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Index of chlorophyll content in common winter wheat (*Tr. aestivum L.*) genotypes at grain filling stage

Dimitrina Nikolova[1]*, Galina Mihova[1], Radoslav Chipilski[2]

[1]Dobrudzha Agricultural institute – General Toshevo, Agricultural Academy – Sofia, Bulgaria

[2]Institute of Plant and Genetic Resources – Sadovo, Agricultural Academy – Sofia, Bulgaria

*Corresponding author: *didilak@abv.bg*

Dimitrina Nikolova ORCID: 0009-0002-2966-7880

Gallina Mihova ORCID: 0000-0001-9808-5767

Radoslav Chipilski ORCID: 0000-0001-6314-5715

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Abstract: Chlorophyll content is directly related to the rate of photosynthesis. Changes in the photosynthesis due to reduced amount of chlorophyll affect the weight and quality of grain. A two-year field trial was carried out (2022-2023, 2023-2024) with 12 varieties and lines of common winter wheat with the aim to determine the relationship of chlorophyll concentration during grain filling with yield and its components. The index of chlorophyll content was determined using Chlorophyll Content Meter Model CCM-200 Plus. The measurements were done at stage grain filling, the accessions being grouped according to their date to heading. Chlorophyll content was most variable in the early heading genotypes. The results were unidirectional over years. During the first vegetative growth season, a significant positive correlation with yield was determined, while during the second vegetative growth season it was negative but low. A low correlation of chlorophyll concentration with test weight and protein content in grain was found. The established significant differences in the chlorophyll concentration between the individual genotypes confirmed the possibility of using this precise tool for differentiation of the lines during the breeding process. The identified genotypes with high chlorophyll content were suitable to use as potential germplasm in the breeding programs.

Key words: winter wheat; chlorophyll concentration; grain filling; yield

INTRODUCTION

The photosynthetic capacity, the duration and dynamics of grain filling and post-anthesis leaf senescence determine the yield formation in common winter wheat (Xu et al., 2015). The chlorophyll content in the leaves is a key factor for determining the rate of photosynthesis and dry matter accumulation (Ghosh et al., 2004, Ahmadi-Lahijani & Emam, 2016). It provides information on the physiological status of the plants (Gitelson et al., 2003), serves as a criterion for the need of macro and micro elements during the vegetative growth of the plants (Aregui et al., 2006) and for prediction of yield (Jin et al., 2012); it is also used as an index of soil fertility (Gao et al., 2024) and for correction of some technological elements of production. The screening and evaluation of the genotypes in the breeding process allows selecting those, which possess improved biological and economic properties (Ullah, 2021). The methods based on vegetation indices through non-destructive assessment of chlorophyll content in leaves by using technologies for distant monitoring are often applied in practice (Cui et al., 2019). Fast and non-invasive, they provide indirect measurement of the photosynthetic response of the plants. They are useful for monitoring of nitrogen content and allow evaluating the structural and functional dynamics of the vegetation (Shah et al., 2019). The manual chlorophyll meters are conventional devices and ensure fast results for the purposes of diagnostics (Ravier et al., 2017). The chlorophyll meter measures the transmittance of red and near-infrared radiation through the leaves and evaluates chlorophyll as an index by defined arithmetic operations (Lunagaria et al., 2015). The readings of the chlorophyll meters on the status vary depending on the specificity of the genotype, the used agronomy practices and the factors of the environment (Wood et al., 1993). It is considered also, that the readings of the chlorophyll meters can be affected by a number of external factors, such as pests and diseases, leaf thickness, solar radiation, water status, etc. (Samborski et al., 2009, Ravier et al., 2017).

Zhang et al. (2009) reported a genetic correlation between grain yield and chlorophyll content in leaves. This report was supported by establishing several loci, which control simultaneously the yield and the chlorophyll content. Reynolds et al. (1994) determined a significant correlation of the photosynthetic rate and the conductivity of stomata with the yield. Low was the correlation between chlorophyll content under irrigation conditions during three key stages of development – booting, heading and grain filling. According to Hoel (1998), the correlation between the values of chlorophyll meter and the nitrogen content, grain yield and protein content in grain was higher immediately before heading, GS 49 (according to Zadoks et al., 1974), in comparison to GS 31. Similar results were reported by Bavec & Bavec (2001), who established strong correlation ($r = 0{,}538$) at stage GS 45-50, while at GS 31-32 it was low ($r = 0{,}134$).

Researchers (Talebi, 2011; Javed et al., 2022) have reported a positive correlation between chlorophyll content and yield both under irrigation and drought. The leaf chlorophyll status is a key factor, which determines high rate of photosynthesis under stress (Roy et al., 2021). Lower chlorophyll content under high temperatures and drought contributes to reducing the negative effect of stress due to lower stomata conductivity as an adaptation mechanism (Mohhammadi et al., 2009). Chipilksi et al. (2019) found a low positive correlation between chlorophyll content and seed yield at heading stage under drought and a moderate negative correlation under stress conditions at grain filling stage.

Parallel changes in photosynthesis during grain filling, chlorophyll content and grain weight have been reported by Guendouz & Maamari (2012).

The aim of this study was to determine the variation of the chlorophyll concentration in the common winter wheat genotypes at grain filling stage and its correlation with yield and its elements.

MATERIAL AND METHODS

The experiment was carried out in the trial field of Dobrudzha Agricultural Institute – General Toshevo, Bulgaria. It included 12 new varieties, now being introduced in practice, and lines of common winter wheat, which were compared to the standards used in state varietal testing (Table

Table 1. Common winter wheat genotypes included in the study

Genotype	Pedigree		Genotype	Pedigree
Anapurna	Standard		Kasidi	Goritsa / OAC Montres
Avenue	Standard		Shibil	Enola / Leda
Enola	Standard		Yulita	Anna / Laska // Lazarka
Andronia	Iveta / Mechura		Zara	Azametly / Aleksa
Boil	Enola / Todora // Vitta		GT 14-12	Albena / Viza
Chudomira	Enola / Medeya		GT 15-35	Dragana x Boema
Fedora	Litera / Enola		14/15-43	Mv Kolompos x Soissons
Indzhe	Enola / Aglika // Simonida			

1). The study encompassed harvest years 2022-2023 and 2023-2024. The experiment was designed in two replications, with plot size 25 m^2. The sowing rate was 550 germinating seeds / m^2. When the vegetative growth of plants resumed at the beginning of March, nitrogen nutrition with 8 $t.ha^{-1}$ active matter was applied. The used agronomy practices were in accordance with the technology appropriate for the crop.

The relative value of chlorophyll (Chlorophyll content index – CCI) was determined by the precise tool Chlorophyll Content Meter Model CCM-200 Plus GPS. The measurements were done on 15 intact leaves from different parts of the trial plot at grain filling stage. In 2023, the plants were tested at stage beginning of milk maturity (**GS 73**), and in 2024 – at late milk maturity (**GS 77**). The phenological stages were read according to Zadoks et al. (1974).

The following parameters were analyzed: days to heading (DH), number of days (from 1st January); plant height (PH), cm; productive tillers per 1 m^2 (NPT), number; thousand kernel weight (TKW), g; test weight (HW), kg/hl, protein content (PC), %, and grain yield (YG) $t.ha^{-1}$.

The level of chlorophyll concentration was represented by a median. To differentiate the genotypes, one-way analysis of variance (ANOVA) was applied. The relationships between the relative value of chlorophyll and some parameters of productivity were determined through correlations (r). The experimental data were processes with the help of Microsoft Excel^{xp} and STATISTICA, release 7.0 (StatSoft Inc., 2004).

RESULTS AND DISCUSSION

Agrometeorological conditions

The years of investigation were characterized by comparatively mild autumn-winter periods and late spring frost. The combination of meteorological factors at the beginning of the reproductive stage was specific (Figure 1). May and June of 2023, when the stages of heading, anthesis and grain filling occurred, were with precipitation below the norm. The registered mean monthly temperatures were close to or below the average for the long-term period. The conditions were favorable for mass occurrence of yellow rust. During the recent years, this disease has been one of the limiting factors of grain production in the region of Dobrudzha. In 2024, the booting stage occurred under severe drought and dynamic temperature amplitudes. Accelerated phenological development was observed, as well as unusual early beginning of the heading stage. The rainfalls in April and May were favorable for the progress of the next growth stages and for the formation of high productivity.

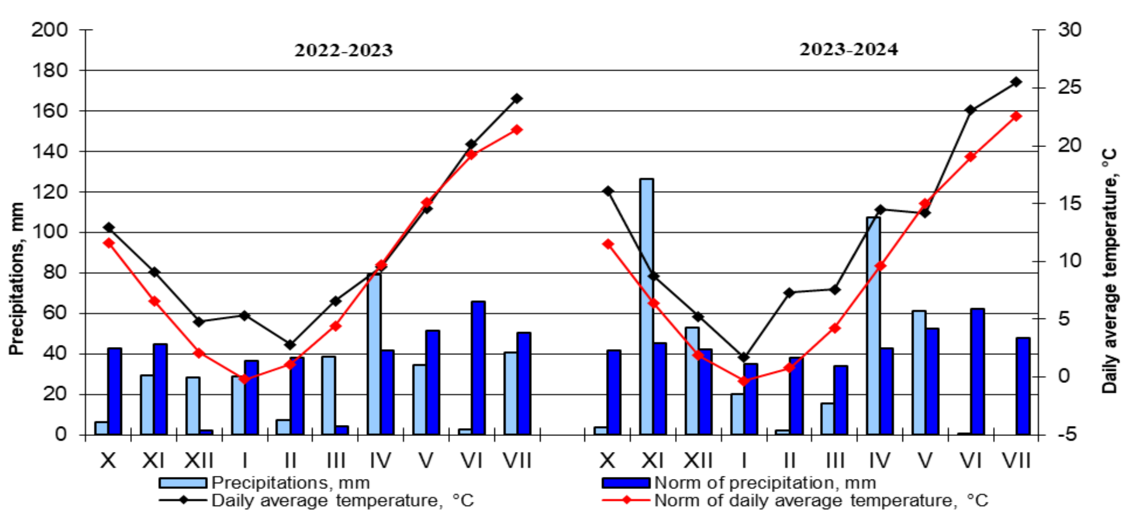

Figure 1. Meteorological characteristics of the investigated years

Chlorophyll content in leaves

The differences in the indices of chlorophyll content between the genotypes and over vegetative growth seasons were statistically significant (Table 2). During the first year of testing, the values were between 5,9 and 26,1, with an average of 13,0 for all varieties. During the second year, in spite of the more advanced developmental stage at the time of measurements, higher values were read, but the variation was within a narrower range, between 14,4 and 25,9, respectively, or 22,9 on the average.

Grouped according to their date to heading, the tested samples revealed significant differences. Varieties Avenue, Enola, Fedora and Zara belonged to the group with shorter date to heading in 2023. Variety Zara was with the highest relative chlorophyll value (25,7), while Enola was with the lowest (5,9). The next varieties forming a separate group were also with high differences in their values – from 7,1 of variety Indzhe to 22,4 for variety Anapurna. In 2024, the highest was the variation in the group of the earliest heading genotypes, in this group the highest and the lowest readings of chlorophyll concentration were determined. Variety Indzhe was with the lowest relative value (14,4), while Fedora was with the highest (25,9). No significant differences were observed in the rest of the varieties.

Yield and yield components

In harvest year 2022 – 2023, high number of productive tillers was registered, but the yields were comparatively lower, with an averaged for the investigated accessions: 6,40 t.ha^{-1} (Table 3). The main reason for this were the deteriorated conditions during grain filling and the resulting 1000 kernel weight was comparatively low, between 33,2 and 45,4 g, with an average of 41,2 g. The combination of meteorological factors at the beginning of the next vegetative growth period 2023 – 2024 was not favorable for the normal development of the plants and the number of productive tillers remained low. The shorter interphases were directly related to the lower biomass and lower plant height. The favorable conditions

Table 2. Date to heading, time of measurement and relative chlorophyll value over years

Genotype	DH	Test day	CCI	Genotype	DH	Test day	CCI
	2022-2023				2023-2024		
Anapurna	136	17	26,1	Andronia	118	40	22,6
Avenue	127	26	18,0	Boil	119	39	24,2
Enola	135	18	5,9	Chudomira	120	38	22,2
Andronia	137	16	10,7	Fedora	116	42	25,9
Boil	136	17	9,1	Indzhe	116	42	14,4
Chudomira	137	16	6,9	Kasidi	118	40	23,1
Fedora	135	18	8,2	Shibil	116	42	16,8
Indzhe	136	17	6,9	Yulita	116	42	22,7
Kasidi	136	17	16,7	Zara	118	40	25,5
Shibil	136	17	12,3	GT 14-12	120	38	20,7
Yulita	136	17	10,1	GT 15-35	121	37	23,7
Zara	135	18	25,7	14/15-43	121	37	22,1
Mean value			13,0				22,0
LSD 5 %			2,95				5,23
LSD 1 %			3,89				6,95
LSD 0.1 %			4,99				8,96

Test day – number of days after heading at a later stage allowed normal grain formation and filling. The absolute weight varied within 41,6 – 49,7, with an average of 46,3 g. High mean yields were registered – between 8,52 and 9,54 t.ha^{-1}.

The variation of protein content and test weight among the investigated genotypes was higher during the first year of testing, while in the second year the differences were lower. Protein content in grain was from 11, 1 % to 14,0 % in 2023, and from 12,6 to 14,3 % in 2024. The mean values of test weight over the years of study were similar.

Correlation between chlorophyll content and grain yield

The correlations between the Chlorophyll content index and some productivity parameters

Table 3. Level of the studied parameters over years

Genotype	GY	NPT	PH	TKW	PC	HW	Genotype	GY	NPT	PH	TKW	PC	HW
	2022-2023							2023-2024					
Anapurna	7,44	600	93	38,2	12,1	77,3	Andronia	9,03	520	82	45,4	13,3	77,8
Avenue	6,68	584	77	33,2	11,1	76,4	Boil	8,79	532	90	48,2	13,2	77,5
Enola	4,52	432	100	41,5	12,1	79,7	Chudomira	8,84	456	93	44,2	12,6	78,0
Andronia	6,06	546	97	41,2	13,2	78,4	Fedora	9,53	428	98	41,6	13,0	77,6
Boil	7,12	500	103	43,7	12,1	79,3	Indzhe	8,91	440	90	46,9	13,4	76,9
Chudomira	5,92	476	100	40,1	13,1	76,6	Kasidi	8,68	512	85	46,5	13,1	78,0
Fedora	6,13	498	107	37,7	12,3	78,4	Shibil	9,54	496	98	47,3	12,7	78,5
Indzhe	6,70	500	103	42,1	13,7	76,1	Yulita	8,81	584	90	49,5	13,6	76,6
Kasidi	6,92	570	95	42,4	12,9	79,6	Zara	8,81	532	95	49,7	14,3	79,9
Shibil	6,52	536	105	43,7	11,6	79,5	GT 14-12	8,52	584	93	47,2	13,3	78,4
Yulita	6,20	554	99	45,2	11,9	77,7	GT 15-35	8,83	480	94	43,0	12,2	79,6
Zara	6,60	540	111	45,5	14,0	80,8	14/15-43	9,31	500	80	45,9	13,4	79,6
Mean value	**6,40**	**528**	**99**	**41,2**	**12,5**	**78,3**	Mean value	**8,97**	**505**	**91**	**46,3**	**13,2**	**78,2**
Min	4,52	432	77	33	11	76	Min	8,52	428	80	41,6	12	77
Max	7,44	600	111	46	14	81	Max	9,54	584	98	49,7	14	80
Sd	**74,4**	**48,3**	**8,6**	**3,5**	**0,9**	**1,5**	Sd	**32,5**	**50,0**	**5,8**	**2,4**	**0,5**	**1,1**
VC%	**10,0**	**8,1**	**7,8**	**7,7**	**6,2**	**1,9**	VC%	**3,4**	**8,6**	**5,9**	**4,9**	**3,7**	**1,3**

Table 4. Correlations of chlorophyll concentrations with the studied traits

	GY	PH	TKW	HW	PC	CCI	NPT	DH
GY		0,16	-0,39	0,07	-0,19	-0,11	-0,53	-0,36
PH	-0,16		-0,12	0,03	-0,23	0,00	-0,21	-0,32
TKW	-0,05	0,73**		-0,09	0,70*	-0,19	0,71**	-0,22
HW	-0,15	0,54	0,58*		-0,01	0,28	0,01	0,62*
PC	0,00	0,53	0,43	0,15		0,13	0,45	-0,27
CCI	0,58*	-0,21	-0,10	0,20	0,04		0,17	0,30
NPT	0,73**	-0,50	-0,21	-0,15	-0,20	0,75*		0,13
DH	-0,06	0,70*	0,68*	0,27	0,51	-0,26	-0,30	

**p*<0.05; ***p*<0.01

are presented in Table 4, below and above the diagonal for 2022-2023 and 2023-2024. In the first year of the study, the results showed a positive correlation of the relative value of chlorophyll with yields (r = 0, 58, significant at level 0,05). Under the conditions of 2024, a low negative correlation was registered. Such traits as plant height and 1000 kernel weight were in low negative but not significant correlation in both years of the investigation.

The precise predicting of the nitrogen content in the leaves of plants grown under different conditions of the environment is difficult due to the different ways of its uptake and accumulation. On the one hand, this is the nitrogen taken up from soil after heading stage, and on the other – the remobilized nitrogen from other organs prior to the beginning of this stage (Aranguren et al., 2021). The level of chlorophyll content is suitable for evaluation and respective correction of the nitrogen nutrition, when necessary, especially with regard to the realization of the qualitative parameters (Matsunaka et al., 1997).

Hoel (2002) found out that the percent of protein in grain usually increased with the higher readings of a portable chlorophyll meter, but due to the variation over years, it is not recommended to use the measurements for prognosis of its level. This is supported by the results from present study. The correlation between CCI and protein content was positive, low and easy to break. The results were unidirectional over vegetative growth periods. The duration of the grain filling stage and its rate were determining for dry matter accumulation and grain weight (Khan et al., 2014). The effect of the environment was significant (Brdar, 2008). Pireivatlou et al. (2011) reported that the high rate of grain filling was with higher weight for accumulation of assimilates and formation of productivity. The results were confirmed both under irrigation conditions and drought. A similar tendency, under different climatic conditions, has been reported by Jockovich et al. (2014) and Wu et al. (2018). The positive correlation between the photosynthetic rate and the grain yield indicates that after anthesis the high level of photosynthesis is important for higher number and weight of grains (Djanaguiraman et al., 2020). The chlorophyll content in the leaves of wheat reaches its highest level at heading stage (Jin et al., 2012), then it starts decreasing. In this relation, the genotypes identified as having high chlorophyll content at grain filling stage can be considered potential germplasm for breeding improvement work.

CONCLUSION

The highest variation in the values of the relative chlorophyll content was observed in the group of genotypes with shorter date to heading. The results were unidirectional over years. The significant differences established in the Chlorophyll content index confirmed that it is possible to determine the physiological status of the plants and differentiate them during the breeding process.

The year conditions were determining for the strength of the correlation of chlorophyll content with productivity. The correlations with protein content and test weight were positive but low and statistically not significant. The differences established over years did not allow an objective assessment of the levels of the investigated parameters based on the measurements with chlorophyll meter.

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