and rice (Ladha *et al.,* 2007). SPAD chlorophyll meter was confirmed to provide a quick, simple, and nondestructive estimation of leaf chlorophyll content (Chapman and Barreto, 1997). The use of SPAD with grain (Ramesh *et al.,* 2002) and vegetables (cabbage and carrot) has recently been tried (Westerveld *et al.,* 2004). Normalized difference vegetation index (NDVI) as a spectral vegetation index is useful for acquiring crop information indirectly, such as productivity potential, photosynthetic efficiency, and potential yield (Raun *et al.,* 2001; Inman *et al.,* 2007, Thind *et al.,* 2017).

Both NIR and red irradiance are strongly influenced by plant cover. NIR irradiance increases and red irradiance decreases with increasing plant cover. Given these relationships, NDVI from vegetated surfaces is heavily influenced by chlorophyll content in the vegetation. A deficiency in nutrients such as nitrogen decreases pigment formation, which subsequently increases red reflectance (Jones *et al.,* 2007).

NDVI is a broadband index that has a good correlation with green biomass and leaf area index (Pen˜uelas *et al.,* 1994). Raun *et al.* (2001) showed that the expected yield determined from NDVI had a strong relation with actual grain yield in winter wheat. Bausch and Diker (2001) investigated the remote sensing techniques to increase the nitrogen use efficiency of corn. Their results showed that the NIR could well predict the plant N at the 9-leaf to 12-leaf crop growth stages. However, the effects of the soil background on reflectance had a negative effect on these relations.

Site-specific N management strategy using GreenSeeker™ optical sensor (GS) was evaluated in dry direct-seeded rice (DDSR) in north-western India. N use efficiency was improved by more than 12% when N fertilizer management was guided by GS as compared to when the general N fertilizer recommendation was followed (Ali *et al.,* 2014).

Sharma *et al.* (2011) observed that high N use efficiency in irrigated wheat grown in Northwest India can be achieved by replacing general fertilizer recommendation with an optical sensor-based N management strategy. Enciso *et al.* (2017) evaluated current commercially available sensor technology for use in a ground-based platform for plant phenol typing and crop management decisions. Results showed that the Normalized Difference Vegetation Index (NDVI) data collected using the GreenSeeker sensors were more consistent and presented less variability when compared to the Decagon SRS sensor.

Currently, most of the methods available for measuring leaf area index are based on manual measurements, which are time-consuming, laborious, and destructive (Fuentes *et al.,* 2014). Aerial and ground-based remote sensors have emerged as an important source of information on vegetative canopy through vegetation indices. The NDVI is related to the quantitative biomass and can be used to monitor vegetative growth and to determine biophysical variables such as leaf area (Junges *et al.,* 2019). Ter-Mikaelian and Parker (2000) estimated the biomass of white spruce seedlings with vertical photo imagery. The accuracy of this technique was comparable to the traditional methods using seedling basal diameter. Lukina *et al.* (1999) evaluated the use of a digital image to estimate vegetation coverage and a multispectral radiometer to measure NDVI index in winter wheat. The results of this study showed a strong correlation between NDVI and vegetation coverage (r^2 = 0.66 to 0.96). In addition, NDVI have a strong correlation with dry biomass (r^2 = 0.52) and with nitrogen content (r^2 = 0.66).

Spinach (*Spinacia oleracea*) is a leafy green flowering plant native to central and western Asia. Its leaves are a common edible vegetable consumed either fresh, or after storage using preservation techniques by canning, freezing, or dehydration. Spinach is rich with vitamins such as vitamin C, vitamin A, vitamin E, minerals like magnesium, manganese, iron, calcium, and folic acid. Spinach is also a good source of chlorophyll, which is known to aid in digestion (FAO, 2020). Along with these advantages, to increase crop yield, it needs high N fertilizer and in the commercial production of this plant, the recovery of N is poor, which may result in environmental contamination. To increase spinach yield and decrease its environmental consequence, there is a need to optimize nitrogen consumption (Navarrete *et al.*, 2016). Therefore, this study aimed to investigate the relationship of nitrogen rate, leaf area index, and biomass with NDVI to find an effective, fast and non-destructive way to estimate leaf N in spinach plants and to test the potential linkage between FVC and biomass with NDVI of GS.

Materials and Methods

Plant material and experimental setup

This study was conducted at the agricultural research station, faculty of agriculture, university of Bu-Ali Sina (35°1’ N, 48°31’ E, 1690 m alt) during the 2019 growing season. This site has a semi-arid and cold climate, an average annual rainfall of 333 mm and an average temperature of 24° C in the warmest month (Hamzei *et al.*, 2012).

Spinach seeds ‘native cultivar of Nahavand’ was planted on March 10th with 20 plants per m^2 density. The experiment was laid out as Randomized Complete Block Design with three replications including four levels of nitrogen (0, 75, 150, and 300 kg ha^{-1}). Each experimental unit contained six lines a distance of 30 cm and a length of 6 m, and the distance between each block was 1 m. Three random soil sample cores were obtained from each plot prior to fieldwork using a 3 cm diameter hand probe to a depth of 0-15 cm for potassium (K), phosphorus (P), pH, and organic matter and 0-30 cm in depth for nitrate. Soil samples were air-dried, ground to pass through a 2 mm screen, and were mixed before analysis for soil pH, available P, K, and organic matter. Soil pH was measured in a 1:1 ratio of soil to deionized H_2O solution (Watson and Brown, 1998), P by the Olsen method (Olsen *et al.*, 1954), K was analyzed using the 1-N ammonium acetate method (Thomas, 1982), soil nitrogen was determined using O'Brain and Flore (1962) method and organic matter was measured using the loss following ignition method (Schulte and Hopkins, 1996). Plots in all experiments were irrigated with flood irrigation. The result of soil analysis was presented in Table 1.

Table 1- The result of soil analysis in the research site

Soil characteristic	Quantity	Unit
Texture	sandy clay loam	-
pH	7.15	-
Organic carbon	2.38	%
Organic matter	4.10	%
Total N	0.20	%
Available P	57.84	mg kg^{-1}
Available K	703.30	mg kg^{-1}

NDVI and SPAD Value Measurements

Canopy reflectance was measured during 20 to 56 days after emergence with a

GreenSeeker hand-held optical sensor unit (Trimble Navigation Ltd., Sunnyvale, California, USA) over 12 plots of field. According to the Instructions for Use (Figure 1), the GreenSeeker was held at 65 cm above the crop canopy using an adjustable shoulder harness. Readings were taken for the defined area of each plot every four days throughout the experimental period. Spectral measurements were collected from each plot by moving the sensor across the center of each plot with an area of 1.8×2 m^2. To analyze the chlorophyll content of leaves, leaf samples were taken from areas located at both sides of each plot with a length of 1.5 m along the length. The SPAD readings were taken as an average of three different leaf readings located in the middle to the upper level of the plant excluding the midrib. In this study, a portable chlorophyll meter SPAD-502 (Minolta Co., LTD. Japan) was used to assess the nitrogen status of spinach leaves at various growth stages.

Fig.1. The Handheld Greemseeker and height of the sensor

Determination of Chlorophylls and total Carotenoids in Leaves

After reading the NDVI of the central section of each plot with the GreenSeeker sensor and imaging with the visual imaging system, one leaf located in the middle to the upper level of the four plants at the inside of the two side sections were hand-harvested. The spinach samples were placed in plastic bags, weighed, labeled, carefully closed, and then refrigerated for later processing in the laboratory. In the laboratory, samples were washed, frozen, and dried. The chlorophyll and carotenoid pigments were extracted in 99% acetone by macerating the leaves with a mortar and pestle. The absorption of the extracts at wavelengths of 470 nm, 645 nm, and 664 nm was measured by spectrophotometer UV/visible (Varian-carry 100) according to the spectrophotometric method of Inskeep and Bloom (1985).

Measuring crop biomass

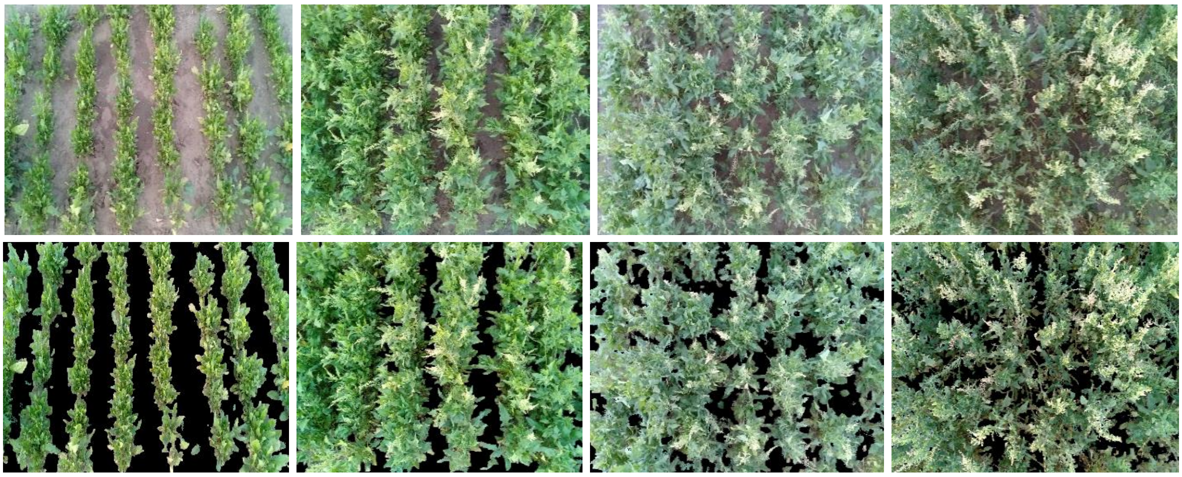
The main objective of this measuring was to estimate final crop biomass by NDVI of GS in the period of growth stages. At each sampling location, spinach biomass was measured by cutting all plants at ground level from within a 1-m^2 quadrat at the end of growth stages. Biomass samples were transported to the laboratory where fresh weight was recorded.

Acquiring visual images

After reading, the NDVI of the sample area using the GreenSeeker sensor, a corresponding visual image was acquired using a Samsung digital camera with a resolution of 8 Mpixels (3264×2248). The imaging system was mounted to a pole on a platform held horizontally 1.5 m above the ground. For each plot, three photographs were taken and the area photographed was approx. 1.8×2 m^2. All the images were taken between at cloudy conditions or on areas shaded with a sheet to eliminate the effect of sunlight on image quality. All images were saved in JPEG-Format. The images were processed by LabView 2014 (National Instruments Corporation, Austin, TX, USA) and MatLab 2016a (MathWorks, Inc., Natick, Massachusetts, US).

Image segmentation

This section aims to separate the soil background from the canopy representing green plant parts. This allowed determining the ground cover of living plant leaves. Several methods have been developed for segmenting crop canopy images. The common segmentation technologies used for this purpose are color index-based segmentation, threshold-based segmentation, and learning-based segmentation (Hamuda *et al.,* 2016). Most researchers have used color to separate soil from a plant (Meyer and Camargo-Neto, 2008; Kirk *et al.,* 2009). In a recent study at the early stage of growing, according to Woebbecke *et al.* (1995), the G-B (green-blue) index was used as a color index-based segmentation to discriminate leaves from soil background. Nevertheless, in the flowering period, the Excess Green (ExG), according to Equation 1, the index showed good results than G-B (Soontranon *et al.*, 2014). Because of unreliable results in the auto threshold, fixed threshold values were used for each series of images, which were taken under similar light conditions. After segmentation of images, the fractional vegetation cover (FVC) was calculated as the ratio of the number of pixels of all vegetation to the total number of pixels in the image (Song *et al.,* 2015). The temporal segmentation results of the crops were shown in Figure 2. In this Figure, it can be observed that the color indices of G-B and ExG perform well in segregating this crop from its background.



a (G-B) b (G-B) c (ExG) d (ExG)

Fig.2. The segmentation results of the crop at a different stage of growth. a and b are early stage, c and d are the late stages of growing

$$\mathrm{ExG} = 2\mathrm{G} - (\mathrm{R} + \mathrm{B}) \quad (1)$$

Results and Discussion

Changing NDVI during growing stages

The change of GS readings in the canopy of spinach was shown in Figure 3. The GS readings for all treatments (N0 to N300) increased until 44 DAG and then decreased. With increasing the length of growth period, spinach leaves with a high concentration of N fertilizer always had higher GS values than those with a low concentration of N fertilizer. For all days of growth period value of treatment, N300 was higher than that of other N treatments. These changes indicated that it is possible to predict the nitrogen level of the canopy for all days of the growth stage. This Figure also shows that for all nitrogen levels, the GS reading peaking at 44 DAG. After 44 days, for all treatment, GS readings were dramatically declined due to the maturation of plants. Changes in SPAD readings in leaves of spinach were shown in Figure 3. Similar to GS readings, the SPAD readings have high variance at the early stage of plant growth, whereas at the late stage of growing, these data have low variance. Data collection overlapped with plants' anthesis, which interfered with the GreenSeeker™ NDVI values and decreased readings in the later phases of growing (Basyouni *et al.*, 2015). Additionally, the flowering progress might have consumed leaf N, which decreased sensor readings at later stages (Lawrie and Wheeler, 1974).

Liu *et al.* (2006) reported similar results for the SPAD reading changes during the growth stage of 25 to 35 days after sowing. However, they did not investigate the SPAD reading changes at the late stage of growth. Junges *et al.* (2019) studied a vineyard in Brazil using the GreenSeeker remote sensor and reported similar results for the GS readings. The index increases rapidly at first (September to November), followed by a relative stabilization (December to February), and decreases in the final stage (March to May).

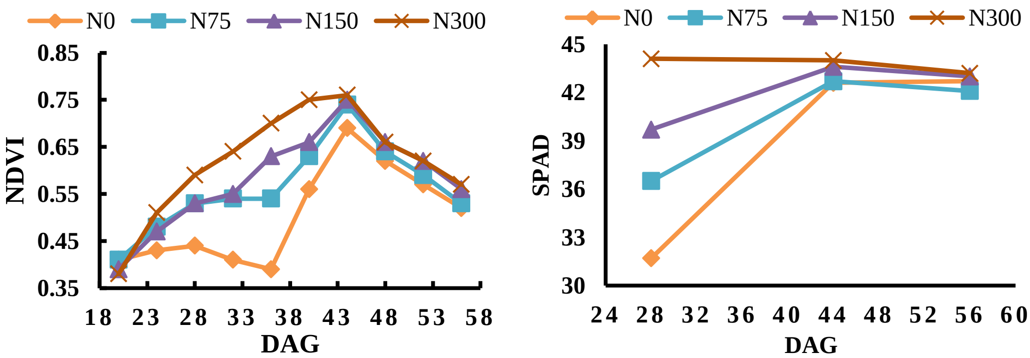


Fig.3. Effects of N levels on GreenSeeker and SPAD values at different days after emergence

Table 2 demonstrates the Pearson's correlation coefficients between GreenSeeker NDVI and the other variables. These results showed that NDVI has a good correlation with chlorophyll yield and biomass with correlation coefficients (R) ranging from 0.61 to 0.91 and from 0.69 to 0.87, respectively. The highest correlation between these variables was obtained at the growth period of 32-40 days. After 44 days, nutrition deficiency in the plant leads to yellowing leaves. These changes in leaves color and preliminary mature of spinach plants can be the main reason for the decreasing of correlation coefficients between NDVI and these variables. This relationship concurs with work done by Raun *et al.* (1998) and Basyouni *et al.* (2016).

As Table 2 shows, NDVI correlations with leaf N and fertilizer rate were not significant ($P < 0.05$) at 20 and 24 DAG. This can be related to the small size of plants at the early stages of growth, which results in background noise interfering with the NDVI readings (Basyouni *et al.*, 2015). As plants grew and filled the plots with time, these correlations were strong. This corresponds with the results of Wang *et al.* (2012), Dunn and Goad (2015), and Ali *et al.* (2020) showed a strong correlation between GreenSeeker NDVI readings and chlorophyll yield in geranium and ornamental cabbage and wheat.

Table 2- Mean correlation coefficient of plots between GS NDVI- chlorophyll yield, NDVI - Biomass, and NDVI - FVC during the growth period

Day After Emergence	NDVI vs. chlorophyll yield	NDVI vs. biomass
20	0.47	0.51
24	0.74**	0.53
28	0.88**	0.73**
32	0.90**	0.76**
36	0.91**	0.85**
40	0.86**	0.87**
44	0.61*	0.69*
48	0.41	0.45
52	0.42	0.46
56	0.42	0.46

*, **, representing correlation coefficient (r), significant at $P \leq 0.05$, $P \leq 0.01$, respectively.

NDVI correlations with biomass were also not significant at the early stage of establishment. In addition, there was no significant correlation between these variables at the last stage of plant growth. The decreasing NDVI values at the end of the cycle were expected due to the senescence and yellowing of the plant leaves. This result was similar to previous literature findings in dianthus (Basyouni *et al.,* 2016) and grape (Junges *et al.,* 2017).

Effects of fertilizer rate on NDVI

Table 3 shows GS readings during the growth followed linear trends as fertilizer rates increased. With increasing N rate, the linear trends of NDVI and leaf N rates were significant. Comparing the mean value of the GreenSeeker™ NDVI indicated that there are significant differences among N rates. However, after 44 DAG there are no significant differences between N treatments. For the early growth stage, the result of this study was similar to the result of Basyouni *et al.* (2016) that investigated the use of non-destructive sensors to assess nitrogen status in potted dianthus production. However, during the late growth stage, these results are in contrast to their finding.

Table 3- GreenSeeker™ NDVI means, and trend analysis at six dates of days after emergence (DAG) and five N fertilizer rates

Total N applied (kg ha^{-1})	DAG						
	20	24	28	36	44	52	56
0	0.415	0.430^{a}	0.441^{a}	0.390^{a}	0.690	0.575	0.525
75	0.415	0.481^{ab}	0.530^{b}	0.540^{b}	0.745	0.591	0.535
150	0.39	0.475^{ab}	0.535^{b}	0.635^{c}	0.752	0.620	0.565
300	0.385	0.515^{b}	0.595^{c}	0.710^{d}	0.761	0.625	0.575
	N.S	L*	L**	L**	N.S	N.S	N.S

*, **, linear (L) response across treatments at $P \leq 0.05$, $P \leq 0.01$, respectively. N.S: Non-Significant difference

Estimate FVC using NDVI of GS

The traditional method of estimating FVC is to harvest vegetation and measure all the one-sided leaf areas directly. In this study, the relation between FVC and GS NDVI was done to investigate the capability of GS to measure FVC in spinach. As Figure 4 shows, at the early stage of the growing period from 28 to 40 DAG, there were significant correlations between GS-NDVI and FVC extracted by the low-cost camera. However, before 28 and after 44 DAG there were no significant correlations between these variables. Figure 5 showed GS NDVI and FVC regression at 28, 40, and 52 DAG. As this figure showed, although the FVC was higher at the late growth stage, because of N deficiency in leaves the GS NDVI and FVC correlation was low. Results indicated that at the early stage of growth, the GS NDVI was a good index for the estimation of canopy leaf area. Whereas at the late period of growing, it is not a good estimator of canopy leaf area. This result was similar to the previous study that investigated the relationship between NDVI and FVC in semiarid grassland (Fan *et al.*, 2009; Tang *et al.*, 2020). Also at the late stage of growth and at the higher ranges of FVC, when the vegetation canopy tends to be closed, NDVI saturates and can no longer be used to detect any differences in FVC (Pontailler *et al.*, 2003). The findings of Lukina *et al.* (1999) and Sembiring *et al.* (1998) on estimating vegetation coverage in wheat using digital images and spectral radiance, respectively, were also supported the results in his work.

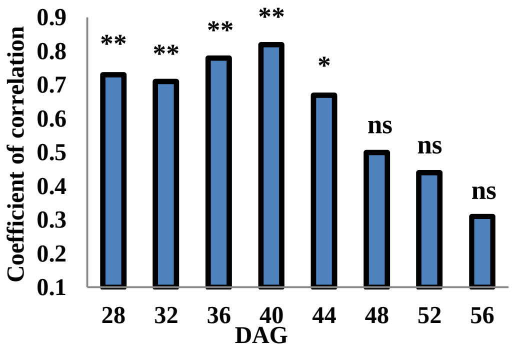


Fig.4. Coefficient of correlations between GS-NDVI and FVC at a different growing stage. (**: significant at P ≤ 0.01, ns: non-significant)

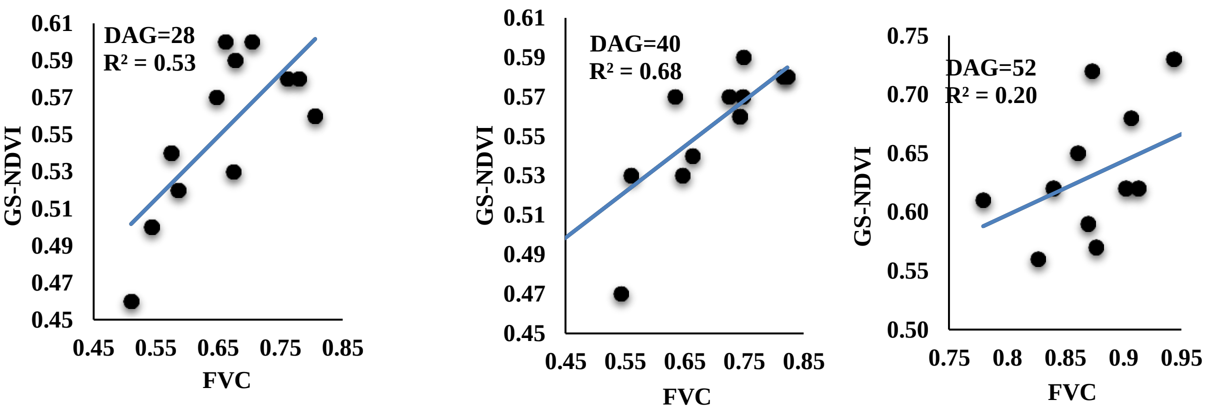


Fig.5. NDVI from sensor vs. FVC measured by the visual camera at three growth stages (22, 40, and 52 DAG)

Conclusion

The NDVI data acquired using the GS sensor and the visual imaging system were sensitive to changes in plant chlorophyll yield and plant biomass in row crop spinach. Correlations between NDVI and biomass were approximately the same as the correlation of NDVI and chlorophyll yield in the period of growth. Each growing stage demonstrated a different response relationship between sensor response and plant characteristics. At the early and late stages of growth, there were no significant correlations among NDVI with biomass, chlorophyll yield, and FVC. The best growth stage for investigating and estimating the chlorophyll yield and biomass using GS was the period of 28 to 40 DAG, in this period the high values of coefficient of correlation were obtained between NDVI of GS with chlorophyll yield and biomass. To estimate the FVC of spinach, the period of 36 to 40 DAG was considered as a recommended period for measuring FVC using GS. Results of this study showed that the GreenSeeker has high reliability and capability for the estimation of chlorophyll yield, biomass, and FVC in the middle of the plant growth period.

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مقاله پژوهشی

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ارزیابی قابلیت حسگر Greenseeker در برآورد وضعیت نیتروژن و تخمین مقدار شاخص FVC گیاه اسفناج

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چکیده

شاخص پوشش گیاهی سبز کسری (FVC) و شاخص نرمال شده تفاضل پوشش گیاهی (NDVI) از شاخص بسیار مهم سبزینگی می‌باشند و ارتباط بسیار قوی با زیست‌توده سبز دارند. هدف اصلی این پژوهش، ارزیابی شاخص NDVI حاصل از حسگر دستی Greesnseeker (GS) در تخمین مقدار زیست‌توده، کلروفیل و شاخص FVC در گیاه اسفناج می‌باشد. در این پژوهش برای جداسازی مناسب زمینه خاک از گیاه از شاخص‌های رنگی G-B و ExG استفاده شد. در طول دوره رشد ۲۸ تا ۴۴ روز بعد از جوانه‌زنی گیاه، نتایج تحقیق نشان داد که NDVI حاصل از GS ارتباط خوبی با کلروفیل داشته (R = 0.61 to 0.91) و ارتباط بین این شاخص با زیست‌توده نیز معنی‌دار بود. علاوه بر این، نتایج نشان داد که در این دوره رشد ارتباط خوبی بین شاخص NDVI حاصل از GS با شاخص FVC وجود دارد (R = 0.67 to 0.82). در حسگر در ارزیابی تاثیر نرخ نیتروژن بر شاخص NDVI، مشخص شد که تنها در دوره کوتاه ۲۸ تا ۳۶ روز پس از جوانه‌زنی ارتباط خطی معنی‌داری بین این دو متغیر وجود دارد. نتایج نشان داد که حسگر Greenseedke توانایی خوبی در تخمین کلروفیل و مقدار زیست‌توده گیاه دارد و از آن می‌توان در میانه رشد گیاه، مقدار شاخص پوشش گیاهی سبز کسری را به‌خوبی برآورد کرد.

واژه‌های کلیدی: پوشش گیاهی، حسگر نوری، قطعه‌بندی، کلروفیل

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