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IDENTIFYING GENETIC VARIATION IN 12 WHEAT CULTIVARS FOR NITROGEN USE EFFICIENCY

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Abstract

*The article introduces Normalized Difference Vegetation Index (NDVI), Flag-leaf Chlorophyll Content, which was performed for 12 Triticum aestivum wheat (Shuha-4, Attila50, Seri.1B*2, Jawahir-1, Seri.1B, KBG-01, Flaf-3, Kauz, Hubara-3, Adana99 and Arehane). The two rate of nitrogen fertilizer (high-200 kg N ha-1 and low-0 kg N ha-1) was applied to the wheat varieties. Measurements were recorded in pre and post anthesis for both high and low nitrogen input plots. The NDVI value for (Aras) was the lowest (0.62) and (Seri.1B and Kauz/Pastor) were the highest (0.78) under LN condition. Significant differences were detected at post anthesis stage, Flag-3 had the lowest flag-leaf chlorophyll content (37), while (Seri.1B*2) had the highest flag-leaf chlorophyll (50). And under HN condition, KBG-01 had the lowest flag-leaf chlorophyll content (43), and (Seri.1B*2) had the highest flag-leaf chlorophyll (51). At pre anthesis stage, the genotypes differed in NDVI, it ranged from 0.64 (Flag-3) to 0.81 (Kauz/Pastor) under LN condition, and the range was 0.73 (Hubara-3*2) to 0.82 (Adana99) under HN condition.*

***Key words**: Normalized Difference Vegetation Index, fag-leaf chlorophyll content.*

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the major cereal crops and the most widely grown crop (Sakin et al., 2015). It is essential to increase the grain yield in order to supply the rest demand on food due to growing world population (Asrar et al., 1984). The intensive cultivation and the modern wheat cultivars require a high amount of fertilizers to be applied to increase the grain yield, especially nitrogen fertilizers (Colwell, 1963; Blackshaw et al., 2002). Large amounts of nitrogen fertilizer inputs have a great impact on the environment, health and cost on the production (Zhu and Chan, 2002; Quiao et al., 2015). Improving nitrogen use efficiency through identifying related traits such as stay-green might be a possible mechanism to reduce the environmental impacts of nitrogen input (Hawkesford, 2014). The aim of this study is to determine the effects of the pre and post anthesis of wheat on the effects of low and high nitrogen input.

MATERIALS AND METHODS

This study was carried out at Directoriate of Agriculture Research in Bakrajo province of Iraq. The soil type was clay loam. 12 strains of aestivum wheat (Shuha-4, Attila50, Seri.1B*2, Jawahir-1, Seri.1B, KBG-01, Flaf-3, Kauz, Hubara-3, Adana99 and Arehane), and Arass cultivar as local check, were sown by applying two rates of nitrogen fertilizer; high N (200 kg $N.ha^{-1}$) and Low N (0 kg $N.ha^{-1}$).

The experimental design was a split plot design with two nitrogen fertilizer rates, High (200 kg $N.ha^{-1}$) and low nitrogen (0 kg $N.ha^{-1}$), and 160 kg/h daimio phosphate, randomised on the main-plots and 12 genotypes randomised on sub-plots in three replicates. The plot size was 2.5 m x 0.8 m with 4 rows with 0.2 m between rows. The genotypes within the experiment were sown at the same seeding rate 120 kg/h.

Normalized difference vegetation index (NDVI)

Spectral reflectance indices are important tools for evaluating photosynthetic traits (Sims and Gamon, 2002; Gitelson et al., 2003). The Normalized Difference Vegetation Index (NDVI) is one of the most widely used vegetation indices as an indicator of canopy green area and it is associated with grain yield as well (Reynolds et al., 2007; Tanrıverdi,

2010). It is based on the difference between the maximum absorption of radiation in the red spectral band and the maximum reflection of radiation in the near-infrared spectral bands (Ghorbani et al., 2012; Tucker et al., 2005; Carlson and Ripley, 1997). Canopy NDVI values are between –1.0 and +1.0 and for soil and vegetation are usually positive values. The healthy green plant absorbs most of the incident red light and they reflect a large rate of the near infrared radiation (high NDVI value) while in non-green plants the absorption of red light is less (low NDVI value). Hence, this difference in adsorption and reflection of the light waves is measured using the NDVI equation: NDVI = (NIR - RED)/ (NIR + RED) or (R900-R680)/R900-R680) (Moriondoa et al., 2007).
NDVI was measured on two occasions at pre and post anthesis using in each plot under high nitrogen HN and low nitrogen LN conditions in three replicates using GreenSeeker HandHeld (Greenseeker, USA). This instrument measures the amount of near infrared (NIR) and red light reflected by the canopy.

Flag-Leaf Chlorophyll Content

Chlorophyll content is used for the determination of stay green or leaf senescence in agricultural research (Bahar, 2015). Flag-leaf chlorophyll concentration is an important parameter that is frequently measured as an indicator of chloroplast development, photosynthetic capacity, leaf nitrogen content or general plant health (Ling et al., 2011). Flag-leaf greenness was measured using the SPAD 502 meter (Minolta Japan) which is a hand-held device that is widely used for rapid, accurate and non-destructive measurement of leaf chlorophyll concentration. This device is based on two light-emitting diodes and a silicon photodiode receptor, that measures leaf transmittance in the red (650 nm; the measuring wavelength) and infrared (940 nm; a reference wavelength) regions of the electromagnetic spectrum (Uddling et al., 2007). These transmittance values are used by the device to derive a relative SPAD meter value that is proportional to the amount of chlorophyll in the sample.
In this experiment, samples of three fertile shoots (those with an ear) were randomly measured before and after anthesis stage from each plot in the HN treatment in all replicates. Three measurements were taken from each flag-leaf at the base, middle and top of the flag leaf.
The most significant event when the leaf start to senesce is the changes that happens in the leaf chloroplast where photosynthesis occur. Decline in photosynthetic activity is associated with reducing in dark reactions of the Calvin cycle which is mainly due to the degradation of Rubisco disassembly of the photosynthetic apparatus (Lu et al., 2002).
High flag-leaf chlorophyll content has been known to be related with high photosynthesis rate as higher amounts of chlorophyll increase the photosynthesis rate. More light will be absorbed by the flag leaf and this will improve the radiation use efficiency and hence enhancing photosynthesis rate (Foulkes et al., 2009; Makino, 2011; Garnett and Rebetzke, 2013).

RESULTS AND DISCUSSIONS

Normalized Difference Vegetation Index (NDVI)

This measurement was determined using the GreenSeeker HandHeld where the equipment sensor was held about 50 cm above the canopy. Two measurements were recorded in pre and post anthesis for both high and low nitrogen input plots.
At pre anthesis stage, the genotypes differed in NDVI, it ranged from 0.64 (Flag-3) to 0.81 (Kauz/Pastor) under LN condition, and the range was 0.73 (Hubara-3*2) to 0.82 (Adana99) under HN condition (Table 1). However, there were no significant differences among the genotypes and the N x G interaction (Table 2).
Significant differences were detected among cultivars at post anthesis stage (Table 3). The NDVI value for (Aras) was the lowest (0.62) and (Seri.1B and Kauz/Pastor) were the highest (0.78) under LN condition. The value of NDVI was the lowest for (Hubara-3*2) 0.68, and the highest value was 0.78 for (Seri.1B) (Table1).

Flag-Leaf Chlorophyll Content

Flag-leaf chlorophyll content was determined at GS61 using a SPAD 502 meter (Minolta, Japan). Three different points on the flag-leaf were measured for 3 fertile shoots per plot under LN and HN conditions.

The genotypes differed in flag-leaf chlorophyll content but the variation was non-significant (Table 5); at pre anthesis stage the flag-leaf chlorophyll content ranged from 36 (Flag-3) to 54 (Hubara-3*2) under LN condition, and from 41 (Attila 50y) to 54 (Kauz/Pastor) under HN condition (Table 4).

Significant differences were observed at post anthesis stage (Table 6), Flag-3 had the lowest flag-leaf chlorophyll content (37), while (Seri.1B*2) had the highest flag-leaf chlorophyll (50).

And under HN condition, KBG-01 had the lowest flag-leaf chlorophyll content (43), and (Seri.1B*2) had the highest flag-leaf chlorophyll (51) (Table 4).

Table 1. Mean data for NDVI at pre and post an thesis stage

Genotypes	PRE		POST	
	LN	HN	LN	HN
Shuha-7	0.76	0.78	0.67	0.72
Attila 50y	0.73	0.79	0.68	0.72
Seri.1B*2	0.78	0.80	0.73	0.74
Jawahir-1	0.73	0.77	0.69	0.73
Seri.1B	0.80	0.80	0.75	0.78
KBG-01	0.80	0.78	0.72	0.74
Flag-3	0.64	0.76	0.67	0.74
Kauz/Pastor	0.81	0.77	0.75	0.74
Hubara-3*2	0.71	0.73	0.67	0.68
Adana99	0.75	0.82	0.72	0.75
Arehane	0.74	0.76	0.70	0.73
Aras	0.76	0.78	0.62	0.70

Nguyen et al., 2016, are conducted a study on 15 wheat varieties, grown under three N levels, were phenotyped for NUE-related traits under field conditions. Significant genotypic differences were observed in varieties having low to high responsiveness to N applications. The results suggest that basal low N can be used to screen wheat varieties that are less responsive to N, whereas N supply from 80 to 160 kg $N.ha^{-1}$ could be used to screen high N-responsive varieties. Normalised difference vegetation index (NDVI) measured by using Crop Circle.

Gaju et al., 2016, found that flag-leaf chlorophyll content was positively associated with at anthesis leaf Amax (R2 = 0.74) amongst 15 wheat genotypes under high N conditions. Schlemmer et al., 2013, carry out that leaf nitrogen and chlorophyll content were retrieved accurately from leaf reflectance spectra. Canopy nitrogen and chlorophyll content were closely related, vegetation indices using green and red-edge spectral bands were used for accurate chlorophyll and nitrogen estimation at canopy level, optimal spectral bands found for nitrogen and chlorophyll estimation match well-spectral bands of near future space systems. Kapp et al., 2016, state that wheat grain yield was high and it did not follow the gains in the production.

Table 2. Statistical analysis for NDVI at pre anthesis stage

S.O.V	d.f	SS	MS	F cal.	F 0.05
Blocks	r-1=2	0.007	0.003	1.505	
A	a-1 = 1	0.014	0.014	6.156	18.513
Error (a)	(r-1)(a-1)= 2	0.005	0.002		
B	b-1= 11	0.060	0.005	1.064	2.014
AB	(a-1)(b-1) = 11	0.030	0.003	0.527	2.014
Error (b)	a(b-1)(r-1) = 44	0.225	0.005		
Total	abr-1 = 71	0.340			

A lot of research has been done about the wheat which is the most important agricultural product of the world. In all studies, it was observed that nitrogen fertilization was effected on wheat production, the effect of chlorophyll content and spectral reflection. In addition, Normalized Difference Vegetation Index measurements pre and post anthesis were used by different researchers.

Table 3. Statistical analysis for NDVI at post an thesis stage

S.O.V	d.f	SS	MS	F cal.	F 0.05
Blocks	r-1=2	0.015	0.008	13.302	
A	a-1 = 1	0.002	0.002	4.230	18.513
Error (a)	(r-1)(a-1)= 2	0.001	0.001		
B	b-1= 11	0.118	0.011	5.880	2.014
AB	(a-1)(b-1) = 11	0.004	0.000	0.220	2.014
Error (b)	a(b-1)(r-1) = 44	0.080	0.002		
Total	abr-1 = 71	0.222			

Table 4. Mean data for flag-leaf chlorophyll content at pre and post an thesis stage

Genotypes	PRE		POST	
	LN	HN	LN	HN
Shuha-7	48	48	44	44
Attila 50y	40	41	41	44
Seri.1B*2	43	48	50	51
Jawahir-1	41	49	41	47
Seri.1B	47	48	44	47
KBG-01	48	53	48	43
Flag-3	36	43	37	40
Kauz/Pastor	45	54	47	48
Hubara-3*2	54	50	47	50
Adana99	39	43	43	45
Arehane	43	48	40	46
Aras	44	51	46	45

Table 5 shows the mean values for flag-leaf chlorophyll content at pre and post anthesis stages.

Statistical analyzes of these data were carried out and the results are given in Table 5 and Table 6. In many genotypes the average flag-leaf chlorophyll content shows differences between pre and post anthesis. However, in some genotypes the average flag-leaf chlorophyll content showed differences. The significance levels of these differences are given below as a statistical analysis table.

Table 5. Statistical analysis for flag-leaf chlorophyll content at pre an thesis stage

S.O.V	d.f	SS	MS	F cal.	F 0.05
Blocks	r-1=2	366.667	183.334	179.421	
A	a-1 = 1	277.694	277.694	271.768	18.513
Error (a)	(r-1)(a-1)= 2	2.044	1.022		
B	b-1= 11	1115.693	101.427	1.669	2.014
AB	(a-1)(b-1) = 11	201.546	18.322	0.301	2.014
Error (b)	a(b-1)(r-1) = 44	2674.676	60.788		
Total	abr-1 = 71	4638.320			

Table 6. Statistical analysis for flag-leaf chlorophyll content at post an thesis stage

S.O.V	d.f	SS	MS	F cal.	F 0.05
Blocks	r-1=2	535.947	267.973	26.907	
A	a-1 = 1	50.167	50.167	5.037	18.513
Error (a)	(r-1)(a-1)= 2	19.919	9.959		
B	b-1= 11	632.942	57.540	2.134	2.014
AB	(a-1)(b-1) = 11	140.272	12.752	0.473	2.014
Error (b)	a(b-1)(r-1) = 44	1186.501	26.966		
Total	abr-1 = 71	2565.747			

CONCLUSIONS

Chlorophyll retention or stay-green is regard as an indicator of the plant performance under stress conditions such as nitrogen.

The improvement of genotypes for nitrogen use efficiency and photosynthesis are requiring identification associated traits among various genotypes and select the superior.

The genotypes showed variation in their flag leaf chlorophyll content as the value of SPAD. The genotype (Kauz/Pastor) had the advantage in NDVI values as it was significantly different from all other genotypes under low nitrogen condition and post anthesis measurements.

The genotype with higher NDVI had a greater potential for grater grain filling.

This is an indicator that the plants have better nitrogen utilization and they are capable of retain their tissues green for longer period of time, more nitrogen will be accumulated from the soil and more active in their photosynthesis.

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