DUFED 4(1) (2015) 1-5

Dicle University Institute of Natural and Applied Science Journal

journal homepage: http://www.dufed.org

Relationships among flag leaf chlorophyll content, agronomical traits, and some physiological traits of winter wheat genotypes*

Kışlık buğdayda, bayrak yaprak klorofil içeriği ile tarımsal özellikler ve bazı fizyolojik özellikler arasındaki ilişkiler

Bilge Bahar

Department of Food Engineering, Faculty of Engineering and Natural Sciences, University of Gumushane, 29100 Gumushane, Turkey

ARTICLE INFO

Received: 15 December 2014
Received in revised form: 1 January 2015
Accepted: 14 January 2015
Available online: 30 December 2015
Printing: 20 January 2016

ABSTRACT

In this study, relationships among flag leaf chlorophyll contents of some winter wheat genotypes, agronomical traits, and some physiological characters such as canopy temperature, membrane thermal stability, membrane injury, and relative water content of flag leaf were evaluated. The study was conducted in the Application and Research Area of Siran Vocational School of Gumushane University in the growth season of 2010-2011. Chlorophyll content of genotypes were measured by a portable chlorophyll meter at the start of anthesis (ZGS 60) and the early milky stage (ZGS 73). The mean chlorophyll content of the tested genotypes at ZGS 60 was 45.6 as SPAD unit, and ranged from 39.1 for line 51 to 54.0 for line 42. Chlorophyll content as the mean of all genotypes at ZGS 73 was 41.8 as SPAD unit. Mean chlorophyll content of the genotypes at this growth stage ranged between 35.2 for line 27 and 50.9 for line 44. The mean pigment loss was the percent of 8.3 as an average of all genotypes. The chlorophyll loss ranged between 1.7 % for line 75 and 19.2 % for line 32. The statistically significant correlations between chlorophyll contents and main yield components like grain number per spike and spike yield were obtained at both measuring stages. The significant correlation between chlorophyll loss and chlorophyll content was positive at ZGS 60, but negative at ZGS 73. These results show that determination of flag leaf chlorophyll content in winter wheat is important selection criteria for yield components in breeding programs.

Keywords: Chlorophyll content, Senescence, MTS, RWC, Winter wheat

ÖZET

Bu çalışmada, kışlık ekmeklik buğdayda bayrak yaprak klorofil içerikleri ile tarımsal özellikler ve membrane termostabilitesi, membrane hasar indeksi ve bayrak yaprak oransal su içeriği arasındaki ilişkiler incelenmiş olup; çalışma, 2010-2011 yetiştirme sezonunda Gümüşhane Üniversitesi, Şiran Mustafa Beyaz MYO uygulama alanında yapılmıştır. Genotiplerin klorofil içeriği, klorofil metre ile çiçeklenme başlangıcı (ZGS 60) ve erken süt olum (ZGS 73) dönemlerinde ölçülmüştür. Genotiplerin korofil içerikleri ortalaması ZGS 60 döneminde 45.6 SPAD birimi olarak ölçülmüş olup; genotiplere göre 39.1 (line 51) ile 54.0 (line 42) arasında değişim göstermiştir. ZGS 73 döneminde ise genotiplerin klorofil içeriği ortalaması 41.8 olup; genotiplere göre 35.2 (line 27) ile 50.9 (line 44) arasında değişmiştir. Genotiplerin klorofil kaybı, ortalama %8.3 olup; genotiplere göre %1.7 (line 75) ile %19.2 (line 32) arasında dağılım göstermiştir. Her iki ölçüm döneminde, klorofil içerikleri ile başakta dane sayısı ve başak verimi gibi verim ana komponentleri arasında istatistiksel olarak önemli ilişkiler bulunmuştur. Klorofil içerikleri ile klorofil kaybı arasındaki ilişki, ZGS 60'da olumlu, ZGS 73'te olumsuz bulunmuştur. Sonuçlar, kışlık buğdayda bayrak yaprak klorofil içeriği tayininin ıslah programlarında verim komponentleriyle ilişkisi bakımından önemli bir seleksiyon kriteri olduğunu göstermektedir.

Anahtar sözcükler: Klorofil içeriği, Yaşlanma, MTS, RWC, Kışlık buğday

* *This research was presented as oral presentation in the "7th International Congress on Pigments in Food" by the support of TUBITAK-International Meetings Grant Programmes.*

Corresponding address: Bilge Bahar
Tel. +90 456 233 10 32 E-mail: bilgebahar@gmail.com

1. Introduction

Chlorophyll is an important photoreceptor pigment that absorbs light energy and transfers energy to the photosynthetic system [1]. Chlorophyll content is used for the determination of stay green or leaf senescence in agricultural research. It has noted that stay green or delayed senescence traits of wheat play an important key role in grain filling under limited assimilation conditions that are affected by drought and heat stress [2]. Some researcher suggested that healthy stay-green crops had the capacity to produce high yields [3]. The stay green characteristic in wheat, which is related to maintaining green leaf area, [4] has been improved to increase the grain yield in wheat. It has reported that modern bread wheat lines, which lack natural stay green characteristics, become senescent more rapidly than ancient wheat lines that had high stay green characteristics; thus, the authors stressed the utility of ancient wheat strains in improving the stay green capabilities of modern wheat lines [5].

Many methods can be used to determine crop greenness. Initially, organic extraction and spectrophotometry was used to identify chlorophyll concentration measured in units of mg g^{-1} [6]. Later, digital camera which was based on the calculation of the Green/Red index for the estimation of the crop greenness was used [7]. Numerical image analysis (NIA) technique was used with the similar goal in evaluating leaf greenness [8]. Several researchers used chlorophyll meters to determine the greenness index [9-11]. Nondestructive optical instruments such as Soil Plant Analysis Development (SPAD) 502 and Chlorophyll Content Meter (CCM 200) provide a chlorophyll index that is expressed in relative chlorophyll content, but do not provide a quantitative result per unit leaf area or per area of fresh leaf tissue. These methods are non-destructive, less expensive, rapid, and adaptable to field conditions [12] and they were also used to determine chlorophyll content in various crops such as corn [12], rice [13], soybeans [12], tobacco [14], wheat [10,15], and potatoes [16].

In this study, the effects of changes in chlorophyll content at the beginning of anthesis to early milky developmental stages were evaluated in relation to agronomical (grain yield, heading time, plant height, spike length, grain number per spike, spike yield, thousand kernel weight) and physiological (relative water content, membrane injury, membrane thermal stability) traits.

2. Material and Methods

2.1. Plant materials and growing conditions

Field trial was conducted at the Application and Research Area of the Siran Vocational School, Gumushane University, Turkey during the 2010-2011 growing season. Sixty winter bread wheat genotypes which included 59 lines and a standard (Gun-91 cv) were used as research material. Wheat lines were provided from CIMMYT (International Maize and Wheat Improvement Center). Sowing was done by hand with 2 m row length and 22.5 cm row space per genotype on September 25, 2010. Plants were grown under organic (minimum input) conditions. According to the meteorological data, wheat genotypes received 79.4 mm rainfall, 20.45 °C mean temperature, and 56.2% relative humidity during the heading to grain filling period and total rainfall was 451.4 mm during the growth season.

2.2. Data Collection

Phenology of the crops was observed and recorded using the Zadoks Growth Scale (ZGS), which was developed by [17]. Agronomical traits (heading time, plant height, spike length, grain numbers per spike, grain yield, thousand kernel weight) were determined according to the standard methods.

Chlorophyll content was measured as SPAD units with a handheld chlorophyll meter (SPAD 502 Plus) at two growth stages (beginning of anthesis, ZGS 60; 16 June, 2011 and early milk stage, ZGS 73; 5 July, 2011). Both were determined at the mid-point of each intact flag leaf from ten main stems in each genotype and recorded by chlorophyll meter in SPAD units. Canopy temperatures of the genotypes were determined as the mean of three measurements by handheld infrared thermometer within the day (11:00, 12:00 a.m., and 13:00 p.m.; on 5 July, 2011) at ZGS 73. Relative water content (RWC) was calculate by the following standard formula:

$$\text{RWC (\%)} = \text{(Fresh Weight - Dry Weight)} * 100 / \text{(Turgid Weight - Dry Weight)} \quad \textbf{(1)}$$

Membrane thermal stability was measured by two different equations of [18].

a) $\text{Membrane Injury (MI, \%)} = 1-[(1-(T_1/T_2))/(1-(C_1/C_2))*100 \quad \textbf{(2)}$

b) $\text{Membrane Thermal Stability (MTS, \%)} = [1-(T_1/T_2)]*100 \quad \textbf{(3)}$

On the upper equations [(2,3)], while C and T refer to electrical conductivity (EC) of control and heat treated samples, 1 and 2 refer to EC readings before and after boiling.

2.3. Data Analysis

Mean value, ranges (max and min values), and standard deviations for each trait were calculated on the excel program. Also, correlations among all traits were determined by JMP.

3. Results and Discussion

The mean chlorophyll content of the tested genotypes at ZGS 60 was 45.6 as SPAD unit, and ranged from 39.1 for line 51 to 54.0 for line 42. Chlorophyll content as the mean of all genotypes at ZGS 73 was 41.8 as SPAD unit. Mean chlorophyll content of the genotypes at this growth stage ranged between 35.2 for line 27 and 50.9 for line 44. The mean pigment loss was 8.3 % as an average of all genotypes and it ranged between 1.7 % for line 75 and 19.2 % for line 32 (Table 1).

The statistically positive significant correlations were obtained between chlorophyll contents and main yield components like grain number per spike ($r = 0.338^{**}$, $P \leq 0.01$ at ZGS 60; $r = 0.255^{*}$, $P \leq 0.05$ at ZGS 73) and spike yield ($r = 0.266^{*}$, $P \leq 0.05$ at ZGS 60; $r = 0.288^{*}$, $P \leq 0.05$ at ZGS 73) (Table 2). The significant positive correlations between SPAD values and grain numbers per spike at heading time and milky grain development stage of durum wheat under rainfed conditions were reported by [19]. Correlation of the chlorophyll content with chlorophyll loss was positive ($r = 0.330^{**}$, $P \leq 0.01$) at ZGS 60, but negative ($r = -0.293^{*}$, $P \leq 0.05$) at ZGS 73 (Table 2). This result clearly shows that the genotypes with slow senescence and the lowest chlorophyll loss were able to retain their stay green characteristics best; in other words, for high stay green or slow senescence, genotypes with lower Spad values at ZGS 60 (beginning of anthesis) or with higher Spad values at ZGS 73 (early milky ripeness) must be selected in breeding programs. Chlorophyll loss was also negatively associated with relative water content ($r = -0.407^{**}$, $P \leq 0.01$) and plant height ($r = -0.319^{*}$, $P \leq 0.01$). Plant height was also negatively correlated with chlorophyll contents at ZGS 60 ($r = -0.460^{**}$, $P \leq 0.001$) and ZGS 73 ($r = -0.267^{*}$, $P \leq 0.05$) (Table 2). Also, canopy temperature showed negative significant correlations with grain yield ($r = -0.304^{*}$, $P \leq 0.05$) and plant height (-0.269^{*}, $P \leq 0.05$). The strong negative significant associations ($r = -0.647^{***}$, $P \leq 0.001$) between canopy temperature and grain yield of spring bread wheat were found by [20]. Apart from all these, membrane thermal stability a heat tolerance parameter was positive significantly associated with thousand kernel weight ($r = 0.252^{*}$, $P \leq 0.05$).

Table 1: Mean values, ranges, and standart deviations of 60 winter bread wheat genotypes for chlorophyll based traits, agronomical and physiological characters.

Traits	Mean of genotypes	Range		Standard Deviation
		Min.	Max.	
Chl – I (SPAD Unit)	45.6	39.1	54.0	3.63
Chl – II (SPAD Unit)	41.8	35.2	50.9	3.29
Chlorophyll Loss (%)	8.3	1.7	19.2	4.44
Heading Time (days)	161	154	171	3.57
Plant Height (cm)	87.5	65.8	137.0	12.68
Spike Length (cm)	9.7	7.3	14.4	1.18
Grain no per Spike (no)	46.0	24.0	66.0	9.98
Spike Yield (g)	2.01	0.94	8.09	0.96
Thousand Kernel Weight (g)	40.88	31.80	49.17	3.24
Grain Yield (t ha^{-1})	6.19	2.01	11.44	2.25
Canopy Temperature (^{o}C)	24.71	22.45	27.05	1.07
Relative Water Content (%)	88.0	66.7	95.4	5.36
Membrane Injury (%)	73.05	30.48	92.8	14.98
Membrane Thermal Stability (%)	24.44	6.89	64.96	13.77

Chl – I, chlorophyll content at ZGS 60; Chl – II, chlorophyll content at ZGS 73.

Table 2: Correlation coefficients between chlorophyll content, chlorophyll loss, agronomical and physiological traits (n = 60).

Traits	Chl-II	CL	HT	PH	SL	GNPS	SY	TKW	GY	CT	RWC	MI	MTS
Chl-I	.805**	.330**	.137	- .460**	.204	.338**	.266*	.088	.102	.058	.136	.215	.017
Chl-II		- .293*	.229	- .267*	.212	.255*	.288*	.067	- .176	.066	.121	.159	- .014
CL			- .132	- .319*	- .016	.143	- .021	.044	.109	- .019	- .407**	.107	.000
HT				- .123	.274*	.156	- .083	- .112	- .095	- .094	.217	.229	- .080
PH					.005	- .169	- .208	- .088	.066	- .269*	.166	- .103	- .028
SL						.640**	.168	- .117	- .108	- .063	.045	- .077	- .061
GNPS							.438**	- .086	- .255*	.027	- .154	043	.009
SY								.044	- .163	.057	- .201	.048	.000
TKW									- .202	- .151	- .027	- .244	.252*
GY										- .304*	.156	- .004	- .150
CT											- .182	.186	.089
RWC												- .034	- .198
MI													.070

Chl – I, chlorophyll content at ZGS 60; Chl – II, chlorophyll content at ZGS 73; CL, chlorophyll loss; HT, heading time; PH, plant height; SL, spike length; GNPS, grain number per spike; SY, spike yield; TKW, thousand kernel weight; GY, grain yield; CT, canopy temperature; RWC, relative water content; MI, membrane injury; MTS, membrane thermal stability; $^{*}p \leq 0.05$; $^{**}p \leq 0.01$.

4. Conclusion

In this study which has examined the relationships among flag leaf chlorophyll contents of some winter wheat genotypes, agronomical traits, and some physiological characters such as canopy temperature, membrane thermal stability, membrane injury, and relative water content of flag leaf. The statistically significant correlations between chlorophyll contents and main yield components like grain number per spike and spike yield shows the importance of chlorophyll pigment of the wheat genotypes. Also, it is clearly understood from the findings that the genotypes with slow senescence and the lowest chlorophyll loss were able to retain their stay green characteristics best. Chlorophyll loss was also negatively associated with relative water content and plant height. Thus, the study exhibits the utility of chlorophyll content into wheat breeding programs. Also, canopy temperature, membrane thermal stability and relative water content must be preferred as breeding parameters.

References

1. B. Demmig-Adams, W.W. Adams, The role of xanthophyll cycle carotenoids in the protection of photosynthesis. Trends in Plant Science 1, 21-27, (1996).
2. U. Kumar, A.K. Joshi, M. Kumari, R. Paliwal, S. Kuma, M.S. Roder, Identification of QTLs for stay green trait in wheat (*Triticum aestivum* L.) in the 'Chirya 3' × 'Sonalika' population, Euphytica 174, 437-445, (2010).
3. H. Thomas, C.M. Smart, Crops that stay green, Annals of Applied Biology 123, 193-201, (1993).
4. G. Spano, N. Di Fonzo, C. Perrotta, C. Platani, G. Ronga, D.W. Lawlor, J.A. Napier, P.R. Shewry, Physiological characterization of stay green mutants in durum wheat, Journal of Experimental Botany 54, 1415-1420, (2003).
5. D.L. Sparkes, Are 'ancient wheat species' more adapted to hostile environments than modern bread wheat? South African Journal of Plant and Soil 27, 331-334, (2010).
6. D.I. Arnon, Copper enzymes in isolated chloroplast polyphenol oxidase in Beta vulgaris, Plant Physiology 24, 1-15 (1949).
7. F.G. Adamsen, P.J. Pinter, E.M. Barnes, R.L. La Morte, G.W. Wall, S.W. Leavitt, B.A. Kimball, Measuring wheat senescence with a digital camera, Crop Science 39, 719-724, (1999).
8. M. Hafsi, W. Mechmeche, L. Bouamama, A. Djekoune, M. Zaharieva, P. Monneveux, Flag leaf senescence, as evaluated by numerical image analysis, and its relationship with yield under drought in durum wheat, Journal of Agronomy and Crop Science 185, 275-280, (2000).
9. P. Janaki, T.M. Thiyagarajan, Effect of SPAD techniques and planting density on 'Y' leaf nitrogen concentration in transplanted rice, Acta Agronomica Hungarica 52, 95-104, (2004).
10. M. Lopes, M.P. Reynolds, Stay-green in spring bread wheat can be determined by spectral reflectance measurements (normalized difference vegetation index) independently from phenology, Journal of Experimental Botany 63, 3789-3798, (2012).
11. J.P.G. Rigon, S. Capuani1, N.E.M. Beltrao, J.F.B. Neto, V. Sofiatti, F.V. França, Non-destructive determination of photosynthetic pigments in the leaves of castor oil plants, Acta Scientarum Agronomy 34, 325-329, (2012).

12. J. Markwell, J.C. Osterman, J.L. Mitchell, Calibration of the Minolta SPAD-502 leaf chlorophyll meter, Photosynthesis Research 46, 467-472, (1995).
13. O.A. Monje, B. Bugbee, Inherent limitations of nondestructive chlorophyll meters: A comparison of two types of meters, Horticultural Science 27, 69-71, (1992).
14. F. Castelli, R. Contillo, F. Miceli, Non-destructive determination of leaf chlorophyll content in four crop species, Journal of Agronomy and Crop Science 177, 275-283, (1996).
15. A. Guendouz, S. Guessoum, K. Maamari, M. Hafsi, Predicting the efficiency of using the RGB (Red, Green and Blue) reflectance for estimating leaf chlorophyll content of Durum wheat (*Triticum durum* Desf.) genotypes under semi arid conditions, American-Eurasian Journal of Sustainable Agriculture 6, 102-106, (2012).
16. J. Uddling, J. Gelang-Alfredsson, K. Piikki, H. Pleijel, Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings, Photosynthesis Research 91, 37-46, (2007).
17. J.C. Zadoks, T.T. Chang, C.F. Konzak, A decimal code for the growth stages of cereals, Weeds Research 14, 412-415 (1974).
18. A. Blum, A. Ebercon, Cell membrane stability as a measure of drought and heat tolerance in wheat, Crop Science 21, 43-47, (1981).
19. M. Yildirim, H. Kilic, E. Kendal, T. Karahan, Applicability of chlorophyll meter readings as yield predictor in durum wheat, Journal of Plant Nutrition 34, 151-164, (2011).
20. B. Bahar, M. Yildirim, C. Yucel, Heat and drought resistance criteria in spring bread wheat (*Triticum aestivum* L.): Morpho-physiological parameters for heat tolerance, Scientific Research and Essays 6 (10), 2212-2220, (2011).