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Wheat genotypic variability in utilizing nitrogen fertilizer for a cooler canopy under a heat-stressed irrigated environment

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Canopy cooling in dry heat-stressed areas is regarded as one of the most physiologically efficient way to attain high grain yields in wheat. Twelve wheat genotypes were grown with four N levels in a 2-year field trial to investigate their efficiency to utilize addition of nitrogen for canopy cooling and high chlorophyll accumulation under a dry but irrigated hot environment in Sudan. Both canopy temperature depression (CTD) and chlorophyll content (CC) increased significantly with addition of 43 (N₄₃), 86 (N₈₆) and 129 (N₁₂₉) kg N/ha compared to zero N (N₀) treatment. The two-season average increases in CTD were 33, 59 and 67% at N₄₃, N₈₆ and N₁₂₉, respectively, relative to N₀. The average increases in CC were 15, 22 and 23% at N₄₃, N₈₆ and N₁₂₉, respectively. However, genotypes showed a wide range of responses to addition of N especially for CTD. The combined genotypic ranges in percent increase of CTD and CC were 19 to 63% and 4 to 22% at N₄₃, 38 to 88% and 13 to 36% at N₈₆, and 34 to 113% and 15 to 28% at N₁₂₉, respectively. Strong association was found between CTD and CC, which in-turn, was highly and significantly associated with biomass and grain yield. On the other hand, CTD showed significant negative association with harvest index. The results indicate that application of N fertilizer was reflected in a cooler canopy under the dry heat stress conditions of this study. However, genotypic variation in utilizing N fertilizer in canopy cooling necessitate that this should be done in accordance with the response and efficiency of each cultivar.

Key words: Nitrogen fertilizer, canopy temperature depression, chlorophyll content, heat stress, wheat.

INTRODUCTION

Wheat (*Triticum aestivum* L.) production in arid and semi-arid regions is constrained by many factors including high temperatures, moisture deficit and low soil fertility. High temperature that repeatedly occurs throughout the cropping season represents a major challenge to wheat production in these areas. It causes reduction in grain yield via alteration of many physiological processes and acceleration of vegetative growth and reduction in grain filling rate and duration (Shpiler and Blum, 1991; Wardlaw and Moncur, 1995; Tahir et al., 2006).

Besides breeding heat stress tolerant genotypes, wheat yield in hot environments could be improved by

modifying the crop micro-climate through some cultural practices such as mulching, frequent irrigation and nitrogen fertilizer application (Ageeb, 1994; Badaruddin et al., 1999). Addition of N fertilizers to wheat increases aboveground dry matter production and grain yield via increased leaf area index and duration, ground cover and green spikes area. Application of nitrogen under heat stress condition significantly increased canopy temperature depression, biomass production and grain yield (Badaruddin et al., 1999; Ali, 2000). The positive correlation of canopy temperature depression, the difference between the crop canopy temperature and the air temperature, and chlorophyll content with grain yield and other related traits under dry, hot and irrigated conditions has drawn major attention to these traits as avenues for increasing grain yield under these environments (Amani et al., 1996; Reynolds et al., 1998).

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Table 1. Maximum, minimum and mean temperatures (°C) of the two cropping seasons (2006/2007 and 2007/2008) at the Gezira Research Station Farm, Wad Medani, Sudan.

Period	First season (2006/2007)			Second season (2007/2008)			Crop growth stage
	Maximum	Minimum	Mean	Maximum	Minimum	Mean	
1st -15th Nov.	37.2	18.2	27.7	39.3	22.1	30.7	Sowing
16th -30th Nov.	37.5	19.8	28.6	38.1	19.4	28.7	1st irrigation
1st -15th Dec.	37.6	18.7	28.2	37.3	17.6	27.5	Vegetative
16th-31st Dec.	36.1	18.9	27.5	35.3	16.8	26.0	Vegetative
1st -15th Jan.	36.5	18.3	27.4	35.5	17.1	26.3	Vegetative
16th -31st Jan.	35.3	18.7	27.0	31.7	15.1	23.4	Vegetative/heading
1st -15th Feb.	37.2	18.2	27.7	35.0	15.9	25.5	Heading/grain filling
16th -28th Feb.	36.7	15.1	25.9	34.0	15.6	24.8	Grain filling
1st -15th Mar.	38.4	20.7	29.5	40.4	20.5	30.5	Grain filling/maturity
16th -31st Mar.	38.4	20.7	29.5	41.1	19.7	30.4	Maturity

Genotypic variation in canopy temperature depression and chlorophyll content has been verified; however, at present, very little is known about how different genotypes will respond to different cultural practices, mainly N fertilization, for improved physiological traits that are expected to increase grain yield under heat stress. Therefore, this study investigated genotypic variation in utilizing added N for better canopy cooling and higher chlorophyll content under dry, hot irrigated conditions.

MATERIALS AND METHODS

A field experiment was conducted during 2006/2007 and 2007/2008 cropping seasons at the Gezira Research Station Farm (GRSF), Wad Medani, located in the central clay plain of Sudan (14°44'N; 33°29'E; 411 m asl). The soil of the experimental site is characterized as fine, heavy cracking montmorillonitic, isohyperthermic soil with pH of 8.0 to 8.5 and a rooting depth up to about 40 cm. This soil is poor in organic matter (0.3%), deficient in total nitrogen (0.03 to 0.05%) and low in available phosphorus (4 to 5 ppm; Olsen extractable P). For more details on chemical and fertility attributes of the soil at the experimental site refer to Ibrahim et al. (2010). Daily maximum, minimum and mean temperatures during the two cropping seasons were obtained from the meteorological station located at about 500 to 750 m from the experimental sites.

Twelve bread wheat genotypes were selected to represent a historical set of varieties released during the last five decades for cultivation in dry, hot irrigated environment of Sudan. The genotypes were tested under four nitrogen levels; 0 kg N/ha (N_0), 43 kg N/ha (N_{43}), 86 kg N/ha (N_{86} , the recommended dose) and 129 kg N/ha (N_{129}) split-applied in two equal batches as urea at three-leaf stage (second irrigation) and tillering stage (fourth irrigation). The recommended dose of phosphorus fertilizer in the form of super phosphate at the rate of 43 kg P_2O_5 /ha was applied by furrow placement prior to seeding.

The experiment was conducted during the third and fourth weeks of November using a randomized complete block design with three replications. Seeding was done mechanically using a seed rate of 120 kg ha⁻¹. Plot size was 8 rows, 5 m long and 0.2 m apart. Irrigation by gravity was carried out at 10 to 12 days intervals to avoid any water deficit stress. Standard cultural practices recommended for wheat production in Sudan were followed as described in more details in Tahir et al. (2006).

Canopy temperature depression was measured using a hand held infrared thermometer (Everest Inter Science, INC, USA). Measurements were taken in the afternoon (13:00 to 14:00) of full sunshine conditions. The viewing angle was around 45° to the horizontal line above the canopy so as to avoid the confounding effect of soil temperature as described earlier (Amani et al., 1996). Readings were taken 3 to 5 days after irrigation during the grain filling period.

The flag leaf chlorophyll content was indirectly assessed after anthesis using a chlorophyll meter (Minolta SPAD meter: Minolta Camera Co., Tokyo, Japan). Average of five measurements (SPAD unit) was taken in each plot. Biomass and grain yield were measured from a hand harvested area of 4.5 m². Harvest index was calculated as the ratio of grain yield to biomass.

Standard analysis of variance was done using GenStat Discovery Edition (GenStat- Seventh Edition (DE3). 2008. VSN International Ltd, Hemel Hempstead, UK) for each season separately as well as the combined analysis of the two seasons after the error mean squares were tested for homogeneity of variance. The relationship of canopy temperature depression and chlorophyll content with biomass, grain yield and harvest index were calculated using the means of genotypes across all N levels over the two seasons (n=48).

RESULTS

Growing conditions and analysis of variance

Maximum, minimum and mean temperatures of the two cropping seasons are shown in Table 1. The maximum, minimum and mean temperatures during November were higher in the second season. During December, January and February, the temperatures of the first season were always higher than that of the second season. However, during March the maximum temperatures were always above 40°C in the second season.

The combined analysis of the canopy temperature depression data for both seasons revealed significant differences between seasons, N levels, genotypes and season × N level interaction ($P < 0.001$). However, season × genotype, genotype × N level and season × N level × genotype interactions were not significant. Similarly, the combined analysis of the chlorophyll

Table 2. Canopy temperature depression (°C) of 12 bread wheat genotypes grown at four N levels for two seasons at Gezira Research Farm, Wad Medani, Sudan.

Genotype	Season 2006/2007				Season 2007/2008			
	N ₀	N ₄₃	N ₈₆	N ₁₂₉	N ₀	N ₄₃	N ₈₆	N ₁₂₉
Beladi 60	3.5	5.2 (49)	5.7 (65)	5.9 (69)	4.3	5.0 (18)	6.5 (53)	7.9 (86)
Giza 155 71	2.4	4.8 (100)	4.9 (108)	5.6 (137)	4.0	5.0 (25)	6.4 (58)	7.6 (88)
Condor 78	2.4	3.2 (33)	4.2 (76)	4.5 (86)	3.8	4.8 (26)	4.9 (29)	6.5 (70)
Debeira 82	3.7	4.5 (22)	4.9 (33)	5.3 (43)	4.5	5.3 (17)	6.5 (45)	8.0 (78)
Wadi Elneel 87	2.8	4.5 (58)	4.9 (72)	4.7 (66)	4.4	5.1 (17)	6.4 (46)	7.4 (67)
Elnielain 90	3.4	4.9 (44)	5.1 (50)	4.7 (36)	5.1	5.9 (14)	6.5 (27)	6.7 (31)
Nesser 96	2.7	3.7 (36)	5.4 (99)	4.2 (57)	3.9	4.9 (25)	6.9 (77)	6.5 (66)
Argine 98	3.0	4.3 (44)	5.3 (80)	4.7 (58)	5.1	5.5 (9)	7.0 (37)	8.1 (60)
Imam 00	3.7	4.9 (30)	5.1 (37)	5.4 (44)	4.2	5.3 (26)	6.6 (57)	6.7 (59)
Tagana 04	3.4	5.1 (49)	5.5 (61)	5.4 (56)	4.3	5.4 (24)	8.0 (85)	9.2 (112)
Khalifa 04	3.4	5.1 (52)	5.6 (65)	5.9 (75)	5.4	5.5 (1)	7.3 (36)	8.2 (52)
Bohaine 06	2.8	3.7 (31)	4.4 (56)	4.6 (62)	3.8	5.1 (33)	6.2 (62)	6.1 (60)
Mean	3.1	4.5 (46)	5.1 (67)	5.1 (66)	4.4	5.2 (20)	6.6 (51)	7.4 (69)
Range	1.3	2.0 (78)	1.5 (75)	1.7 (101)	1.6	1.1 (32)	3.1 (58)	3.1 (81)
Variance analysis								
N level (N)		***				***		
Genotype (G)		***				***		
N x G		NS				NS		
SE mean (N x G)		0.32				0.43		
CV %		12.4				12.7		

The numerical suffix (in bold) to the genotype name refers to the year of its release in Sudan. Number in parentheses is the percentage increase relative to the N₀.*** Significant at the P ≤ 0.001. NS = not significant.

content data revealed significant differences between seasons (P < 0.001), N levels (P < 0.001), genotypes (P < 0.05) and season × N level interaction (P < 0.001). The season × genotype, genotype × N level and season × N level × genotypes interactions were not significant. Therefore, the data of canopy temperature depression and chlorophyll content of each season will be presented.

Canopy temperature depression

Significant differences in canopy temperature depression (CTD) were found across N levels and genotypes (P < 0.001) in both seasons (Table 2). The CTD was generally higher in the second season compared with that of the first season.

In the first season, the mean CTD increased from 3.1 °C at N₀ to 4.5 and 5.1 °C at N₄₃ and N₈₆, respectively. However, the mean CTD at N₁₂₉ did not increase compared to that at N₈₆ despite the differences among genotypes observed (Table 2). The mean CTD in the second season increased by 20, 51 and 69% at N₄₃, N₈₆ and N₁₂₉, respectively, compared with N₀. Genotypes responded very differently to addition of different N rates in terms of percent increase in the CTD relative to that at N₀. For example, addition of 43 kg N in the first season

resulted in CTD increase ranging from 22% in Debeira to 100% in Giza 155. Other genotypes that showed above-average percent increase included Wadi Elneel, Khalifa, Beladi and Tagana. Similarly, addition of 86 kg N increased the CTD of Debeira and Giza 155 by 33 and 108%, respectively, while addition of 129 kg N increased the CTD of Elnielain and Giza 155 by 36 and 137%, respectively (Table 2).

In the second season, the percentage increase in the CTD at N₄₃ ranged from 1% in Khalifa to 33% in Bohaine. Genotypes that showed above-average percent increase included Condor, Imam, Giza 155, Nesser and Tagana. At N₈₆, the CTD of Elnielain and Tagana increased by 27 and 85%, respectively. Genotypes Nesser, Bohaine, Giza 155, Imam and Beladi also showed above-average percent increase in the CTD at N₈₆. At N₁₂₉, the percentage increase in CTD ranged from 31% in Elnielain to 112% in Tagana, and genotypes Giza 155, Beladi, Debeira and Condor showed above-average percent increase (Table 2).

Addition of more N consistently increased the CTD of some genotypes such as Beladi and Giza 155 despite the differences in the trend in the two seasons. On the other hand, no or slight increments in CTD of some genotypes were observed with addition of more N such as that of Elnielain and Imam.

Table 3. Chlorophyll content (SPAD) of 12 bread wheat genotypes grown at four N levels for two seasons at Gezira Research Farm, Wad Medani, Sudan.

Genotype	Season 2006/2007				Season 2007/2008			
	N ₀	N ₄₃	N ₈₆	N ₁₂₉	N ₀	N ₄₃	N ₈₆	N ₁₂₉
Beladi 60	32.3	44.0 (36)	46.5 (44)	43.9 (36)	43.0	45.9 (7)	51.5 (20)	45.1 (5)
Giza 155 71	39.3	41.3 (5)	44.9 (14)	43.1 (10)	40.1	41.5 (3)	49.8 (24)	48.2 (20)
Condor 78	31.1	37.6 (21)	43.7 (41)	38.2 (23)	37.8	41.4 (10)	49.9 (32)	50.1 (33)
Debeira 82	34.1	40.3 (18)	40.4 (19)	43.2 (27)	39.7	42.0 (6)	45.7 (15)	46.2 (16)
Wadi Elneel 87	33.7	43.1 (28)	45.0 (33)	44.3 (31)	40.3	45.1 (12)	46.2 (15)	48.7 (21)
Elnielain 90	35.5	42.1 (19)	43.7 (23)	44.3 (25)	37.8	45.3 (20)	47.8 (26)	48.6 (29)
Nesser 96	31.8	41.9 (32)	42.7 (34)	44.1 (39)	43.7	43.8 (0)	46.5 (6)	48.1 (10)
Argine 98	36.2	42.0 (16)	39.6 (9)	45.4 (25)	39.0	44.8 (15)	49.6 (27)	47.8 (23)
Imam 00	31.8	40.7 (28)	42.7 (34)	45.3 (43)	43.9	44.5 (1)	49.0 (12)	48.8 (11)
Tagana 04	35.5	39.4 (11)	41.7 (17)	43.3 (22)	42.1	45.5 (8)	46.0 (9)	49.4 (17)
Khalifa 04	35.2	46.1 (31)	42.1 (20)	44.2 (26)	42.7	44.6 (4)	47.9 (12)	50.5 (18)
Bohaine 06	32.1	40.8 (27)	41.5 (29)	40.8 (27)	40.6	42.8 (5)	47.0 (16)	47.6 (17)
Mean	34.0	41.6 (23)	42.9 (27)	43.3 (28)	40.9	43.9 (8)	48.1 (18)	48.3 (18)
Range	8.2	8.5 (31)	6.9 (35)	7.2 (33)	6.1	4.5 (20)	5.8 (26)	5.4 (27)
Variance analysis								
N level (N)			***				***	
Genotype (G)			*				NS	
N x G			NS				NS	
SE mean (N x G)			1.77				1.84	
CV %			7.6				7.0	

The numerical suffix (in bold) to the genotype name refers to the year of its release in Sudan. Number in parentheses is the percentage increase relative to the N₀.*,*** Significant at the P ≤ 0.05 and P ≤ 0.001, respectively. NS = not significant.

Chlorophyll content

Significant differences in chlorophyll content (CC) of the flag leaf were found among N levels in both seasons. The differences among genotypes was significant in 2006/2007 but not in 2007/2008 (Table 3). In the first season, average CC increased by 23, 27 and 28% at N₄₃, N₈₆ and N₁₂₉, respectively, compared to the N₀. At N₄₃, the chlorophyll content of Giza 155 increased by 5% while that of Beladi increased by 36%. Application of 86 kg N/ha resulted in chlorophyll increases ranging from 9% in Argine to 44% in Beladi. At N₁₂₉, the CC of Giza 155 increased by 10% while that of Imam increased by 43% relative to that at N₀.

In the second season, the percentage CC increases were 8, 18 and 18 at N₄₃, N₈₆ and N₁₂₉, respectively, compared to the N₀. The percentage increase in CC of the genotypes at N₄₃ was lower than that of the first season and ranged from less than 1% in Nesser to 20% in Elnielain. Addition of 86 kg N/ha resulted in CC increases ranging from 4% in Imam to 32% in Condor while addition of 129 kg N/ha increased the CC of Beladi and Condor by 5 and 33%, respectively (Table 3).

Trait association

Strong association was found between canopy temperature

depression and chlorophyll content ($R^2 = 0.689$, $P \leq 0.001$) across different N levels (Figure 1). Both canopy temperature depression and chlorophyll content showed highly significant association with biomass ($R^2 = 0.853$, $P \leq 0.001$ and $R^2 = 0.683$, $P \leq 0.001$, respectively) across the four N levels (Figure 2). Similarly, highly significant associations were found between grain yield and both CTD ($R^2 = 0.603$, $P \leq 0.001$) and CC ($R^2 = 0.565$, $P \leq 0.001$) across different N levels (Figure 3). On the other hand, harvest index showed negative significant association with canopy temperature depression ($R^2 = 0.162$, $P \leq 0.01$) while its association with chlorophyll content was not significant ($R^2 = 0.079$, $P = 0.052$) though tended to be negative (Figure 4). These negative associations were mostly due to the negative relationship between harvest index and biomass (data not shown) especially for the old tall genotypes at high N levels.

DISCUSSION

Wheat genotypes used in this study showed wide variability in utilizing applied N for cooling the canopy temperature and accumulating more chlorophyll in the flag leaves under the heat-stressed irrigated conditions of Sudan. Earlier reports have shown clear associations of CTD and CC with grain yield in warm environments with

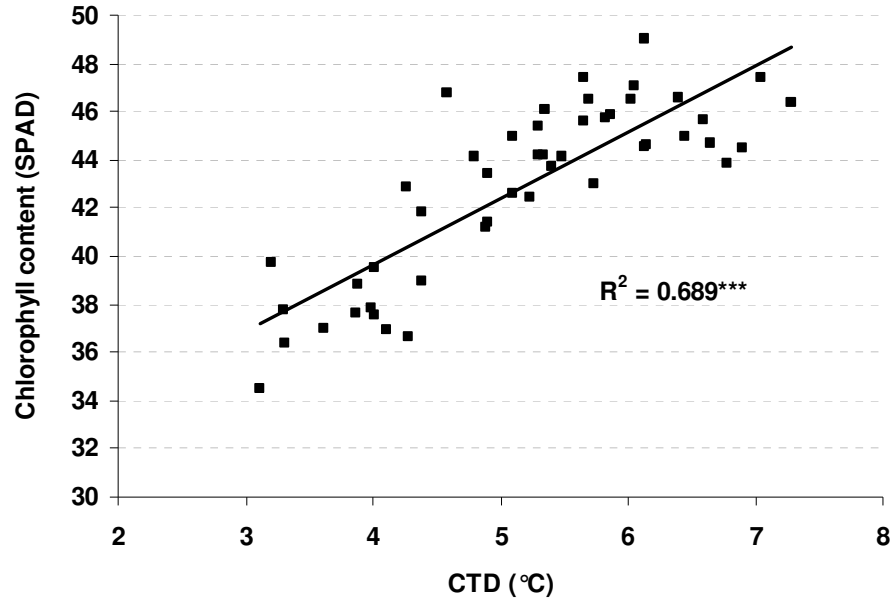


Figure 1. The relationship between canopy temperature depression (CTD) and chlorophyll content of 12 wheat genotypes grown under four N levels at the Gezira Research Station Farm, Wad Medani, Sudan.

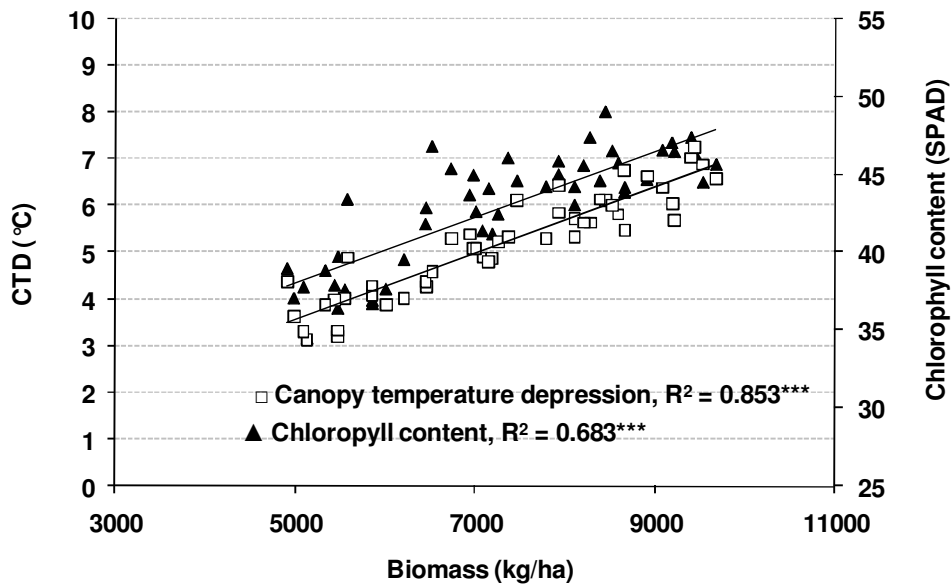


Figure 2. The relationship of biomass with canopy temperature depression (CTD) and chlorophyll content of 12 wheat genotypes grown under four N levels at the Gezira Research Station Farm, Wad Medani, Sudan.

low relative humidity such as those of wheat growing areas in Sudan (Amani et al., 1996; Reynolds et al., 1998, 2001). CTD is a function of a number of environmental factors such as soil water status, air temperature, relative humidity, and incident radiation (Reynolds et al., 2001). The trait is best expressed at high vapor pressure deficit

conditions associated with low relative humidity and warm air temperature (Amani et al., 1996). Under irrigated field condition, the canopy cooling alleviated some of the negative effects of heat stress (Amani et al., 1996). Similarly, chlorophyll content positively correlated with grain yield under heat stress conditions, and the loss

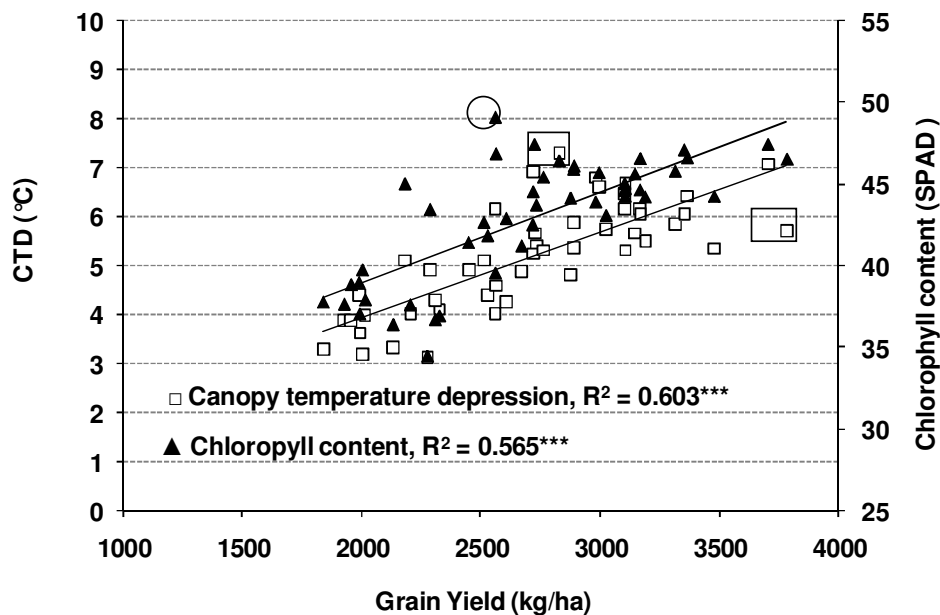


Figure 3. The relationship of grain yield with canopy temperature depression (CTD) and chlorophyll content (CC) of 12 wheat genotypes grown under four N levels at the Gezira Research Station Farm, Wad Medani, Sudan. The data point marked with an oval shape shows an example of a big deviation of CC from the regression trend line. Likewise, data points marked with rectangles are examples of deviation of CTD observed values from the trend line.

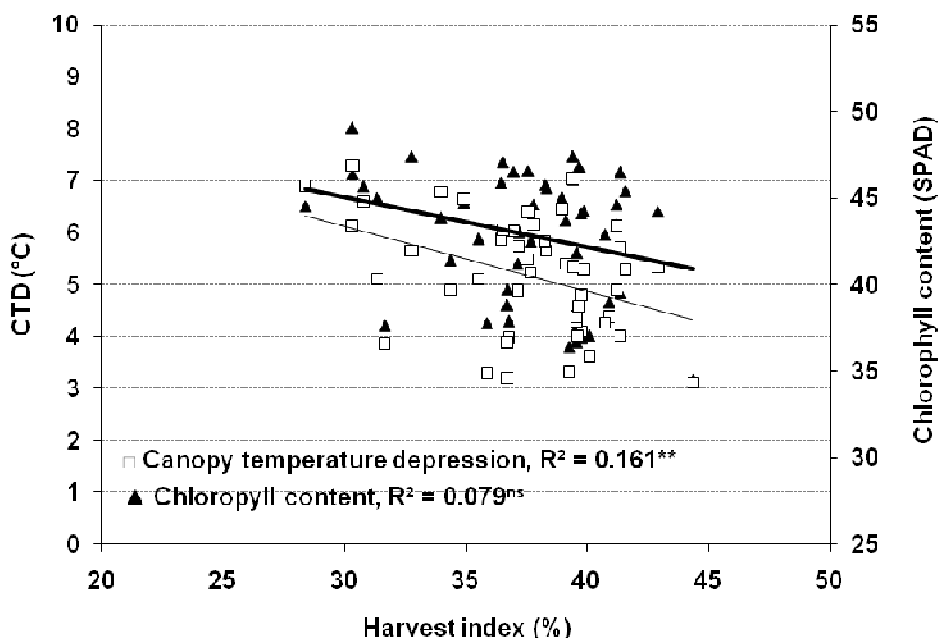


Figure 4. The relationship of harvest index with canopy temperature depression (CTD) and chlorophyll content (SPAD) of 12 wheat genotypes grown under four N levels at the Gezira Research Station Farm, Wad Medani, Sudan.

of chlorophyll during grain filling, which results in alteration of photosynthesis, is associated with reduced grain yield under heat-stressed field conditions (Reynolds

et al., 1994, 1998). However, remobilization of N from the stem of wheat during grain filling under heat-stressed conditions, which is associated with the loss of leaf

chlorophyll, should be considered (Tahir and Nakata, 2005).

In the light of ensuing climate change, maintaining crop productivity while minimizing harmful environmental impacts of increased N fertilizer application is a major challenge that agriculture faces. The most economically beneficial to farmers and environmentally-friendly approach could be development of N-efficient wheat cultivars (Foulkes et al., 2009). Such wheat cultivars will best-utilize the N management in a most cost-effective way. Considerable genetic diversity in wheat for nitrogen uptake, utilization and remobilization efficiencies has been reported (Ortiz-Monasterio et al., 1997; Le Gouis et al., 2000; Guarda et al., 2004; Tahir and Nakata 2005; Barraclough et al., 2010).

Badaruddin et al. (1999) reported that wheat yields in warm environments can be raised significantly by modifying agronomic practices such as application of animal manure and straw mulch, as well as from increased levels of inorganic nutrients and irrigation frequency. Physiological traits such as light interception, canopy temperature depression, and flag leaf chlorophyll content were also improved by these agronomic practices. However, a heat-tolerant genotype was generally more responsive to additional inputs compared to a heat-sensitive genotype (Badaruddin et al., 1999). Therefore, even modification of agronomic practices would be better when a more N-use efficient and heat tolerant wheat genotype is used. Nitrogen use efficiency is genetically controlled, however; some environmental factors such as drought and heat stresses could modify the genetic effects. N utilization and remobilization from the stems of wheat was reduced by high temperatures during grain filling although differential genotypic response was found (Tahir and Nakata, 2005).

In our study, addition of more N fertilizer resulted in high canopy temperature depression and chlorophyll content, which were associated with high biomass and grain yield. However, it has been observed that some genotypes did not efficiently utilize their high CTD and CC for higher grain yields (Figure 3). This can be noticed from the large deviation of the observed CTD and CC values of some genotypes from the trend line. For example, the average high CTD of the genotype Tagana at N129 was not associated with high mean grain yield. In contrast, the genotype Elnielain gave high mean grain yield at N129 despite its low mean CTD (Figure 3, the data points marked with rectangles). Similarly, the average high CC of the genotype Beladi at N86 was not matched with high grain yield (Figure 3, the data point marked with an oval shape).

Both CTD and CC tended to have negative association with harvest index across different N levels. Although increased CTD and CC resulted in higher biomass, some wheat genotypes seemed to be inefficient in partitioning the assimilates into the grain production. Wheat genotypes with high harvest index and low forage yield

have low plant N loss and increased N use efficiency (Kanampiu et al., 1997). However, production of more biomass is important under the heat-stressed conditions of Sudan. A wheat genotype that is capable of striking a balance between high biomass accumulation, efficient use of N and effective assimilate partitioning to grain would best fit in such a stressful environment.

The results of this study indicate that application of N fertilizer reflected in cooler canopy and higher chlorophyll contents under the dry heat-stressed condition of Sudan. However, genotypic variation in utilizing N fertilizer in canopy cooling and accumulating more chlorophyll in the flag leaves necessitate that application of more N should be done in accordance with the response and efficiency of each cultivar.

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