

## Use of Hyperspectral Data for Chlorophyll Estimation Based on Leaf Area Index (LAI) in Wheat

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### Keywords

Wheat  
Hyperspectral data  
Index  
Chlorophyll estimate  
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### ABSTRACT

Spectral Indices are frequently used in the estimation of green parts of plants, usually developed to reduce the spectral effects of external factors such as atmosphere and soil. The aim of this study was to evaluate the ability of different spectral indices to estimate chlorophyll in wheat according to phenological developmental stages and to calculate their optimal band combinations. In this study, chlorophyll-pigment related indices primarily used in chlorophyll estimation, as well as structural and red edge indices were used. Spectral reflectance values obtained for different phenological periods were correlated with SPAD (Minolta-502) values and Partial Least Square (PLS) model was used to calculate the prominent hyperspectral indices and their optimal band combinations. In this study, the responses and sensitivities of different spectral indices for chlorophyll estimation against LAI change in phenological periods were investigated. As a result, the indices that were least and most affected by saturation changes were revealed. Thus, the power of the indices to predict the chlorophyll content of the canopy was demonstrated. In chlorophyll estimation, NDVI (705,750) was the least affected by the saturation change due to the increasing LAI value in the early period and showed a high correlation (LAI= 2.63,  $R^2= 0.554$ ). This was followed by Red Edge (740-720), (LAI= 2.63,1.722), NDVI (550,780), (LAI= 2.63,0.733), SRPI (430,680) (LAI= 2.63,0.661), LCCI (705,750) (LAI= 2.63,0.554), and NPCI (430,680) (LAI= 2.63, 0.203). These indices, which showed high correlation in the early period, were in the range of  $R^2=0.836-0.761$ . In Haymana in the late period between 2013-2014 (26 May,04-12-24 June 2014) LAI values vary between 0.63-3.38 and correlation values are between  $R^2=0.892-0.862$ . MSR (705,750) was the least affected by the saturation change due to the increasing LAI value in the late period and showed high correlation (LAI=1.904, 0.906). This was followed by NDVI<sub>670</sub> (LAI=1.904,0.703), NDVI<sub>550</sub> (LAI=1.904,0.651) and LCCI (LAI=1.904,0.448).

## Buğdayda Yaprak Alanı İndekslerine (LAI) Dayalı Klorofil Tahmini İçin Çok Bantlı (Hiperspektral) Verilerin Kullanımı

### Anahtar Kelimeler:

Buğday  
Çok bantlı veri  
İndeks  
Klorofil tahmini  
Yaprak alan indeksi (LAI)

### ÖZ

Spektral indeksler bitkide yeşil kısmın tahmin edilmesinde sıkça kullanılmaktadır. Bunlar genellikle atmosfer ve toprak gibi harici faktörlerin spektral etkilerini azaltmak için geliştirilmiştir. Bu çalışmanın amacı buğday'da fenolojik gelişim evrelerine göre farklı spektral indekslerin klorofil tahmin edebilme yeteneğini ortaya koymak ve bu indekslerin optimal band kombinasyonlarını hesaplamaktır. Çalışmada öncelikli olarak klorofil tahmininde kullanılan klorofil-pigment ilişkili indeksler ve bunun yanı sıra strüktürel ve kırmızı kenar (Red Edge) indeksler kullanılmıştır. Farklı fenolojik dönemler için elde edilen spektral yansıma değerleri SPAD (Minolta-502) değerleri ile korelasyona tabi tutulmuş, öne çıkan hiperspektral indeksleri ve onların optimal band kombinasyonlarını hesaplamak için ise "En Küçük Kısmi Kareler toplamı" (PLS) modeli kullanılmıştır. Bu çalışmada, farklı Spektral İndekslerin klorofil tahmininde fenolojik dönemlerdeki LAI değişimine karşın, tepkileri ve hassasiyetleri incelenmiştir. Sonuçta saturasyon değişiminden en az ve fazla etkilenen indeksler ortaya çıkarılmıştır. Böylece indekslerin kanopinin klorofil kapsamını tahmin etme gücü ortaya konmuştur. Klorofil tahmininde erken dönemde artan LAI değerine bağlı olarak, saturasyon

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değişiminden en az etkilenen ve yüksek korelasyon gösteren indeks NDVI<sub>(705,750)</sub> 'dir (LAI= 2.63, R<sup>2</sup>= 0.554). Bunu sırasıyla Red Edge<sub>(740-720)</sub>, (LAI= 2.63,1.722), NDVI<sub>(550,780)</sub>, (LAI= 2.63,0.733), SRPI<sub>(430,680)</sub> (LAI= 2.63,0.661), LCCI<sub>(705,750)</sub> (LAI= 2.63, 0.554) ve NPCI<sub>(430,680)</sub> (LAI= 2.63, 0.203) takip etmiştir. Erken dönemde yüksek korelasyon gösteren bu indeksler R<sup>2</sup>=0.836-0.761 korelasyon aralığında yer almıştır. Haymana 2013-2014 yılları arasında geç dönemde (26 Mayıs,04-12-24 Haziran 2014) LAI değerleri 0.63-3.38 arasında değişmekte korelasyon değerleri ise R<sup>2</sup> =0.892-0.862 arasında yer almaktadır. Geç dönemde artan LAI değerine bağlı olarak saturasyon değişiminden en az etkilenen ve yüksek korelasyon gösteren indeks MSR<sub>(705,750)</sub>'dir. (LAI=1.904, 0.906). Bunu sırasıyla NDVI<sub>670</sub> (LAI=1.904,0.703), NDVI<sub>550</sub> (LAI=1.904, 0.651) ve LCCI<sub>(705,750)</sub> (LAI=1.904, 0.448) takip etmiştir.

## 1. INTRODUCTION

The use of remote sensing technology to determine nitrogen levels in plants is not new. Since it is known that leaf nitrogen concentration depends on the amount of chlorophyll in the plant, studies have focused on determining the leaf concentration of the plant (Haboudane et al. 2008). However, the spectral absorption of chlorophyll and its encapsulation by other plant pigments make it difficult to accurately estimate plant nitrogen levels using remote sensing technology (Hatfield et al.). Plant chlorophyll coverage is affected by other stress factors such as water, light, disease and other plant nutrient deficiencies or toxicity (Penuelas and Filella 1998; Chaerle & Straeten 2000; Barraclough and Kyle 2001). Advanced research on plant indices has been obtained with different combinations of narrow bands among themselves. Field-based studies using hyperspectral reflectance values obtained with handheld spectroradiometry have focused on different plants, both on the leaf and canopy (Blackburn, 1998a,b; Thenkabail et al., 2001; White, Trotter, Brown, & Scott, 2000; Yoder & Pettigrew-Crosby, 1995).

Increasing the sensitivity of the vegetation indices to be used in the calculation of chlorophyll and other pigments is possible with the correct selection of sensitive band regions in hyperspectral imaging (Blackburn, 1998b). As a consequence of the different measurement conditions, there is disagreement on the choice of wavelengths to be used in the determination of plant parameters. Hyperspectral reflectance values recorded under natural conditions in productive crops with high input costs have been published, albeit in limited numbers (Broge & Mortensen, 2002; Filella, Serrano, Serra, & Penuelas, 1995; Serrano et al., 2000).

The performance of vegetation indices (VI) used to determine the nitrogen and chlorophyll concentration of plants is related to variables such as the biomass and chlorophyll density of the field and nitrogen uptake Hansen & Schjoerring (2003); Feng et al. (2008); Fava et al. (2009). Therefore, it has become necessary to develop new approaches to determine the nitrogen concentration of the plant, especially in the early stages. Therefore, improved multiple linear regression models using more bands (Miao et al. 2009), partial sum of least squares (PLS) (Hansen & Schjoerring 2003), artificial neural networks (Yi et al., 2007) have been developed.

Red-Edge is important in determining the Chlorophyll content in plants (Clevers et al., 2001; Curran, 1989; Dash & Curran, 2004). Recently, red edge bands on Sentinel-2 have been used in simulation studies to estimate LAI and Chlorophyll (Delegido et al., 2011; Herrmann et al., 2011). Studies have shown that ratio indice (RVI) and normalized differential indice (NDI) using Red Edge bands give very good results in chlorophyll and nitrogen estimation. Gitelson et al., 2003, 2006 developed two different indices called Red Edge (CI<sub>red-edge</sub> = R<sub>800</sub>/R<sub>710</sub> - 1) and Green-Chlorophyll Indice (CI<sub>green</sub> = R<sub>800</sub>/R<sub>550</sub> - 1) for chlorophyll estimation using NIR (800 nm) bands and red edge bands (710 nm). It was found important that these indices provide accurate approaches by eliminating the negative effects of saturation. Studies have shown that plants show the most typical reflection in the near infrared region (400-1100 nm). Therefore, spectroradiometric measurements in plants are concentrated in the near infrared region (Başyigit & Dinç, 2001). The fact that the reflectance characteristics of plants are directly related to leaf chlorophyll content and mineral content has led to the idea that nutrient deficiencies can also be determined by spectral methods.

The decrease in chlorophyll concentrations in the leaf is due to nitrogen deficiency (Penuelas et al. 1994). In case of severe nitrogen deficiency, plants show more reflection in the red reflection region. Increases in reflectance in the red region indicate chlorophyll deficiency caused by nitrogen deficiency, while decreases in reflectance in the near-infrared region indicate decreases in leaf area index and green biomass (Filella et al. 1995). This situation has been determined by many researchers, especially in cereals (Asrar et al. 1984; Jensen et al. 1990).

To summarise the main objective of the research; the amount of nitrogen in plants is directly related to chlorophyll. Today, classical deterministic classical methods are used for the determination of chlorophyll in plants. This is time-consuming and expensive. It has become necessary to utilise remote sensing technology to estimate the chlorophyll in the plant in a short time and accurately by reducing the effects of factors affecting chlorophyll uptake in the plant. For this purpose, improved narrow band spectral indices were used and their linear regression equations were developed.

**2. MATERIALS and METHOD**

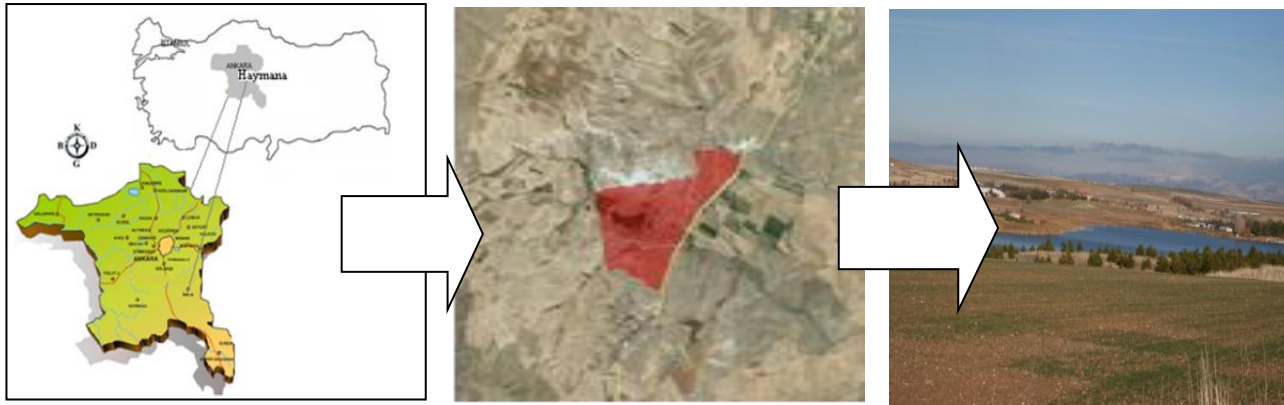
**2.1. Material**

**2.1.1. Study area**

The study were carried out for one year during the 2013-2014 production season at the İközce / Haymana Research-Practice Farm of the Ankara Field Crops Central Research Institute, located in the south-west of Ankara, in an area with a continental climate, dry and hot in summer and cold and rainy in winter (Figure 1). The test area is between 39' 12" -

43' 6" north latitude and 35' 58" -37' 44" east longitude.

The project area is located in the south of Ankara province, within the borders of Haymana district center, at the 22nd km of Haymana-Gölbashi State Highway, with Topaklı village in the northwest and İközce village in the southwest, and covers an area of 968.3 ha. The slope of the land varies between 2-15%. The altitude of the area where the meteorological station in the farm is located is 1070 m. The land is between 1028-1132 meters above sea level. The main crops grown in the region are wheat and barley.



**Figure 1.** Study area

**2.1.2. Climate characteristics of study area**

To evaluate the climate of the study area, according to the 20-year data of İközce station, the average annual temperature of the area is 10.0 °C. The average highest temperature is 18.5 °C in August and the average lowest temperature is -5.2 °C in January. Annual precipitation is 398.7 mm. The wettest month is December with 53.8 mm and the driest month is August with 13.8 mm. Annual precipitation distribution is 133.8 mm in spring (33.6%), 58.2 mm (14.6%) in summer (14.6%), 84

mm (21.1%) in autumn (21.1%), and 122.7 mm (30.8%) in winter (Anonymous, 2005).

Frost is quite severe in Ankara and its region. The average number of frosty days is 85 days. Early and late spring and fall frosts coincide with certain calendar days. These days are likely to be April 20 in the spring and every day in November in the fall. Hail usually falls in the spring, in April and May. It can be large and severe enough to damage fruit and other crops. The long-term average climate data for the experimental area are given in Table 1 (DMİ, 2011).

**Table 1.** Long-year average climate data for the region (1975 – 2010)

Climate data	MONTHS												Mean
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
Average temperature °C	0.3	2.1	6.2	11.3	16.0	20.2	23.5	23.2	18.7	13.0	6.8	2.2	11.9
Max. temperature, °C	4.3	6.7	11.9	17.2	22.2	26.6	30.2	30.2	26.0	19.6	12.3	6.1	17.7
Min. temperature, °C	-3.1	-2.0	1.1	5.7	9.7	13.1	16.1	15.2	11.9	7.5	2.3	-0.9	6.8
Precipitation, mm	39.2	33.6	36.1	50.0	49.7	35.1	16.0	12.4	18.9	32.5	36.0	42.6	33.5
Average sunshine duration, h	11.1	10.4	10.6	12.1	12.3	9.3	4.1	3.2	4.2	7.5	8.9	11.0	8.7
Relative humidity, mm	72	71	60	58	58	50	37	35	41	57	70	79	57.3
Evaporation, mm	-	-	-	103.0	146.6	200.0	254.2	244.0	167	95.2	44.3	-	104.5
Wind speed, m s <sup>-1</sup>	1.2	1.3	1.4	1.2	1.3	1.2	1.2	1.2	1.1	1.2	1.3	1.3	1.2

**2.1.3. Soil characteristics of experiment area**

The soil of İközce farm lands belongs to the brown soil group. Soil analysis was carried out by Ankara Soil and Water Resources Central Research Institute. According to the results; the soil texture of the test area has a clayey-loamy structure and the

average water holding capacity was found to be 62.50%. EC (Ds m<sup>-1</sup>) value was calculated as 0.610, Total Salinity 0.025%, Ph 7.57 (neutral values), Lime % was calculated as 37.81 (high lime). Organic matter content was low (average 1.22%) and organic carbon content was low (average 0.71%).

## 2.2. Methods

Field studies were carried out in an area representing dry conditions in the experimental field located in the Haymana enterprise of the Central Research Institute of Field Crops, which was left fallow in the previous year. The experiment was carried out on 15 plots by applying 5 different fertilizer doses (0-4-8-12-16 kg/da.) on the data obtained from the sampling points on the plots formed according to the random blocks experimental design with 3 replications in rainfall-dependent irrigation under dry conditions. One control plot without fertilizer was left in each replicate.

The experiment was started by obtaining the trial results obtained between 2013 and 2014 for the first year. The experimental area consists of a total of 15 plots with 3 replications. The width of each parcel is planned as 6 m. and the length as 30 m. The area of each parcel is 180 m<sup>2</sup>. DAP was calculated as 12 kg./da (378 g/31.5 m<sup>2</sup>) per decare as base fertilizer and applied to the plots. In the spring, 33% ammonium nitrate fertilizer was used as top fertilizer, and additional application was made by calculating the amount to be given to the plot based on pure matter. IKIZCE wheat seed was applied at 20 kg/da (378 gr/ 31,5 m<sup>2</sup>). Before planting, soil samples were taken from the experimental area and nutrient and physical analysis were performed.

The experimental plots were arranged in three replications as 6 m.\*30 m. = 180 m<sup>2</sup> and 20 kg (0.630 gr/31.5 m<sup>2</sup>) of IKIZCE wheat per decare was sown with a 24-row pneumatic seeder with a row spacing of 13 cm. Half of the 10 m. plot length determined for each plot was reserved for agronomic sampling (% cover, LAI calculation, wet-dry biomass per m<sup>2</sup>, etc.) to be taken at different growth stages. The remaining half (5 m) was allocated for the calculation of grain yield.

### 2.2.1. Collection and evaluation of hyperspectral data

Spectroradiometric canopy reflectance measurements were made using a portable handheld spectroradiometer between 11 am and 3 pm, when the sun's rays were perpendicular to the earth's surface and there was no cloud cover. With the help of the spectral sensor, the spectral reflections in the plant leaves were performed every 3 nm in the band range from 331 nm to 1141 nm. Measurements were taken from a height of 70 cm. at an angle of 250' to the earth's surface. Before each reading, the spectroradiometer was first calibrated and white and black calibrations were performed.

Before spectral reflectance measurements, the spectroradiometer was calibrated with a standard white board (Ba<sub>2</sub>SO<sub>4</sub>). This ensured that all reflection values obtained were minimized by the negative effects of noise and atmosphere.

Spectral reflectance measurements of vegetation were taken from Haymana-İkizce experimental area, three measurements were taken from each plot (from the beginning, middle and end), 45 measurements were taken from 15 plots from three replicates, and 3 measurements were taken from the breeding plot and 48 measurements were obtained in total. Measurements were taken at 8 different phenological stages. These measurements were then averaged and used in the calculation. Spectral measurements were taken at different phenological developmental stages of the plants (Zadoks-Feekes) such as tillering, stalk emergence (Z-35), flowering (Z-54), spike (Z-74), grain filling (Z-84) and grain tying (Z-92) (Table 2).

Before the readings, at least three readings were taken from each plot (at the beginning, middle and end of the plot) with the device, which went through signal testing, automatic integration time adjustment and white and black calibration stages, and the collected reflectance values were stored in the device as raw data with .dvp extension. This raw data was then imported into the computer as .csv so that it could be opened and processed in Excel. A special software was used for this. These raw data collected were subjected to an internal organization and the reflectance values obtained from the plots were averaged and made ready to be processed for use in vegetation indice formulas.

**Table 2.** Different growth stages and classification scales sampled during the 2013-2014 vegetation period in Haymana

Phenological Period	Sampling Dates	Period	Zadoks Skalası	Feekes
Early Period	March 19, 2014	Emergence Period	10-19	1
	March 26, 2014	Tillering Period	21-25	2
	April 03, 2014	End of Tillering Period	26-29	3
	April 22, 2014	Beginning of the bolting	30	4-5
	May 13, 2014	Bolting Period	31-39	6-7-8-9
Late Period	Mayıs 22, 2014 - Mayıs 26, 2014	New formation of the flag leaf ,Spiking,,Flowering	40-69	10
	June 04, 2014	Beginning of the Milk Formation Period	70-77	11
	June 12, 2014	Dough Formation Period and hardening of the grain	80-87	11.2
	June 24, 2014	Hardening and Maturation of the Grain , Start of Ripening	91-92	11.3-11.4
	July 22, 2014	Harvest	99	11.4

### 2.2.2. Measurement of nitrogen content by chlorofilmeter (SPAD 502, Minolta)

Fischer (2001) reported that the chlorophyll content of leaves reflects their photosynthetic capacity and Yadava (1986) reported that there was a linear relationship between SPAD values and the amount of chlorophyll contained in the leaves at the time of reading. This system is based on the indirect determination of chlorophyll content and nitrogen content by measuring the green color of the leaves.

Chlorofilmeter (SPAD 502, Minolta, Spectrum Technologies Inc.) was used to determine the chlorophyll content. The SPAD meter emits light at two different wavelengths (650 nm and 950 nm) and is calibrated basis on the uptake of these rays by chlorophyll (Maas and Dunlap 1989, Minolta, 1989).

SPAD has been developed to measure the amount of chlorophyll in the leaves of the plant, so that it is possible to determine the amount of additional fertilizer required. The amount of chlorophyll is an indicator of the amount of nitrogen. An increased SPAD value is an indication of a healthy plant. SPAD measures the relative amount of chlorophyll a plant has using two wavelengths. Chlorophyll readings in the plant reach peak values in the blue region (400-500 nm) and in the red region (600-700 nm).

The basis of the method is to determine the relationship between leaf chlorophyll levels and nitrogen nutrition status of the plant. In this method, the chlorophyll readings obtained by SPAD meter are converted into NSPAD values by normalising the SPAD values due to the changes in environmental factors on the genotypes. Drought or disease or lack of any nutrients in farmers' fields can cause chlorophyll depletion and discoloration. Therefore, in nitrogen fertilizer trials for calibration purposes, the SPAD value should be converted to NSPAD value. In any fertilizer trial, the percentage value obtained by dividing the SPAD reading of the same genotype in the plots fertilized at different amounts within the

trial by the highest SPAD value (chlorophyll reading) reading of the plant obtained by using the highest fertiliser dose in the same plot is called NSPAD value. Calibration equations are obtained from these values. In farmers' practices, control strips with the nitrogen level corresponding to the highest yield level are formed in practice, and the values read from these strips are subjected to normalisation process and the necessary supplementary nitrogen requirement for the remaining parts of the field is determined.

### 2.3. Testing the Performance of Vegetation Indices for Chlorophyll Estimation

PLS (Partial Least Sum of Squares) Regression Model was used to reveal the important wavelengths that stand out in chlorophyll estimation. To determine the chlorophyll concentration in the plant according to different developmental stages (Early-Late-Full Year), the correlation values between the chlorophyll value calculated by using the vegetation indices from the plots in the experiment and the measured chlorophyll (SPAD) concentration were examined. In the experiment, a total of 18 different Vegetation Indices were calculated in different band combinations to be used in chlorophyll estimation in wheat (Table 3). For each indice, the accuracy and performance of the model were tested by calculating the  $r^2$ , Sum of Squares of Error (RMSE) values and Relative Error % (% RE) obtained from the correlation model with leaf chlorophyll (SPAD) concentration.

**Table 3.** Vegetation indices used in chlorophyll estimation

Indice	Formula	Literature
NDVI <sub>670</sub>		Original formula
NDVI <sub>550</sub>		Original formula
NDVI <sub>(705,750)</sub>	$NDVI = (R_{750} - R_{705}) / (R_{750} + R_{705})$	Original formula
MSR <sub>705,750</sub>	$MSR(705,750) = [(R_{750}/R_{705}) - 1] / [(\sqrt{R_{750}/R_{705}} + 1)]$	Original formula
MCARI <sub>(705,750)</sub>	$MCARI(705,750) = (R_{750} - R_{705}) - 0.2(R_{750} - R_{550})(R_{750}/R_{705})$	Original formula
TCARI/OSAVI	$3[(R_{700} - R_{670}) - 0.2(R_{700} - R_{550})(R_{700}/R_{670})] / [(1 + 0.16)(R_{750} - R_{705}) / (R_{750} + R_{705} + 0.16)]$	Haboudane et al. (2002)
OSAVI (Optimized SAVI)	$OSAVI = (1 + 0.16)(R_{750} - R_{705}) / (R_{750} + R_{705} + 0.16)$	Waldner et al. (2015)
TCARI	$TCARI = 3[(R_{700} - R_{670}) - 0.2(R_{700} - R_{550})(R_{700}/R_{670})]$	Haboudane et al. (2002)
MCARI/OSAVI	$[(R_{750} - R_{705}) - 0.2(R_{750} - R_{550})(R_{750}/R_{705})] / [(1 + 0.16)(R_{750} - R_{705}) / (R_{750} + R_{705} + 0.16)]$	Original formula
GREEN INDEKS (G)(Green Indice)	$GI = R_{554} / R_{677}$	Smith et al. (1995)
SRPI (Simple Ratio Pigment Indice)	$SRPI = R_{430} / R_{680}$	Penuelas et al.(1994)
RVI (Ratio Vegetation Indice)	$RVI = (R_{790} : R_{810}) / (R_{640} : R_{660})$	Jacobsen et al. (1998)
RDVI (Renormalized Difference Vegetation Indice)	$RDVI = (R_{800} - R_{670}) / (R_{800} + R_{670})^{0.5}$	Roujean and Breon (1995)
SIPI (Structural insensitive Pigment indice)	$SIPI = ((R_{800} - R_{445}) / (R_{800} + R_{680}))$	Penuelas et al. (1995a)
NPCI ((Normalized Pigment Chlorofil Indice)	$NPCI = (R_{680} - R_{430}) / (R_{680} + R_{430})$	Penuelas et al. (1995b)
LCCI (Leaf and Canopy Chlorophyll Indice)	$LCCI = (R_{750} - R_{705}) / (R_{750} + R_{705})$	Gitelson ve Merzlyak 1994b
NVI (New Vegetation Indice)	$NVI = (R_{777} - R_{747}) / (R_{673})$	Gupta, et. al. 2001
ARI (Antocyanin Reflectance Indice)	$ARI = (1/R_{550}) - (1/R_{700})$	Zarco-Tejada et al. 2005
Red Edge (750-700)	$R_{750} - R_{700}$	Gitelson & Merzlyak (1996)
Red Edge (740-720)	$R_{750} - R_{700}$	Gitelson & Merzlyak (1996)

**2.4. Statistical Analyses And Regression Model Establishment İn Chlorophyll Prediction**

Reflectance readings obtained from the test area every 3 nm in the band range 331-1141 nm. were used to calculate the spectral indices formulated according to single and dual band combination. As a result, the indices obtained from replicates I, II and III from the experimental area, especially the indices used in the literature and used in chlorophyll estimation, were used in the regression model to be developed to calculate the chlorophyll (SPAD) concentration in the plant.

In addition, different vegetation indices developed to reveal the chlorophyll tracking of wheat at different growth stages were used. In this respect, the developmental stages of wheat are analysed in two parts. Firstly, the period of 1-9 weeks (Feeks 1-9) when the plant did not completely cover the soil surface was considered as the early period, and the second period of 40-11 weeks (Feeks 10-11.2) when the canopy completely covered the soil was considered as the late period.

The results were calculated separately for each period, as well as for the whole year. Correlation and regression analyses were performed using SPSS-16.0 software. To evaluate the performance of the model, the correlation (R<sup>2</sup>) differences between the

indices and chlorophyll concentration were compared. The sum of squared errors (RMSE) and relative error percentage (RE %) were calculated. It was concluded that vegetation indices with higher R<sup>2</sup> values and lower RMSE and RE % values were effective in calculating plant chlorophyll concentration. The sum of squared errors (RMSE) (1) and the relative error percentage (RE %) (2) are calculated by the following equation.

*The sum of squared errors (RMSE)*

$$\sqrt{\frac{\sum_{i=1}^n (y_{obs,i} - y_{model,i})^2}{n}} \tag{1}$$

*The relative error percentage RE (%)*

$$\frac{\text{The sum of squared errors (RMSE)}}{y_{mean,i}} * 100 \tag{2}$$

In Formula ;  $y_{obs,i}$  = Observed Value (SPAD)

$y_{model,i}$  = Value estimated from equation

$y_{mean,i}$  = Average Chlorophyll Value

n = Number of Samples Used in the Experiment

3. RESULTS

3.1. Plant indice-chlorophyll relationships according to phenological periods

In the experiment conducted in Haymana location in 2013-2014, the effectiveness of different indices in determining the amount of chlorophyll of the plant was investigated. When the phenological periods were analyzed in terms of months, the highest correlation values were found at the end of the milky stage and the period when the grain started to harden (June 12, 2014-ZADOKS 80-87)

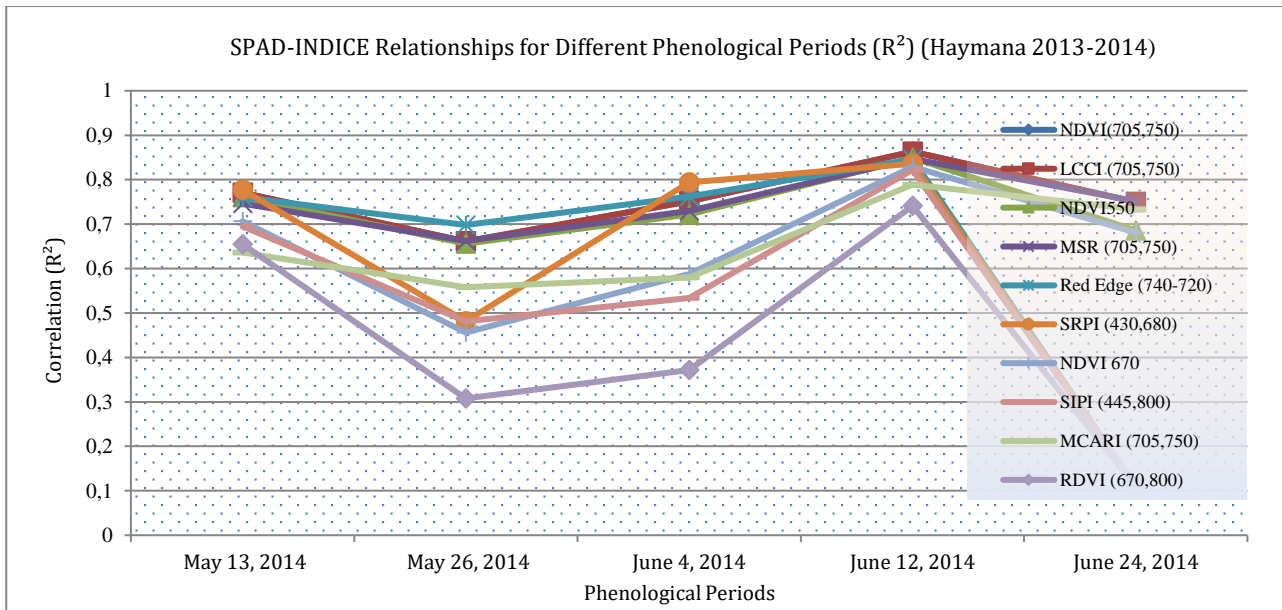
( $R^2= 0.849-0.449$ ). As shown in Table 4, the highest correlation values were found in Leaf Canopy Chlorophyll Index (LCCI) and NDVI ( $705-750$ ), which are structural indices, at N16 nitrogen dose during the hardening period of the grain ( $R^2= 0.863^{**}$ ). This was followed by Ratio Vegetation Indice (RVI) at flowering (May 26, 2014, ZADOKS 40-69) ( $R^2= 0.859^{**}$ ). Modified Simple Ratio MSR( $705-750$ ), Red Edge ( $740-720$ ) and Simple Ratio Pigment Indice (SRPI) of structural indices and chlorophyll pigment indices showed high correlation values ( $R^2= 0.847, 0.846, 0.836$ ) at flowering (June 12, 2014) (Table 4 (Figure 2)).

**Table 4.** Prominent indices according to phenological periods (SPAD-vegetation indice relationships) (Haymana 2013-2014)

Index	Phenological Periods				
	May 13, 2014	May 26, 2014	June 04, 2014	June 12, 2014	June 24, 2014
NDVI <sub>670</sub>	0,707**	0,456	0,588*	0,829**	0,681**
NDVI <sub>I550</sub>	0,761**	0,656**	0,722**	0,849**	0,686**
NDVI <sub>(705,750)</sub>	0,771**	0,661**	0,751**	0,863**	0,750**
MSR 705,750	0,746**	0,662**	0,730**	0,847**	0,751**
MCARI(705,750)	0,636*	0,558**	0,580	0,789**	0,733**
TCARI/OSAVI	-0,743**	-0,742**	-0,787**	-0,837	0,552*
MCARI/OSAVI	0,607*	0,465	0,467	0,735**	0,699**
GREEN INDEKS (G)(Green Indice)	0,378	-0,523*	-0,329	0,449	0,109
SRPI (Simple Ratio Pigment Indice)	0,777**	0,481	0,794**	0,836**	0,109
RVI (Ratio Vegetation Indice)	0,690**	0,859**	0,770**	0,673**	-0,109
RDVI (Renormalized Difference Vegetation Indice)	0,655**	0,308	0,372	0,741**	0,109
SIPi(Structural insensitive Pigment indice)	0,695**	0,481	0,534*	0,822**	0,109
NPCI ((Normalized Pigment Chlorofil Indice)	-0,784**	-0,478	-0,797**	-0,839**	-0,109
LCCI (Leaf and Canopy Chlorophyll Indice)	0,771**	0,661**	0,751**	0,863**	0,750**
NVI (New Vegetation Indice)	0,641*	0,615*	0,613*	0,755**	0,109
ARI (Antocyanin Reflectance Indice)	0,729**	0,779**	0,660**	0,738**	-0,109
Red Edge (750-700)	0,636*	0,355	0,368	0,738**	0,109
Red Edge (740-720)	0,761**	0,698**	0,762**	0,846**	0,108

\* Correlation is at 0.05 significance level.

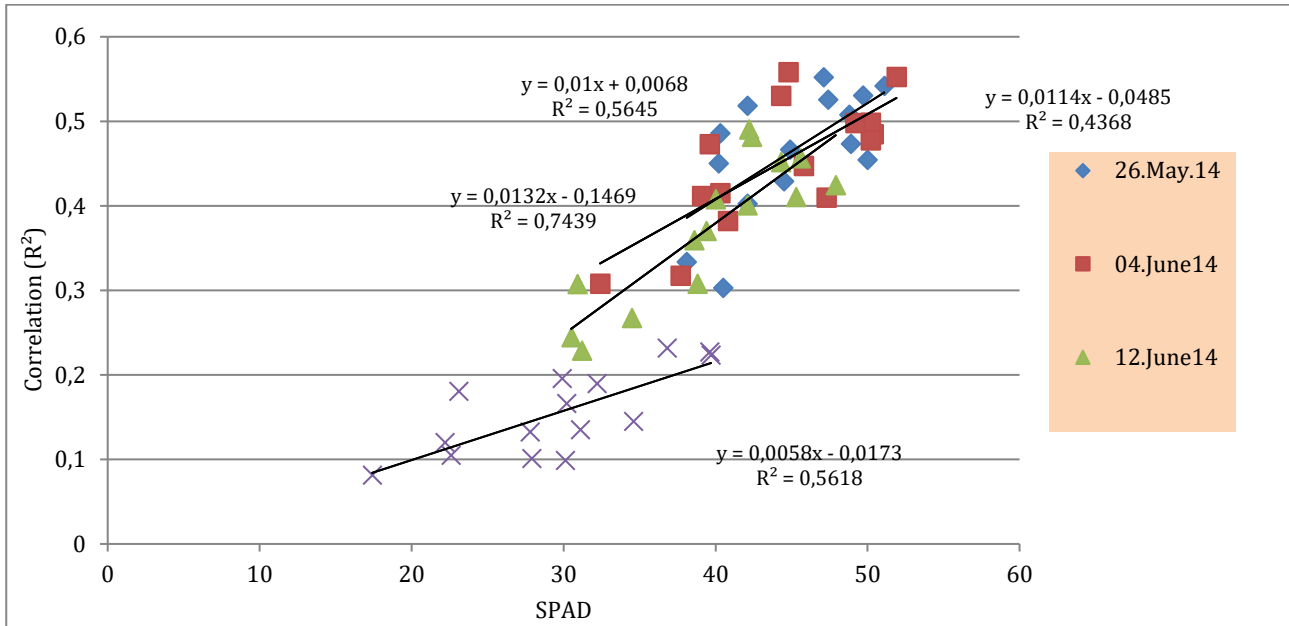
\*\* Correlation is at 0.01 significance level



**Figure 2.** SPAD-indice relationships for different phenological periods (Haymana 2013-2014)

When evaluated according to different phenological periods, the highest correlation values ( $R^2=0.744$ ) were found at the end of the milky stage

and the period when the grain started to harden (12 June 2014-ZADOKS 80-87) (Figure 3).



**Figure 3.** Phenological periods effective in chlorophyll estimation (Haymana 2013-2014)

**3.2. Validation of the Model**

A cross-validation study was carried out between the predicted chlorophyll values and the observed values to evaluate the performance between the indice values and SPAD values according to different growth periods. As a result, high-performing indices are ranked from high to low

according to their correlation ( $R^2$ ) values and from low to high according to their root mean square error of the sum of squares (RMSE) values. The prominent indices in chlorophyll estimation were revealed in the early period (May 13, 2014) (Table 5) and in the late period (May 26, June 04-12-24, 2014) (Table 6).

**Table 5.** Prominent indices for chlorophyll estimation in the early period (May 13, 2014) (Cross-validation Results) (Haymana 2013-2014)

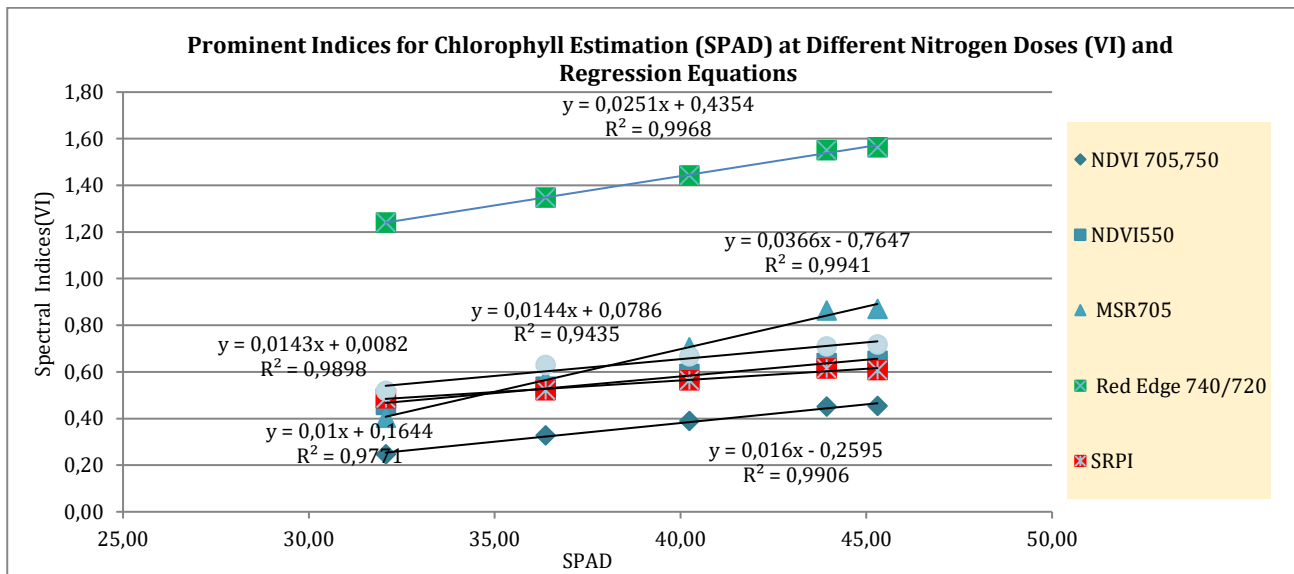
Indice	R <sup>2</sup>	RMSE	% RE
NPCI (680,430)	0,615	1,337	3,068
SRPI (680,430)	0,604	1,354	3,108
LCCI (705,750)	0,594	1,37	3,145
NDVI (705,750)	0,594	1,37	3,145
Red Edge (740/720)	0,579	1,395	3,202
NDVI <sub>550</sub>	0,579	1,397	3,207
MSR	0,557	1,433	3,288
ARI (700,550)	0,531	1,472	3,379
NDVI (670,800)	0,499	1,521	3,491
SIPI	0,483	1,548	3,553
RVI	0,476	1,557	3,574
RDVI	0,429	1,626	3,733
NVI	0,411	1,652	3,791
MCARI	0,404	1,661	3,811
Red Edge <sub>750-700</sub>	0,404	1,661	3,813
MCARI/OSAVI	0,368	1,711	3,927
Green Indice	0,163	1,992	4,572
TCARI/OSAVI	-0,552	1,44	3,306



**Table 6.** Indices that stand out in chlorophyll estimation in the late period (26 May, 04-12-24 June 2014) (Cross-validation Results (Haymana 2013-2014))

Indice	(R <sup>2</sup> )	RMSE	% RE
LCCI	0,796	4,542	7,57
NDVI (705,750)	0,796	5,344	8,906
NDVI <sub>550</sub>	0,781	4,687	7,811
MSR	0,774	5,531	9,218
NDVI <sub>670</sub>	0,743	5,778	9,63
MCARI/OSAVI	0,674	6,168	10,281
MCARI	0,674	6,268	10,447
NVI	0,504	7,623	12,705
RDVI	0,496	7,643	12,739
Red Edge <sub>740/720</sub>	0,494	7,139	11,899
Red Edge <sub>750-700</sub>	0,494	7,596	12,66
SIPI	0,460	8,073	13,455
SRPI	0,412	7,697	12,828
RVI	0,291	7,669	12,782
Green Indice	0,182	9,155	15,259
ARI	0,042	10,331	17,218
TCARI/OSAVI	-0,045	10,05	16,75
NPCI	-0,416	8,203	13,672

The prominent indices (VI) and regression equations for SPAD (Chlorophyll) estimation at different nitrogen doses are shown in Figure 4.



**Figure 4.** Prominent Indexes (VI) and regression equations for chlorophyll estimation (SPAD) at different nitrogen doses

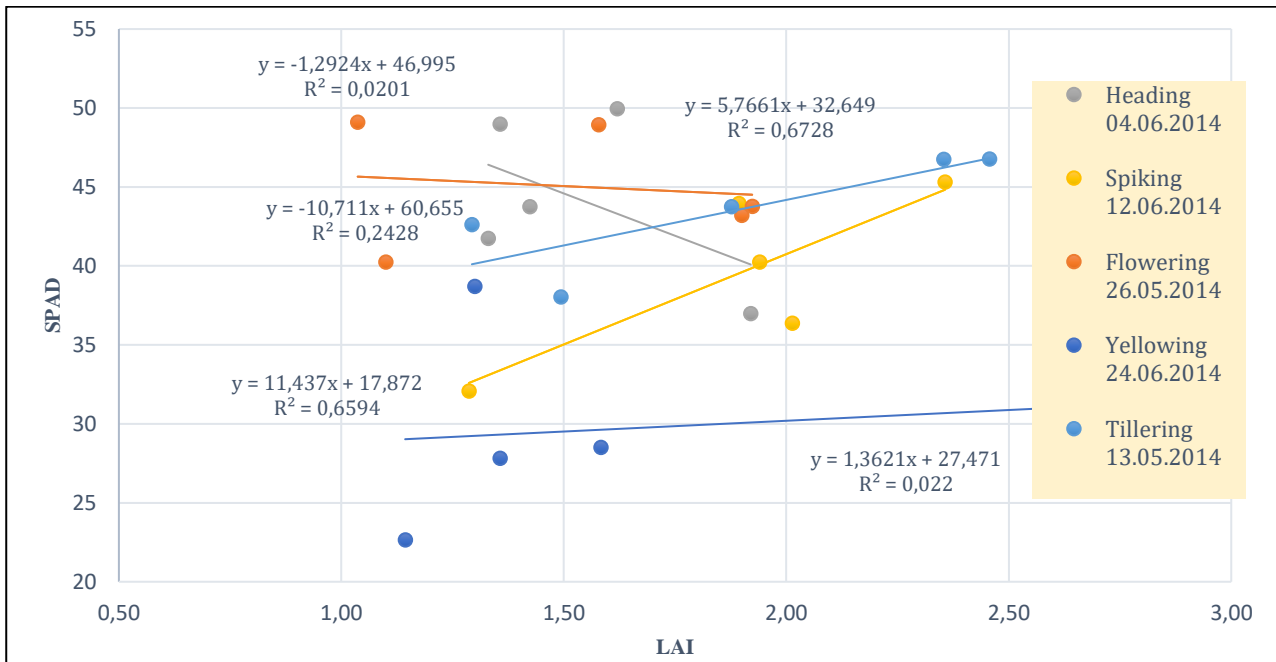
**3.3. Chlorophyll (SPAD) Leaf Area Index (LAI) and Spectral Index Relationships for Different Phenological Periods: (Haymana 2013-2014)**

The relationships between SPAD-LAI were examined according to the replicate averages in different phenological development periods (Table 7), and the highest coefficient correlation value (R<sup>2</sup>=0.673) was reached especially in the estimation

of chlorophyll at early emergence (13 May 2014). Due to the increasing saturation effect of LAI, the correlation showed a decreasing trend during the flowering period (R<sup>2</sup>=0.020) and a slight increase during the grain setting period (04 June 2014) (R<sup>2</sup>=0.243). It increased again (R<sup>2</sup>=0.659) at spike (12 June 2014) and decreased again (R<sup>2</sup>=0.022) at yellowing (Figure 5).

**Table 7.** SPAD-LAI Relationships obtained according to recurrence averages in different phenological periods (Haymana-İkizce, 2013-2014)

Recurrence Average	13.05.2014 (Bolting)		26.05.2014 (Flowering)		04.06.2014 (Heading)		12.06.2014 (Spiking)		24.06.2014 (Ripening)	
	LAI	SPAD	LAI	SPAD	LAI	SPAD	LAI	SPAD	LAI	SPAD
<b>N<sub>0</sub></b>	1,49	38,03	1,100	40,23	1,92	36,97	1,29	32,07	1,14	22,63
<b>N<sub>4</sub></b>	1,29	42,60	1,900	43,20	1,33	41,73	2,01	36,37	1,36	27,80
<b>N<sub>8</sub></b>	1,88	43,73	1,923	43,77	1,42	43,73	1,94	40,24	1,58	28,50
<b>N<sub>12</sub></b>	2,35	46,73	1,578	48,93	1,36	48,97	1,89	43,94	2,73	30,77
<b>N<sub>16</sub></b>	2,46	46,77	1,037	49,10	1,62	49,93	2,36	45,31	1,30	38,70



**Figure 5 .** According to different phenological periods SPAD- LAI relationships in wheat (Ikizce) (Haymana 2013-2014)

Many vegetation indices are strongly influenced by the unfavourable reflection properties of the soil at low LAI. As LAI increases, the predictive power of the spectral indices increases as the saturation effect decreases. In this study, the responses and sensitivities of different spectral indices for chlorophyll estimation against LAI change in phenological periods were investigated. As a result, the indices that are least and most affected by saturation changes were revealed. Thus, the power of plant indices to predict the chlorophyll content of canopy was demonstrated. Leaf Area Index values

obtained from the experimental area in Haymana'da between 2013-2014 varied between 1.08-2.81 in the early period (May 13, 2014). In chlorophyll estimation,  $NDVI_{(705,750)}$  is the indice that is least affected by saturation change due to increasing LAI value in the early period and shows high correlation ( $LAI= 2.63$ ,  $R^2= 0.554$ ). This was followed by Red Edge  $(740-720)$ , ( $LAI= 2.63,1.722$ ),  $NDVI_{(550,780)}$ , ( $LAI= 2.63$ ,  $0.733$ ),  $SRPI_{(430,680)}$  ( $LAI= 2.63$ ,  $0.661$ ),  $LCCI_{(705,750)}$  ( $LAI= 2.63$ ,  $0.554$ ) and  $NPCI_{(430,680)}$  ( $LAI= 2.63$ ,  $0.203$ ). In this early period, highly correlated indices were in the range  $R^2=0.836-0.761$  (Figure 6).

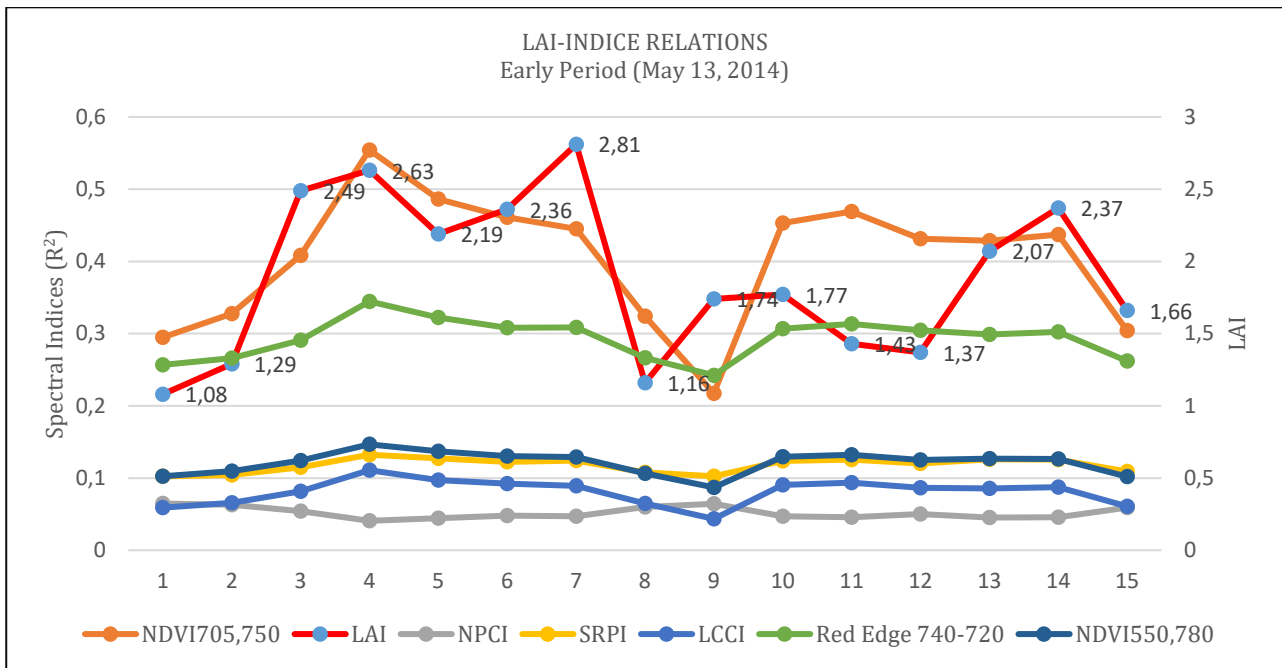


Figure 8. LAI-Spectral indices relations in late period (May 26, June 04-12-24 2014)

When the relationship between SPAD-Index values was examined in the late period (May 26, 04-12-24

June 2014), it was observed that LCCI was the indice most affected by SPAD readings (Figure 9).

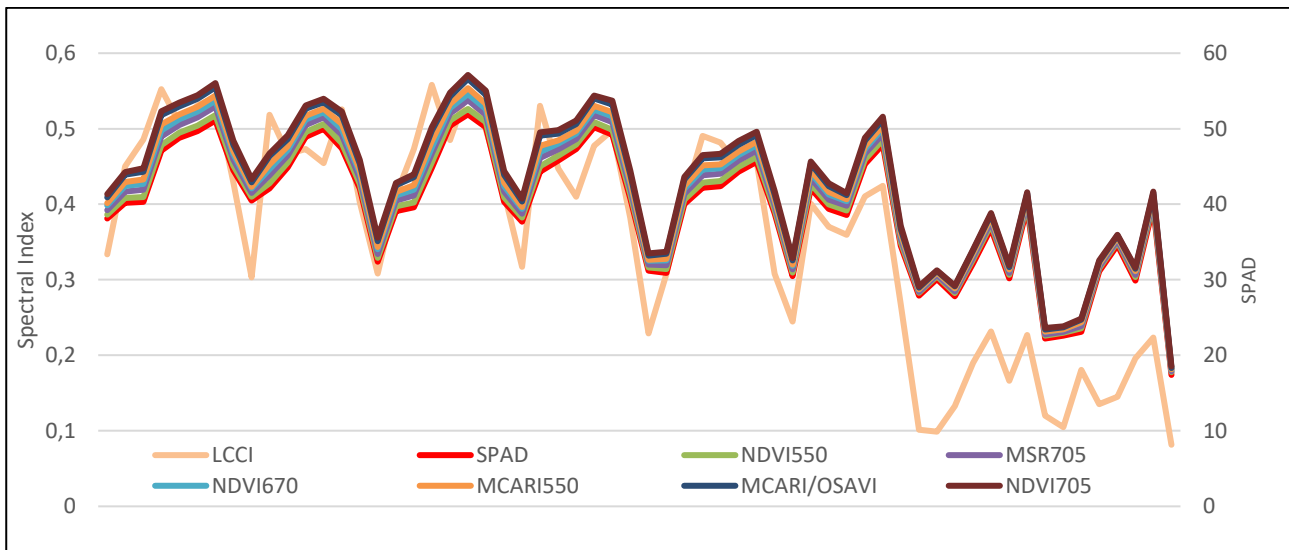


Figure 9. SPAD-Spectral Indices relations in late period (May 26, 04-12-24 June 2014)

#### 4. DISCUSSION and CONCLUSION

##### 4.1. Discussion

In recent years, advances in imaging spectrometry have led to new vegetation indices sensitive to chlorophyll concentration, minimizing spectral confusion caused by soil background and atmosphere, resulting in more accurate approaches ((Collins (1978), Baret, Jacquemoud, Guyot, & Leprieur (1992), Demetriades-Shah, Steven, & Clark (1990), Filella & Pen˘uelas (1994), and Mauser & Bach (1994)).

In the early stages of growth, the change in plant biomass and leaf area index is greater, and their effects on reflectance may obscure the effects of

chlorophyll and nitrogen (Haboudane et al. 2002). In the late stages of growth, the effects of these structural changes are less visible as the canopy of the plant completely covers the soil. Fischer (2001) reported that the chlorophyll content of leaves reflects their photosynthetic capacity and Yadava (1986), reported a linear relationship between SPAD values and the amount of chlorophyll contained in the leaves at the time of reading. This system is based on the indirect determination of chlorophyll content and nitrogen content by measuring the green color of the leaves. Martinez and Guiamet (2004), in a study on wheat and maize, reported that SPAD value increased as the proportional water content of leaves decreased. Spectral vegetation indices (VIs), calculated as combinations of near infrared (NIR)

and red reflectance, correlated well with canopy parameters related to biomass and chlorophyll, where photosynthetic activity (APAR) is absorbed and green leaf area index (GLAI) is rich (Elvidge & Chen, 1995; Myneni & Williams, 1994).

In our study, it was observed that the least affected and highly correlated indices were observed in the early period due to the increasing saturation change in the leaves. As a result, it was observed that the spectral indices calculated in this period were in parallel with the results of previous studies and reached high correlation values. With remote sensing techniques, it has gained great importance to keep up with the rapid changes in the climatic calendar in plant development quickly and quickly and to realise the right applications on time. It has become inevitable that inputs should be used more economically and optimally, and should be applied quickly to the field at the required time and in the required amount. In addition, great contributions will be made to the sensitive agricultural practices that have been carried out in this field recently. With this study, it will be possible to determine the amount of nitrogen and chlorophyll in the plant at an early stage and to plan fertilizer application and recommendation programmes accordingly at an early stage.

#### 4.2. Conclusion

Thanks to this study, the chlorophyll estimation power of the predefined narrow and broadband vegetation indices has been revealed, and by combining the available wavelengths, indices with high estimation power have been obtained. Using all bands of the spectroradiometer, vegetation indices with high predictive power of chlorophyll by phenological periods were derived using least partial squares regression (PLS). At Haymana location, the highest correlations between chlorophyll readings (SPAD) and spectral indices were determined at the end of the milking period and the period when the grain started to harden (June 12, 2014-Zadox 80-87) ( $R^2= 0,849-0,449$ ). The highest correlation values were found in LCCI and  $NDVI_{(705,750)}$  at dose  $N_{16}$  when the grain started to harden ( $R^2= 0,863^{**}$ ). Moreover, RVI indice ( $R^2= 0.859^{**}$ ), structural indice  $MSR_{(705,750)}$ , ( $R^2= 0.847$ ), red edge indice Red Edge $_{(740-720)}$  ( $R^2= 0.846$ ) and chlorophyll pigment indice SRPI showed high correlation values ( $R^2= 0.836$ ) at flowering (June 12, 2014). When the relationships between SPAD and LAI were examined at the same location, the highest correlation value for chlorophyll estimation was found during the heading period (May 13, 2014) ( $R^2=0.673$ ). It showed a decrease ( $R^2=0.020$ ) during the flowering period (May 26, 2014) due to increased saturation and a slight increase ( $R^2=0.243$ ) during the grain setting period (June 04, 2014). It increased during the spiking period (June 12, 2014) ( $R^2=0.659$ ) and decreased again during the yellowing period ( $R^2= 0.022$ ). In chlorophyll

estimation,  $NDVI_{(705,750)}$  ( $LAI= 2.63$ ,  $R^2=0.554$ ) was the least affected by the saturation change due to the increasing LAI value in the early period (May 13, 2014), while  $MSR_{(705,750)}$  ( $LAI=1.904$ ,  $R^2= 0.906$ ) was found in the late period (May 26, June 04-12-24, 2014).

#### Authorship Contribution

The authors contributed equally to the study

#### Conflict of Interest

The authors declare no conflict of interest.

#### Research and publication ethics statement

In the study, the authors declare that there is no violation of research and publication ethics and that the study.

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