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Evaluation of flag leaf chlorophyll content index in 30 spring wheat genotypes under three irrigation regimes

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Abstract

The chlorophyll content in flag leaves reflects photosynthetic activity and yield potential of wheat plants. A two-year field experiment was carried out to evaluate flag leaf chlorophyll content index (CCI) at different growth stages [Feekes 10.5.2 (anthesis), Feekes 11.1 (kernels milky ripe, GF-1), and Feekes 11.2 (kernels mealy ripe, GF-2)] and irrigation regimes [non-irrigated, 50%-evapotranspiration (ET) irrigated, and 100%-ET irrigated] in 30 spring wheat genotypes. The CCI of four groups with different yield performances across irrigation regimes: high-yield genotype (HYG), low-yield genotype (LYG), drought-resistant genotype (DRG) and drought-susceptible genotype (DSG), were compared. Maximum flag leaf CCI was recorded at GF-1 stage for most genotypes under all irrigation regimes. Severe drought stress decreased CCI value while both severe and moderate drought stress accelerated the CCI loss after GF-1 stage. No correlation between CCI and grain yield was found, but CCI decrease (CCID) and grain yield was negatively correlated ($P < 0.05$), especially for the well-watered condition. Compared with the other groups, DRG had much higher CCI, especially CCI evaluated at anthesis under well-watered condition; the CCI of HYG declined either later or slower after GF-1 stage. Results from this study suggest that GF-1 stage may be the optimum time for evaluating flag leaf CCI; the CCID from GF-1 to GF-2 rather than the CCI value could be used as an index to predict grain yield; the CCI value may be considered as an indicator for screening drought resistant genotypes in wheat breeding programs.

Keywords: Chlorophyll content index, Drought stress, Grain yield, Growth stage, Wheat.
Abbreviations: CCI, chlorophyll content index; CCIa, CCI evaluated at anthesis; CCID, CCI decrease from GF-1 to GF-2; $CCIg_1$, CCI evaluated at GF-1 stage; $CCIg_2$, CCI evaluated at GF-2 stage; DRG, drought-resistant genotype; DSG, drought-susceptible genotype; GF-1, Feekes 11.1 (kernels milky ripe); GF-2, Feekes 11.2 (kernels mealy ripe); HYG, high-yield genotype; LYG, low-yield genotype.

Introduction

In cereal crops, the top-most leaf, i.e., flag leaf, is an important source of carbohydrate production. It makes up approximately 75% of the effective leaf area that contributes to grain fill (Miller, 1999). The characteristics of flag leaf have been considered to reflect photosynthetic activity and yield production. Flag leaf is considered to be one of the greatest components in determining grain yield potential in most cereal crops (Hirota et al., 1990). In grain filling period water deficit induces fast senescence in wheat, especially for older leaves. The primary expression of leaf senescence is the breakdown of chlorophyll and the decline of photosynthetic activity. Yellow and old leaves due to loss of chlorophyll lose their photosynthetic power. It is generally accepted that the genotypes which are able to sustain photosynthesis in flag leaf for longer time tend to yield more (Richards, 2000). It has been reported in several studies that there widely exists a difference of chlorophyll content among different wheat genotypes under the identical climatic, soil and farming conditions (Paknejad et al., 2007; Tas and Tas, 2007; Guóth et al., 2009; Keyvan, 2010; Kiliç and Yağbasanlar, 2010). Chlorophyll is the pigment responsible for the green color of plants, as the light capturing molecule in photosystems, it occupies a unique role in the physiology of green plants. Therefore, chlorophyll has been used as a sensitive indicator of plant physiological status and its quantification has always been of special interest to plant scientists. It has been reported that chlorophyll content had changed throughout the growing season of plants, and chlorophyll content of plants begins to decline at the start of aging in plant leaf (Matile et al., 1988; Pulkrabek, 1998). Furthermore, changes in accumulation of chlorophyll in plants are affected by growth conditions, and chlorophyll content reduces in negative conditions (Masuda et al., 2002). Leaf chlorophyll content provides valuable information about physiological status of plants. Photosynthesis is one of the main metabolic processes determining crop production. Some related studies showed that leaf chlorophyll content was positively correlated with photosynthetic capacity (Araus et al., 1997), high chlorophyll content in leaves was considered as a favorable trait in crop production (Teng et al., 2004), and the drought stress caused a faster chlorophyll breakdown (Ommen et al., 1999; Guóth et al., 2009). However, the effect of drought on photosynthesis has long been controversial and it is still not clear whether chlorophyll content parameter is a good indicator for predicting yield and drought resistance.The objectives of this study, therefore, were to: (i) examine CCI of 30 spring wheat genotypes at three different growth stages and three irrigation regimes, (ii) investigate the relationship between CCI and grain yield, and compare CCI for genotypes in the four groups (HYG, LYG, DRG, and DSG), and (iii) determine suitable selection criteria for screening high yielding and drought resistant wheat genotypes.

Results

Analysis of variance

There was no year effect for all CCI (CCIa, $CCIg_1$, and $CCIg_2$) evaluated at different growth stages. The genotype × irrigation (G*I) interaction effects for CCIa, $CCIg_1$, and $CCIg_2$ were not significant (Table 2). Differences among the three irrigation regimes (T1, T2 and T3) were not significant in all CCI (CCIa, $CCIg_1$ and $CCIg_2$) evaluated at different growth stages. Analysis of variance revealed significant differences ($P < 0.001$) among genotypes in CCIa, $CCIg_1$, and $CCIg_2$.

Chlorophyll content index at different growth stages and irrigation regimes

The CCI evaluated at different growth stages differed under all irrigation regimes (T1, T2, and T3), especially for T2 (Fig. 1a). During the growing season, the maximum CCI in flag leaf was reached at GF-1 (Feekes 11.1, kernels milky ripe) for most spring wheat genotypes in different water conditions. The maximum mean CCI of the 30 genotypes were 36.8, 38.3, and 37.6 for T1, T2, and T3, respectively (Table 3), suggesting that moderate drought stress tended to increase the CCI in flag leaves. The greater difference in CCI across irrigation regimes was observed at GF-1 stage (Fig. 1b). Severe drought stress caused a decrease in flag leaf CCI sampled at anthesis and GF-1. The mean CCI of the 30 genotypes under T1, T2, and T3 were 34.7, 35.2, and 35.7 at anthesis, and 36.8, 38.3, and 37.6 at GF-1, respectively (Table 3). The mean CCI sampled at GF-1 ($CCIg_1$) under moderate drought stress (T2) was greater than that under well-watered (T3). However, at a later growth stage (GF-2), the mean CCI under T3 was greater than that under T2. The reason for lacking CCI measurements under the non-irrigated treatment (T1) at GF-2 (Feekes 11.2, kernels mealy ripe) is that the edges of flag leaves were getting rolled and dried, and the flag leaves were not wide enough for measuring. During the period GF-1 to GF-2, genotypes under T2 showed a higher rate of chlorophyll loss than those under T3, indicating that drought stress accelerated the flag leaf chlorophyll breakdown.

Correlations between grain yield and CCI evaluated at different growth stages

No significant correlation between CCI (CCIa, $CCIg_1$, and $CCIg_2$) and grain yield was observed within each irrigation regime or over the three regimes (data not shown). However, correlations between CCID and grain yield were negative and significant, the coefficients were -0.205 and -0.364 ($P < 0.05$) for T2 and T3 irrigation regimes, respectively. Within each of the three irrigation regimes, CCIa was positively correlated with $CCIg_1$ under T1 ($P < 0.001$), CCIa was positively correlated with $CCIg_1$ and $CCIg_2$ under T2 ($P < 0.01$) and T3 ($P < 0.001$). The correlation between CCIa and $CCIg_1$ was more significant than that between CCIa and $CCIg_2$ under all irrigation regimes. No significant relationship was found between $CCIg_1$ and $CCIg_2$ under T2 and T3 (Table 4). The relationships between CCI evaluated at different growth stages and under different irrigation treatments were always significant ($r > 0.4$, $P < 0.05$) except for those between $CCIg_1$ and $CCIg_2$. Among these, the greatest correlation coefficient occurred between CCIa-T2 and CCIa-T3 ($r = 0.899$, $P < 0.001$), followed by $CCIg_1$-T1 and CCIa-T3 ($r = 0.805$, $P < 0.001$), and then $CCIg_1$-T1 and CCIa-T2 ($r = 0.804$, $P < 0.001$).

Chlorophyll content index in different wheat genotypes

Differences among the 30 genotypes were observed for CCI, the mean CCI of 2009 and 2010 evaluated at three different growth stages for 30 genotypes in three irrigation regimes are presented in Table 3. Variation of CCI among 30 genotypes ranged from 25.8 (Klasic under T3 at GF-2) to 51.0 (Alzada under T3 at anthesis). In the 30 genotypes, Alzada, McNeal (DRG), and Jerome had the greatest CCI values, while Vida, Alturas (HYG), Cataldo (LYG), IDO686 (DSG), and Blanca Royale had the smallest ones across different growth stages and irrigation regimes (Table 3). For most genotypes, the flag leaf CCI values were found to decrease from the stage GF-1 to GF-2. However, there were exceptional genotypes (IDO687, McNeal, Blanca Grande, IDO644, UC1600, IDO702, and Alturas) whose CCI values continued to increase after the GF-1 stage in both T2 and T3 treatments. On average, the CCI values decreased 2.5 and 1.5 under T2 and T3, respectively from GF-1 to GF-2, which also indicating that the CCI loss under drought was faster than that under well-watered. Agawam (DRG), Klasic (LYG), IDO694, Louise, and Snowcrest (LYG) showed a faster CCI decrease than other genotypes after GF-1 stage under both T2 and T3. Comparison of the mean CCI of genotypes in each of the four groups (HYG, LYG, DRG, and DSG) and the mean CCI of all 30 genotypes at different growth stages under each of three irrigation regimes based on two years' data is presented in Fig. 2 and Table 5. To show the difference among different kinds of genotypes, the mean CCI of the 30 genotypes was used as a reference. Under the non-irrigated (T1) regime, flag leaf CCI of all groups (HYG, LYG, DRG, and DSG) showed an increase trend from anthesis to GF-1 stage (Fig. 2a). The DRG had the greatest flag leaf CCI value at both stages, which could be used to distinguish DRG from the rest groups. In moderate drought (T2) conditions, DRG had the greatest flag leaf CCI at anthesis and GF-2 stages, and its difference with the other three groups was significant ($P < 0.05$) (Fig. 2b). The flag leaf CCI of HYG, LYG, and DSG showed a first increase then decrease trend along with the more advanced developmental stages (from anthesis to GF-1, and to GF-2). The HYG had smaller CCID while DSG and LYG had greater CCID, which was inversely associated with the yield performance (Table 5). However, the CCI of DRG maintained an increase trend from anthesis to GF-2, which could also be used to distinguish DRG from the other genotypes. Under the well-watered (T3) regime, flag leaf CCI of DRG, LYG, and DSG showed a first increase then decrease trend from anthesis to GF-2. The ranking of CCID was: DSG < DRG < LYG, which was inversely associated with the corresponding yield performance (Fig. 2c, Table 5).

Table 1. Spring wheat cultivars and advanced lines developed by Montana State University (MSU), University of Idaho (U of I), University of California Davis (UCD), Washington State University (WSU), Resource Seeds (RS), and WestBred (WB).

No.	Genotype	Group†	Class‡	Origin	PI No.	Reference
1	Choteau	LYG	HRS	MSU	PI 633974	Lanning et al., 2004
2	Vida		HRS	MSU	PI 642366	Lanning et al., 2006
3	McNeal	DRG	HRS	MSU	PI 574642	Lanning et al., 1994
4	Alzada		Durum	WB	PI 634820	NA§
5	Agawam	DRG	HWS	WB	PI 648027	NA
6	Conan		HRS	WB	PI 607549	NA
7	Hank		HRS	WB	PI 613583	NA
8	WB936		HRS	WB	PI 587200	NA
9	Lassik		HRS	UCD	PI 653535	NA
10	UC1600	LYG	HRS	UCD	Breeding line	NA
11	Louise		SWS	WSU	PI 634865	Kidwell et al., 2006
12	Alpowa	DRG	SWS	WSU	PI 566596	Barrett and Kidwell, 1998
13	WA8039		SWS	WSU	Breeding line	NA
14	UI Winchester		HRS	U of I	PI 642362	NA
15	Jerome		HRS	U of I	PI 632712	Souza et al., 2005
16	IDO702	HYG	HRS	U of I	Breeding line	NA
17	Jefferson		HRS	U of I	PI 603040	Souza et al., 1999
18	Alturas	HYG	SWS	U of I	PI 620631	Souza et al., 2004
19	Cataldo	LYG	SWS	U of I	PI 642361	Chen et al., 2009
20	Lolo	DSG	HWS	U of I	PI 614840	Souza et al., 2003
21	UI Lochsa		HWS	U of I	PI639952	NA
22	IDO694		HWS	U of I	Breeding line	NA
23	IDO686	DSG	SWS	U of I	Breeding line	NA
24	IDO687		SWS	U of I	Breeding line	NA
25	IDO599	HYG	SWS	U of I	Breeding line	NA
26	IDO644		SWS	U of I	Breeding line	NA
27	Klasic	LYG	HWS	RS	PI 486139	Barrett and Kidwell, 1998
28	Snowcrest	LYG	HWS	RS	PI 642376	NA
29	Blanca Grande		HWS	RS	PI 631481	NA
30	Blanca Royale		HWS	RS	PI 655033	NA

† HYG, high-yield genotype; LYG, low-yield genotype; DRG, drought-resistant genotype; DSG, drought-susceptible genotype. ‡ HRS, hard red spring wheat; HWS, hard white spring wheat; SWS, soft white spring wheat. § NA, not available.

Table 2. Analyses of variance for flag leaf chlorophyll content index (CCI) evaluated at anthesis (CCIa), GF-1 (Feekes 11.1, kernels milky ripe; $CCIg_1$), and GF-2 (Feekes 11.2, kernels mealy ripe; $CCIg_2$) in 30 spring wheat genotypes (data from 2009 and 2010 were combined).

Trait	Source of variation	df	Mean square	*F* value	*P*
CCIa	Genotype	29	96.15	9.31	<0.001
	Irrigation	2	13.48	1.3	0.18
	G × I†	58	9.91	0.96	0.56
$CCIg_1$	Genotype	29	83.78	4.99	<0.001
	Irrigation	2	33.61	2	0.09
	G × I	58	19.45	1.16	0.29
$CCIg_2$	Genotype	29	42.3	6.85	<0.001
	Irrigation	1	2.2	0.36	0.55
	G × I	29	1.53	0.31	0.27

† G×I, Genotype × Irrigation interaction.

However, the CCI of HYG showed a consistent increase trend from anthesis to GF-2, which could be used to distinguish HYG from the others. In addition, the CCI of DRG was significantly higher than the other three groups at anthesis, which could be used to distinguish DRG from the others.

Discussion

Accurate field evaluation of physiological traits is critical in the process of agricultural research for understanding the characteristics and mechanism of plants. In the present study, maximum CCI of genotypes was observed at GF-1 stage for most genotypes under all water conditions. The greater difference in CCI across water regimes also occurred at GF-1 stage. Therefore, our study suggests that the GF-1 (Feekes 11.1) stage during grain filling may be the optimal time for flag leaf CCI measurement. Different result was reported by Ommen et al. (1999) that the maximum chlorophyll content of flag leaf reached at anthesis under optimum growth conditions in spring wheat. Therefore, more studies are needed for further confirmation. The relationship between flag leaf CCI value and different drought stress levels was not obvious in the present study, and there was no sufficient evidence for lower CCI value to be associated with higher stress levels. Therefore, it can be inferred that there might be other major factors which can modify flag leaf chlorophyll content besides drought stress, or the flag leaf chlorophyll content possesses little sensitivity to drought stress. However, results from this study indicated that severe drought stress decreased the CCI value, and drought stress (both severe drought and moderate drought) accelerated the CCI reduction

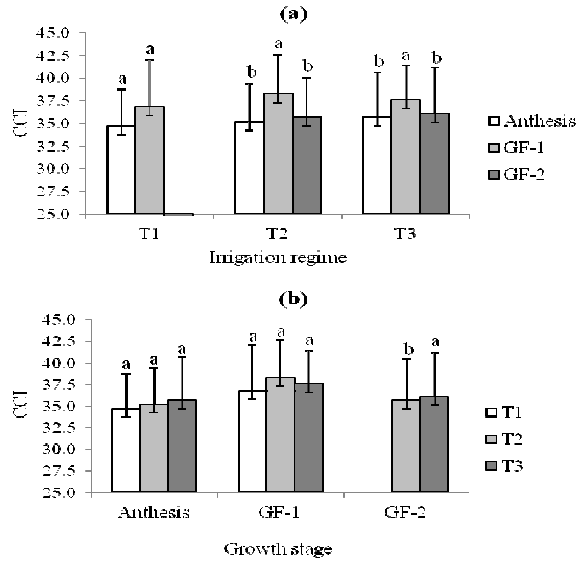

Fig 1. The mean flag leaf chlorophyll content index (CCI) ± SD of 30 spring wheat genotypes evaluated at (a) different growth stages: anthesis, GF-1 (Feekes 11.1, kernels milky ripe), and GF-2 (Feekes 11.2, kernels mealy ripe), and (b) different irrigation regimes: T1 (non-irrigated), T2 (50%-ET irrigated), and T3 (100%-ET irrigated) (ET, evapotranspiration) based on data from the year 2009 and 2010. Means with different letters are significantly ($P < 0.05$) different.

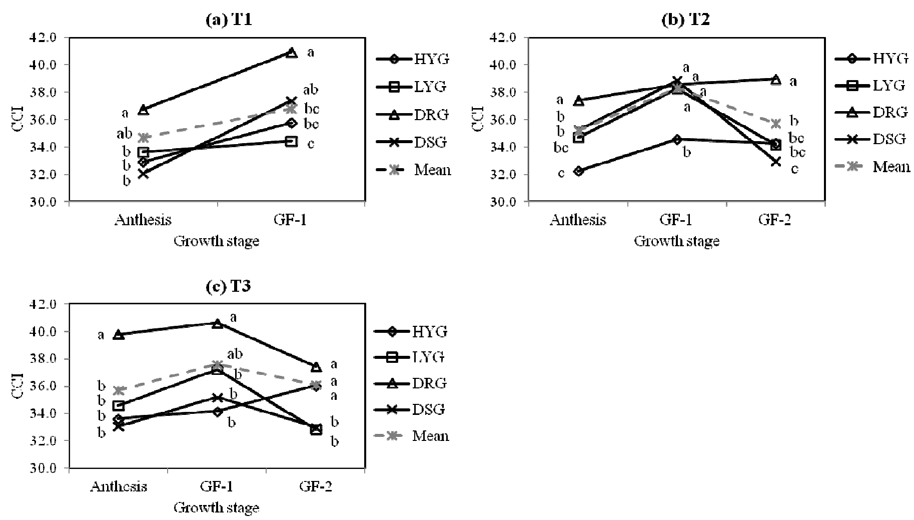

Fig 2. Comparison of the mean flag leaf chlorophyll content index (CCI) of 30 spring wheat genotypes (Mean) and the mean CCI of genotypes in each of the four groups (high-yield genotype (HYG), low-yield genotype (LYG), drought-resistant genotype (DRG), and drought-susceptible genotype (DSG)) evaluated at different growth stages [anthesis, GF-1 (Feekes 11.1, kernels milky ripe), and GF-2 (Feekes 11.2, kernels mealy ripe)] under three irrigation regimes: (a) T1 (non-irrigated), (b) T2 (50%-ET irrigated), and (c) T3 (100%-ET irrigated) (ET, evapotranspiration) based on data from the year 2009 and 2010. Means with different letters are significantly ($P < 0.05$) different.

after GF-1 stage. Similar findings were reported in previous works (Ommen et al., 1999; Tas and Tas, 2007; Keyvan, 2010; Saeedipour, 2011). In this study, the correlation between flag leaf CCI and grain yield was not significant under all water conditions. Therefore, the flag leaf CCI value itself cannot be an index for identifying high yielding wheat genotypes. Similar results were reported by Fischer et al. (1998) that flag leaf greenness was not associated with yield in wheat cultivars; and by Ghobadi et al. (2011) that chlorophyll characteristics were not significantly correlated with grain yield in wheat genotypes. However, different results were also reported that chlorophyll content was positively associated with grain yield under drought conditions in 14 wheat cultivars (Kiliç and Yağbasanlar, 2010), and the correlation between flag leaf chlorophyll content and grain yield under heat and drought stresses was negatively significant in 18 bread wheat genotypes (Mohammadi et al., 2009). However, this study observed that the CCID was reversely associated with grain yield, genotypes with slower CCI loss tended to yield more, which confirmed the previous finding (Richards, 2000). Our results suggest that the CCID from GF-1 to GF-2 could be a more reliable indicator than the CCI value for selecting high yielding wheat genotypes. Compared with the other groups, HYG showed lower CCI value but its CCI declined either slower or later under different water conditions. However, Paknejad et al. (2007) reported that high-yielding varieties had higher chlorophyll content in bread wheat cultivars. Further confirmation study is needed. In the present study, significant difference between DRG and the other groups has been found in CCI value, suggesting that flag leaf CCI value could be used as an indicator in screening drought resistant wheat genotypes. Thereinto, CCI evaluated at anthesis under well-watered condition (T1) is the best for screening drought resistant genotypes, followed by CCI evaluated at anthesis and GF-2 under moderate drought stress (T2). The CCI in DRG was much higher than that in DSG except for $CCIg_1$ under T2, but under the well-watered condition, the rate of CCI declining for DRG was higher than DSG. Similar result was reported by Guóth et al. (2009) that the rate of chlorophyll loss was much higher in the drought-tolerant wheat cultivars.

Materials and methods

Plant materials

Thirty spring wheat genotypes, including 22 cultivars and eight elite breeding lines, were used in this experiment. The 22 cultivars are well adapted in the Pacific Northwest of the U.S. The 30 genotypes are comprised of 12 hard red, nine soft white, eight hard white, and one durum wheat (Table 1). In a previous paper (Li et al., 2012), selected genotypes from the 30 ones were classified into four groups: HYG (high-yield genotype), LYG (low-yield genotype), DRG (drought-resistant genotype), and DSG (drought-susceptible genotype) based on their yield performance across different irrigation regimes. The HYG produced greater grain yield under all irrigation regimes; DRG produced greater grain yield under the non-irrigated regime, and intermediate grain yield under irrigated regimes; DSG produced less grain yield under the non-irrigated regime and greater grain yield under the irrigated regimes; and LYG produced less grain yield than other genotypes under all irrigation regimes.

Experimental conditions

Experiments were carried out in two seasons of 2009 and 2010 at the research field of University of Idaho Aberdeen Research & Extension Center at Aberdeen, ID (42.96° N, 112.82° W, and elevation 1342 m). In this area, the annual precipitation is 203 to 279 mm, the mean annual air temperature is 7.2 to 8.3 ℃, and the frost-free period is 110 to 130 days. The soil at the experimental site was a Declo loam (coarse-loamy, mixed, superactive, mesic, Xeric Haplocalcids) with 0 to 2% slopes and pH of 8.1. Over the growing season, 15.8 and 10.6 g m^{-2} of N and P were applied based on a soil test before planting. Herbicides including Huskie (pyrasulfotole, bromoxynil octanoate, and bromoxynil heptanoate) and Starane (fluroxypyr 1-methylheptyl ester: ((4-amino-3,5-dichloro-6-fluoro-2-pyridinyl)oxy)acetic acid, 1-methylheptyl ester) were applied at the rates of 0.084 and 0.112 g m^{-2}, respectively, during jointing stage. Seeds were planted on 22 April 2009 and 14 April 2010, respectively. Planting depth was 3.8 cm and seeding rate was 300 seeds per m^2. In two seasons, wheat was planted in four-row plots (2009) and seven-row plots (2010), respectively, with the same plot size of 3.048 m long by 1.524 m wide. The experiment was laid out in a split block experimental design, in three replicates, keeping drip irrigation treatments in the fixed main plots and genotypes in sub-plots. Genotypes were randomized within each irrigation main plot. Three irrigation regimes: T1 (non-irrigated, severe drought), T2 (50%-ET irrigated, moderate drought), and T3 (100%-ET irrigated, non-stress) (ET, evapotranspiration) were applied by above-ground drip system and determined based on the crop water use information from the Pacific Northwest Cooperative Agricultural Weather Network recommendations (USBOR, 2009-2010). Irrigation applied once a week, started before heading and during heading for 2009 and 2010 seasons, respectively, and ended at maturity. The amount of water applied per irrigation was determined by the amount of water that plants used and soil surface evaporated (ET) in one week at corresponding growth stages. Irrigated plants received irrigation water and rainfall water, while non-irrigated plants only received rainfall water during the growing season (Apr. to Aug.). During the 2009 growing season, all plots (T1, T2 and T3) received 359 mm of rainfall and irrigated plots (T2 and T3) received an additional 173 mm and 345 mm of irrigation water, respectively. During the 2010 growing season, all plots received 102 mm of rainfall and the T2 and T3 irrigated plots received an additional 248 mm and 452 mm of irrigation water, respectively.

Evaluation of chlorophyll content index

Chlorophyll content was measured in flag leaves of 10 randomly selected fertile shoots (those with an ear) *in situ* in each plot by using a portable chlorophyll content meter (CCM-200, Opti-Sciences Inc., NH, USA), which calculated a chlorophyll content index (CCI) value that is proportional to the amount of chlorophyll. Ten measurements were taken for each plot. The measurements were taken at anthesis and grain filling stages, corresponding to the Feekes growth scale (Miller, 1999) Feekes 10.5.2 (anthesis), Feekes 11.1 (kernels milky ripe, GF-1), and Feekes 11.2 (kernels mealy ripe, GF-2), respectively. The CCI evaluated at three different growth stages (anthesis, GF-1 and GF-2) were expressed as CCIa, $CCIg_1$, and $CCIg_2$, respectively. At Feekes 11.2 (GF-2), flag leaves of plants in the non-irrigated regime (T1) were rolled and greatly lost greenness, which made the CCI measurement unobtainable. The CCI decrease (CCID) from GF-1 to GF-2 was calculated as difference between $CCIg_1$ and $CCIg_2$ ($CCIg_1$- $CCIg_2$).

Grain yield measurement

Plots were harvested using a Wintersteiger Classic small plot combine (1998 Wintersteiger Elite, Wintersteiger Seedmech, Salt Lake City, UT) equipped with a Harvest Master weigh system (HM-400, Juniper Systems, Logan, UT). Grain yield was determined from the grain weight with a moisture content of 12% of each plot for each genotype.

Statistical analysis

Data were analyzed using SAS Version 9.1 (SAS Institute, Cary, NC) and SPSS 17.0 statistical software. Pearsons' correlation was conducted among evaluated traits. Analysis of variance (ANOVA) for CCI was performed using the Proc GLM procedure. The effect of year between 2009 and 2010 was also tested. Significant differences among genotypes and

Table 3. The mean flag leaf chlorophyll content index (CCI) evaluated at different growth stages (anthesis, GF-1, and GF-2) and grain yield (GY, g m^{-2}) of 2009 and 2010 under three irrigation regimes: T1 (non-irrigated), T2 (50%-ET irrigated), and T3 (100%-ET irrigated) (ET, evapotranspiration) for 30 spring wheat genotypes.

No.	Genotype	T1			T2				T3			
		CCIa†	CCIg$_1$†	GY	CCIa	CCIg$_1$	CCIg$_2$†	GY	CCIa	CCIg$_1$	CCIg$_2$	GY
1	Choteau	34.6^{cde}‡	37.8^{def}	154.8^{bcd}	36.2^{cde}	39.4^{cde}	38.3^{cd}	341.5^{c}	36.9^{de}	37.6^{cde}	39.8^{cde}	539.6^{ab}
2	Vida	34.2^{cde}	29.8^{fg}	183^{abcd}	29.1^{h}	33^{gh}	29.3^{fg}	480.4^{ab}	29.8^{gh}	35.8^{ef}	32.6^{fgh}	636.3^{ab}
3	McNeal	41.1^{bc}	43.4^{c}	199.7^{abc}	37.1^{cd}	39.3^{cde}	47.1^{a}	458.8^{ab}	40.8^{c}	38.9^{cd}	44.5^{b}	534.9^{ab}
4	Alzada	42.9^{b}	48.6^{a}	181.6^{abcd}	44.4^{a}	47.7^{a}	43.2^{bc}	420^{ab}	51^{a}	46.8^{a}	46.7^{a}	560.4^{ab}
5	Agawam	37.4^{c}	37.9^{def}	245.1^{ab}	37.7^{cd}	40.2^{cd}	31.7^{def}	483.1^{ab}	39.4^{cd}	44^{b}	30.3^{ghi}	473.2^{b}
6	Conan	31.3^{def}	33.4^{efg}	217.6^{abc}	34.8^{de}	36.6^{def}	35.6^{cde}	429.5^{ab}	36.6^{de}	40.1^{cd}	37.3^{def}	558.4^{ab}
7	Hank	34.9^{cde}	40.5^{de}	209.9^{abc}	34.8^{de}	40.2^{cd}	36.2^{cde}	421.9^{ab}	35.5^{def}	38.5^{cd}	34.1^{fg}	618.8^{ab}
8	WB936	37.2^{c}	39.3^{de}	183.4^{abcd}	38.8^{c}	37de	35.1^{cde}	401.6^{ab}	37de	41^{c}	40.6^{cd}	581.5^{ab}
9	Lassik	33.2^{de}	35.3^{efg}	207.7^{abc}	37.4^{cd}	41.4^{cd}	37.8^{cd}	488.7^{ab}	37.2^{de}	38.9^{cd}	38.3^{de}	634.4^{ab}
10	UC1600	36.2^{cd}	37.7^{def}	149.3^{bcd}	34.7^{de}	35.5^{efg}	37.5^{cd}	406.2^{ab}	34.9^{def}	34.7^{efg}	36.9^{def}	503.4^{b}
11	Louise	38.9^{c}	40.6^{de}	193^{abcd}	39.4^{c}	40.8^{cd}	31.3^{def}	465.3^{ab}	37.8^{cde}	42.6^{bc}	36.8^{def}	574.9^{ab}
12	Alpowa	31.8^{def}	41.5^{d}	237.4^{ab}	37.4^{cd}	36.3^{def}	38.1^{cd}	415.2^{ab}	39.2^{cd}	39^{cd}	37.5^{def}	572.1^{ab}
13	WA8039	36.1^{cd}	37.5^{def}	257.5^{a}	33.2^{efg}	43.8^{bc}	43.3^{bc}	477^{ab}	35.3^{def}	35.9^{ef}	35.2^{efg}	636.6^{ab}
14	UI Winchester	31.9^{def}	34.8^{efg}	203^{abc}	34.9^{de}	37.8^{de}	31.6^{def}	432.5^{ab}	35^{def}	37.8^{cde}	36.9^{def}	648.4^{ab}
15	Jerome	35.5^{cde}	45^{bc}	166.3^{abcd}	44.4^{a}	37.1^{de}	36.2^{cde}	441^{ab}	44.5^{b}	42.8^{bc}	42.4^{c}	576.1^{ab}
16	IDO702	32.9^{de}	37.5^{def}	216.8^{abc}	33.4^{efg}	35.6^{efg}	37.2^{cd}	487.4^{ab}	33.7^{efg}	35.1^{efg}	36.9^{def}	657.9^{ab}
17	Jefferson	32.6^{de}	29.2^{fg}	191.7^{abcd}	31.8^{fgh}	39.6^{cde}	32.9^{def}	476.3^{ab}	31.3^{fg}	39.1^{cd}	35.9^{defg}	545.5^{ab}
18	Alturas	30.8^{ef}	29.9^{fg}	191.6^{abcd}	29.6^{gh}	28.7^{h}	29^{g}	465.8^{ab}	28.6^{hi}	29.2^{h}	34.2^{fg}	659.7^{ab}
19	Cataldo	31.4^{def}	32.5^{efg}	118.4^{d}	29.7^{gh}	33.8^{fg}	32.1^{def}	375.2^{b}	29.5^{gh}	36.7^{de}	29^{hi}	578.3^{ab}
20	Lolo	38.1^{c}	45.7^{b}	145.7^{bcd}	41.9^{b}	42.3^{c}	35.8^{cde}	458.7^{ab}	39.2^{cd}	37de	34.3^{fg}	608.4^{ab}
21	UI Lochsa	32.6^{de}	34.3^{efg}	204.5^{abc}	31.6^{fgh}	46.5^{ab}	33.4^{def}	412.7^{ab}	30.8^{fgh}	36^{ef}	32.7^{fgh}	589.2^{ab}
22	IDO694	45.6^{a}	41.5^{d}	218.8^{abc}	39.1^{c}	43.6^{bc}	36.6^{cde}	472.2^{ab}	35.4^{def}	37.8^{cde}	31.1^{gh}	564.3^{ab}
23	IDO686	26^{g}	29.1^{fg}	132.8^{cd}	28.6^{h}	35.4^{efg}	30.1^{efg}	492.1^{ab}	26.9^{i}	33.4^{fg}	31.7^{gh}	644.8^{ab}
24	IDO687	32.3^{de}	32.5^{efg}	191.2^{abcd}	32.3^{efg}	30.5^{h}	33.8^{def}	444.5^{ab}	31.4^{fg}	29.2^{h}	38.9^{de}	577^{ab}
25	IDO599	35.1^{cde}	40de	240.6^{ab}	33.8^{def}	39.4^{cde}	36.6^{cde}	448.7^{ab}	38.7^{cd}	38.1^{cde}	37^{def}	742.6^{a}
26	IDO644	38.5^{c}	34.2^{efg}	174.7^{abcd}	36^{cde}	37.2^{de}	44.6^{ab}	461.5^{ab}	38.8^{cd}	39^{cd}	41.2^{cd}	645.9^{ab}
27	Klasic	32.5^{de}	27.8^{g}	134.4^{cd}	36^{cde}	41.4^{cd}	29.8^{efg}	349.4^{c}	36.4^{de}	39.5^{cd}	25.8^{i}	467.6^{b}
28	Snowcrest	33.4^{de}	36.4^{ef}	176.9^{abcd}	36.7^{cd}	41.2^{cd}	33.1^{def}	400^{ab}	35.2^{def}	37.5^{cde}	32.8^{fgh}	472.2^{b}
29	Blanca Grande	34.1^{cde}	37.8^{def}	184.4^{abcd}	31.9^{fgh}	34^{fg}	41.5^{bcd}	505.6^{ab}	34.4^{ef}	33.9^{fg}	39de	583.5^{ab}
30	Blanca Royale	30^{f}	33.1^{efg}	186.3^{abcd}	30.1^{gh}	34.1^{fg}	32.5^{def}	535.1^{a}	29.9^{gh}	32.6^{fgh}	32.4^{fgh}	535.4^{ab}
Mean		34.75	36.81	189.9	35.25	38.30	35.70	444.9	35.69	37.63	36.10	584.0
SD		4.02	5.25	33.95	4.17	4.35	4.71	44.84	4.94	3.85	5.13	62.40

† CCIa, CCI evaluated at anthesis; CCIg$_1$, CCI evaluated at GF-1 stage (Feekes 11.1, kernels milky ripe); CCIg$_2$, CCI evaluated at GF-2 stage (Feekes 11.2, kernels mealy ripe).

‡ Means followed by different letters are significantly ($P < 0.05$) different.

Table 4. Pearsons' correlation coefficients among the mean flag leaf chlorophyll content index (CCI) of 2009 and 2010 evaluated at anthesis (CCIa), GF-1 (Feekes 11.1, $CCIg_1$), and GF-2 (Feekes 11.2, $CCIg_2$) in 30 spring wheat genotypes across three irrigation regimes: T1 (non-irrigated), T2 (50%-ET irrigated), and T3 (100%-ET irrigated) (ET, evapotranspiration).

	CCIa-T1	$CCIg_1$-T1	CCIa-T2	$CCIg_1$-T2	$CCIg_2$-T2	CCIa-T3	$CCIg_1$-T3
$CCIg_1$-T1†	$.707^{***}$						
CCIa-T2†	$.682^{***}$	$.804^{***}$					
$CCIg_1$-T2	$.533^{**}$	$.472^{**}$	$.556^{**}$				
$CCIg_2$-T2	$.516^{**}$	$.630^{***}$	$.510^{**}$	ns‡			
CCIa-T3†	$.662^{***}$	$.805^{***}$	$.899^{***}$	$.536^{**}$	$.673^{***}$		
$CCIg_1$-T3	$.530^{**}$	$.536^{**}$	$.751^{***}$	$.628^{***}$	ns	$.793^{***}$	
$CCIg_2$-T3	$.407^{*}$	$.551^{**}$	$.409^{*}$	ns	$.748^{***}$	$.634^{***}$	ns

$^{*}P < 0.05$. $^{**}P < 0.01$. $^{***}P < 0.001$. † T1, T2, and T3 refer to irrigation regimes T1, T2, and T3 respectively. ‡ ns, not significant at $P < 0.05$.

Table 5. The mean flag leaf chlorophyll content index (CCI) and CCI decrease (CCID) of 30 genotypes and genotypes in each of the four groups (HYG, LYG, DRG, and DSG) evaluated at different growth stages (anthesis, GF-1, and GF-2) under the two irrigated regimes: T2 (50%-ET irrigated) and T3 (100%-ET irrigated) (ET, evapotranspiration) based on data from the year 2009 and 2010.

Group†	T2				T3			
	CCIa‡	$CCIg_1$‡	$CCIg_2$‡	CCID‡	CCIa	$CCIg_1$	$CCIg_2$	CCID
HYG	32.3^{c}§	34.6^{b}	34.3^{bc}	0.3^{b}	33.6^{b}	34.2^{b}	36^{a}	-1.8^{b}
LYG	34.7^{bc}	38.2^{a}	34.2^{bc}	4^{a}	34.6^{b}	37.2^{b}	32.9^{b}	4.3^{a}
DRG	37.4^{a}	38.6^{a}	39^{a}	-0.4^{b}	39.8^{a}	40.6^{a}	37.4^{a}	3.2^{ab}
DSG	35.2^{b}	38.8^{a}	33^{c}	5.8^{a}	33.1^{b}	35.2^{b}	33^{b}	2.2^{ab}
Mean of 30	35.2^{b}	38.3^{a}	35.7^{b}	2.6^{ab}	35.7^{b}	37.6^{ab}	36.1^{a}	1.5^{ab}

† HYG, high-yield genotype; LYG, low-yield genotype; DRG, drought-resistant genotype; DSG, drought-susceptible genotype; Mean of 30, the mean value of all 30 genotypes. ‡ CCIa, CCI evaluated at anthesis; $CCIg_1$, CCI evaluated at GF-1 stage (Feekes 11.1, kernels milky ripe); $CCIg_2$, CCI evaluated at GF-2 stage (Feekes 11.2, kernels mealy ripe); CCID, CCI decrease from GF-1 to GF-2. § Means followed by different letters are significantly ($P < 0.05$) different.

irrigation treatments were determined using Fisher's protected LSD at prob. = 0.05.

Conclusions

During the growing season of spring wheat, maximum flag leaf CCI was reached at GF-1 stage for most genotypes across different water conditions. The GF-1 stage was recommended to be the optimum time for measuring flag leaf CCI in wheat genotypes. Severe drought stress decreased the CCI value while both severe drought and moderate drought accelerated the flag leaf CCI reduction. Compared with the other groups, DRG had much higher CCI, especially CCI evaluated at anthesis under the T3 regime; the CCI of HYG declined either later or slower under all different water conditions. The present study suggests that the CCID from GF-1 to GF-2, rather than the CCI value, could be used as an indicator for selecting high yielding wheat genotypes; but the CCI value, especially CCI evaluated at anthesis under well-watered condition, could be considered as an indicator for screening drought resistance in wheat breeding programs.

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