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Association of staygreen trait with canopy temperature depression and yield traits under terminal heat stress in wheat (*Triticum aestivum* L.)

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Abstract The presence or absence of the staygreen trait was screened for 3 consecutive years in 963 wheat lines from various sources, including Indian and CIMMYT germplasm. Staygreen was assessed at the late dough stage by visual scoring (0–9 scale) and the leaf area under greenness (LAUG) measurement. Around 5.5 % of the lines were staygreen, 10.5 % were moderately staygreen, and the remaining lines showed little or no expression of the trait. One hundred lines showing diversity for the staygreen trait were sown under three different sowing dates (timely, late and very late) for 3 consecutive years in three replications to determine the association of staygreen with heat tolerance. There was a decline in yield, biomass, grain filling duration (GFD) and 1,000 grain weight (TGW) under late and very late sowing conditions owing to terminal stress at anthesis and later stages. However, the decline was relatively less in staygreen genotypes compared to the non-staygreen (NSG) ones. The correlation study showed that LAUG and canopy temperature depression (CTD) were strongly correlated. LAUG and CTD were also significantly associated with grain yield, GFD and biomass. To further confirm the association of the staygreen trait with terminal heat stress, individual F_2-derived F_7 progenies from the cross of the 'staygreen' lines with NSG were evaluated for yield and yield traits at the three sowing dates. In each cross, the staygreen progenies showed a significantly smaller decline in yield and TGW under heat stress than the NSG progenies. These results appear to suggest an association between the staygreen trait and terminal heat stress and, thereby, that the staygreen trait could be used as a morphological marker in wheat to screen for heat tolerance.

Keywords Staygreen · CTD · Terminal heat stress · Wheat · *Triticum aestivum*

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Introduction

Terminal heat stress is a major abiotic stress affecting yield in wheat, and genetic diversity for heat tolerance has been reported (Al-Khatib and Paulsen 1990; Joshi et al. 2007a). The photosynthetic process is affected under heat stress conditions, defined by Fischer and Byerlee (1991) to be mean daily temperatures of >17.5 °C in the coolest month, especially during grain filling when demand for assimilates is the greatest. Staygreen is an important trait that allows plants to retain their leaves in an active photosynthetic state when subjected to stress conditions (Rosenow et al. 1983); it contributes to a long grain-filling period and stable yield even when the plant is stressed (Vijayalakshmi et al. 2010). Hence, this trait, which is believed to affect radiation use efficiency and nutrient remobilization, could be important under heat stress conditions (Reynolds et al. 2001; Gregersen et al. 2008). The staygreen trait has been reported in different crops (Thomas and Howarth 2000) and has been widely used in breeding for heat tolerance, partly as an indicator of disease resistance (Kohli et al. 1991; Joshi et al. 2007b; Kumar et al. 2010) and drought resistance (Walulu et al. 1994; Rosenow 1987, 1994). Very few reports are available in wheat providing evidence of substantial variation for the staygreen trait (Joshi et al. 2007b; Ahlawat et al. 2008; Rehman et al. 2009). Staygreen genotypes have been used successfully to select for yield and yield stability of sorghum in the Australian northern cereal wheat belt and also show promise as a selection tool in wheat; for example, Seri M82 exhibits a staygreen phenotype with long grain filling duration (GFD) (Christopher et al. 2008). In durum wheat, a staygreen mutant has been associated with increased leaf area, rate and duration of grain filling and photosynthetic competence (Spano et al. 2003). Staygreen duration of the flag leaf and harvest index showed positive correlations with water use efficiency during the grain formation of wheat (Gorny and Garczynski 2002). It is also reported that green and viable leaves significantly contribute photosynthates to developing grain (Thorne 1982). Since there is a strong association between the duration of photosynthetically active leaf area and grain yield (Rawson et al. 1983), selection for staygreen is expected to have significant implications in terms of wheat productivity, particularly under harsh environments (Reynolds 2002), including those of heat stress.

Although many traits have been suggested as selection criteria to assess heat tolerance, canopy temperature depression (CTD) is considered to be the most effective since one single reading integrates scores of leaves (Reynolds et al. 1994, 1998; Fischer et al. 1998). CTD is highly heritable and very easy to measure using a hand-held infrared thermometer on cloudless days (Reynolds et al. 1994, 1998). Although an association between the staygreen trait and yield and yield traits has been reported in various crops, published studies on a possible association between the staygreen trait and CTD in different crops are scarce. The aim of the study reported here was, therefore, to determine the variation for the staygreen trait in wheat under terminal heat stress conditions, as well as its association with CTD and yield traits.

Materials and methods

Evaluation of germplasm for staygreen trait

The 963 wheat lines tested in our study were obtained from CIMMYT (International Maize and Wheat Improvement Center, Mexico), Directorate of Wheat Research, Karnal, India (DWR) and the National Bureau of Plant Genetic Resources, New Delhi, India (NBPGR). These lines differed from those used in an earlier study by Joshi et al. (2007b) and included cultivars, genetic stocks for different traits and advanced lines being tested in different trials and nurseries: National Genetic Stock Nursery, Leaf Blight Screening Nursery, Drought and Heat Tolerance Nursery, Initial and Advanced Varietal Trials, Yield Component Screening Nursery, Heat Tolerance Wheat Yield Trials, Eastern Gangetic Plains Yield Trials, Eastern Gangetic Plains Screening Nursery, International Nursery on Quality and Yield Trials, National Agriculture Technology Program, Helminthosporium Monitoring Nursery, Salinity trail, Elite International Germplasm Nursery and International Drought and Heat Tolerance Nurseries.

Each germplasm line was hand sown using a randomized complete block design with three replications in a four-row plot with 3-m rows and inter-row spacing of 25 cm. Seeds were sown 5 cm apart. The plant material was tested at the research station of Banaras Hindu University, Varanasi, India (North Eastern Plains Zone, 25.18°N and 83.03°E) for three consecutive seasons (2002, 2003 and 2004). Identical agronomic practices, i.e., those recommended for normal fertility [120 kg nitrogen (N):60 kg phosphorus pentoxide (P_2O_5):40 kg potassium oxide (K_2O) ha^{-1}], were followed in all 3 years. Full doses of K_2O and P_2O_5 were applied at the time of sowing. Nitrogen was supplied in split applications, namely, 60 kg N ha^{-1} at sowing, 30 kg N ha^{-1} at the first irrigation (21 days after sowing) and 30 kg N ha^{-1} at the second irrigation (45 days after sowing). For proper evaluation of the staygreen trait, fungicide Tilt (propiconazole: 1-{[2-(2,4-dichlophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl}-1H-1,2,4-triazole) was applied at 625 g active ingredient/ha at two growth stages (GS; 54 and 69; Zadoks et al. 1974) to prevent spot blotch, the two most important diseases of eastern India.

Evaluation of contrasting lines for terminal heat tolerance

From the lines screened, 100 genotypes showing varying expressions of staygreen were sown for 3 years (2004, 2005 and 2006) in three replications and different sowing dates, namely, timely (normal), late and very late. Timely (normal) sowing was done on 15 November, late sowing on 15 December and very late sowing on 10 January. Mean maximum and minimum temperatures for these months are given in Electronic Supplementary Material (ESM) Table 1. Late sowings were done to expose wheat lines to terminal heat stress. For each line, six rows of 4 m were grown with a row distance of 20 cm. This experiment was also treated with fungicide as described above.

Genetic association between the staygreen trait and terminal heat stress

To confirm the association of the staygreen trait with the terminal heat stress, staygreen lines Chirya 3, Chirya 1, Chirya 7 and Ning 8204 were crossed with the non-staygreen (NSG) cv. Sonalika. F_1 seeds were multiplied in an off-season nursery to obtain F_2 populations. The F_3 generation was obtained by harvesting space-sown random F_2 plants. The F_4, F_5, F_6 and F_7 generations were derived by growing small seed samples of each line and by harvesting one random plant from each line in each generation (Singh and Rajaram 1992; Joshi et al. 2002). Off-season nurseries were used to expedite generation enhancement. In the F_7 generation of each of the four crosses, four parental types homogeneous lines for each of the two categories (staygreen and NSG) were bulk-harvested. The seeds of these lines were multiplied at an off-season nursery and further evaluated for their homogeneity for staygreen expression. Finally, the two most homogeneous lines for each of the parental types (staygreen and NSG) from four crosses were evaluated for 2 years on the three sowing dates in three replications at Varanasi following a split plot design. The five parents were also included in the experiment/analysis. The plots of each line consisted of eight rows of 5 m length with an inter-row spacing of 20 cm. Observations for CTD, yield and yield traits were recorded.

Statistical analysis using different traits of the homozygous/homogeneous staygreen and NSG progeny of F_7 lines was performed using SAS software (SAS Institute, Cary, NC).

Recording of observation for staygreen

The staygreen trait was measured using two approaches: (1) differences in leaf and spike greenness scores on a 0–9 scale at the late dough stage (modified version of the 1–10 scale of Silva et al. 2000), and (2) ‘leaf area under greenness’ (LAUG) (Joshi et al. 2007a). In the first approach, the staygreen trait was recorded on the basis of visual scores (0–9 scale) for both the flag leaf and spike at the late dough stage (SG 87). The measurement scale was from 0 to 9, but for genotype grouping, the difference between the flag leaf and spike scores was considered, which could not exceed the value of 6. The genotypes were grouped as staygreen (SG) (>3–6), moderately staygreen (MSG) (>2–≤ 3), moderately NSG (MNSG) (>1– $\leq$ 2) and NSG (0–$\leq$1).

In the second approach, LAUG was determined as reported by Joshi et al. 2007b). In the LAUG approach scores for the green area of the flag leaf and of spikes were estimated visually on a 0–9 scale at 4-day intervals, starting from the late milk stage to physiological maturity. In the formula used to calculate LAUG, the difference in green area on the spike and flag leaf at time t_i was used as Y_i:

$$\mathrm{LAUG} = \sum_{i=1}^{a} \left[\left\{ \left(Y_i + Y_{(i+1)} \right)/2 \right\} \text{ times } \left(t_{(i+1)} - t_i \right) \right]$$

where, Y_i = difference of green area under spike and flag leaf (0–9 scale) at time t_i with $t_{(i+1)} - t_i$ = time (days) between two consecutive readings.

The green area in the flag leaf and spike was recorded from the late milk stage (GS 77) until physiological maturity, with the latter marked by a complete loss of green colouration in both the flag leaf and spike. For longer duration lines, readings were >5; hence, only the last five readings were considered in the calculations.

Based on the LAUG values, the tested lines were grouped into four categories: 0–<20 as NSG, ≥20–<40 as MNSG, ≥40–<60 as MSG and ≥60–90 as SG.

Recording of CTD and yield traits

Canopy temperature was recorded on a per-plot basis (6-row plot) using a hand-held infrared thermometer on bright sunny days at 1200, 1400 and 1600 hours, at 7-day intervals, starting from anthesis until the late milk stage. At the study location, if wheat is sown late to very late, the terminal heat stress falls at the anthesis stage in the month of March and April (ESM Table 1). CTD was calculated using the following formula:

$$\mathrm{CTD} = \text{Air temperature} - \text{canopy temperature}$$

Yield and yield traits [days to anthesis, days to maturity, 1,000 grain weight (TGW), biomass and grain yield) were also recorded for all the genotypes in all three sowing dates. GFD was calculated as the difference between days to anthesis and days to maturity.

Data analysis

Statistical analysis was done using SAS software (SAS Institute 2003). For the germplasm, analysis of variance (ANOVA) was performed for the staygreen trait. Least significant difference (LSD) was estimated for the staygreen and NSG genotypes. In the second experiment, statistical analysis using LAUG values, CTD, yield and yield traits were performed for 100 genotypes. The simple linear correlation coefficient was calculated to determine the association of CTD and yield traits with the staygreen trait (LAUG) at different sowing dates. The paired *t* test was used to compare the difference between staygreen and NSG genotypes for CTD, LAUG and yield traits.

Results

A significant variation for the staygreen trait measured (0–9 scale) at the late dough stage and LAUG was observed in the tested lines (Table 1). Genotype × year and staygreen × year interaction was also significant, indicating the influence of environment in the expression of this trait. Based on LAUG measurements, around 4.46 % of the germplasm were SG, 10.59 % MSG, 20.26 % MNSG and 64.69 % NSG (Table 2).

In the second experiment, analysis of variance of 100 diverse genotypes showed that there was significant variation among genotypes for staygreen, CTD, yield and yield traits (Table 3). There was also a significant difference in performance over years and sowing dates for the traits studied. However, both the year × genotype interaction and the year × sowing × genotype interaction were not significant for LAUG and CTD. The genotypes fell in same category irrespective of the year and sowing time in which it was screened, indicating that the expression of staygreen is not just environmental but also genetic.

Staygreen genotypes displayed higher CTD values on all of the sowing dates in all 3 study years compared to NSG genotypes (Table 4). CTD values were higher for staygreen genotypes under late and very late sowing conditions, owing to higher temperature stress, than under timely sown conditions (Fig. 1a, b). There was a significant effect on staygreen expression under late sown conditions, but this still offered yield advantage compared to NSG genotypes. Although there was a decline in LAUG values under heat stress, the genotypes fell into the same category as when grouped under normal condition (Fig. 2). The percentage decline in staygreen expression LAUG, yield and yield traits under late and very late conditions relative to the timely sown condition is shown in Fig. 2. The *t* test done to compare the performance of staygreen and NSG genotypes also revealed a significant difference for all of the traits in the 3 study years and under all three sowing dates, except for TGW under the timely sown condition (Tables 4, 5). Compared to the timely sown condition, there was a significant decline in yield, biomass, GFD and TGW in the late and very late sown conditions for all genotypes due to terminal heat stress, but the decline was comparatively lesser in staygreen genotypes and these continued to maintain their superiority under late sown conditions (Tables 4, 5).

The correlation between LAUG and CTD was substantially high and positive ($r = 0.84$–0.90) for all three sowing dates (Table 6). The association of LAUG with grain yield (0.79–0.83), biomass (0.81–0.88), GFD (0.74–0.78) and TGW (0.05–0.57)

Table 1 Analysis of variance of 963 germplasm lines of wheat for the staygreen trait

Source	Degrees of freedom	Mean sum of squares	
		LAUG	Visual score (at late dough stage)
Without staygreen classes			
Year	2	1040.87**	43.43**
Rep (year)	6	1781.31**	56.74**
Genotypes	962	3290.33**	12.20**
Genotype × year	1924	8.18	0.26
Error	5,772	7.96	0.35
With staygreen classes			
Year	2	725.94**	11.54**
Rep (year)	6	1,781.31**	56.74**
Staygreen	3	981,419.08**	3,565.54**
Genotype (staygreen)	959	230.49**	1.08**
Staygreen × year	6	108.53**	3.23**
Error	7,690	7.94	0.33

LAUG leaf area under greenness

*, **Significant at $P < 0.05$ and 0.1, respectively

Table 2 Performance of 963 wheat germplasm lines for staygreen trait

Staygreen class	LAUG			Visual score at late dough stage		
	Mean score	No. of genotypes	% of total	Mean score	No. of genotypes	% of total
NSG	9.46	623	64.69	0.67	636	66.04
MNSG	25.88	195	20.26	2.00	184	19.11
Moderately staygreen	49.84	102	10.59	3.00	89	9.24
Staygreen	66.00	53	4.46	4.67	54	5.61
Total		963	100		963	100
Least significant difference						
1 %	0.327			0.145		
5 %	0.798			0.354		

NSG non-staygreen, *MNSG* moderately non-staygreen

was significant and positive (Table 6). CTD was also found to be positively associated with grain yield (+0.71–79), biomass (+0.72–76), GFD (+0.67–73) and TGW (0.01–0.50). Biomass did not show any significant correlation with TGW in timely and late sown conditions, but was significantly correlated in very late sown conditions (Table 6).

Discussion

Staygreen is the ability of plants to remain green for a longer time than NSG lines, thereby contributing photosynthates for a longer period towards grain development (Thomas and Howarth 2000; Reynolds 2002). The staygreen trait has also been suggested as a useful trait for determining heat tolerance (Reynolds 2002; Joshi et al. 2007a). Germplasm screened in this study showed significant variation for the staygreen trait. LAUG was significantly higher in genotypes displaying the staygreen trait than in those lacking this trait. Visual rating of staygreen is quick to perform in the field on a plot basis using a 0–9 scale; as such, it represents an important tool for use by plant breeders in screening large number of progenies (Xu et al. 2000). However, LAUG is also easy to calculate and appears to be more reliable as it is based on several readings integrated over time and takes into account the decline in degree of greenness of the leaf with respect to spikes over time (Joshi et al. 2007a).

Table 3 Analysis of variance for CTD, LAUG and yield traits tested on 100 diverse wheat germplasm lines for three sowing dates and 3 years

Source of variation	*df*	Mean sum of squares					
		LAUG	CTD	GFD	Grain yield	Biomass	TGW
Year	2	22.75**	1.15**	1,524.49**	892.80**	2,171.74**	19.48**
Sowing	2	62,841.72**	2,365.89**	31,325.05**	100,986.10**	115,323.25**	15,984.40**
Year × sowing	4	139.20**	4.76**	2,982.75**	70.93**	77.48**	94.02**
Rep (year × sowing)	18	6.19	7.46**	198.38**	1,161.40**	225.87**	14.59**
Staygreen	3	438,406.33**	1,191.72**	14,193.54**	45,207.50**	50,866.40**	170.02**
Genotype (staygreen)	96	728.32**	3.92**	50.95**	240.10**	286.54**	9.62**
Sowing × staygreen	6	1,829.87**	54.05**	208.86**	41.00**	70.20**	62.72**
Year × staygreen	6	3.55	0.04	20.97**	19.83**	20.73**	3.50
Year × sowing × staygreen	12	1.42	0.20	36.68**	9.20**	2.31**	3.39
Error	2,550	6.07	0.15	2.77	0.29	0.11	4.46

CTD, canopy temperature depression, *GFD* green filling depression, *TGW* 1,000 grain weight

*, **Significant at $P < 0.05$ and 0.01, respectively

Table 4 Comparison of mean CTD and LAUG of 100 staygreen and NSG wheat genotypes for the 3 study years and three sowing dates

Environment		CTD			LAUG		
Years	Sowing time[a]	Staygreen	NSG	*t* test	Staygreen	NSG	*t* test
2004	Timely	4.86	2.59	26.67**	71.47	8.43	49.05**
	Late	6.53	3.50	33.09**	64.32	3.62	54.08**
	Very late	8.70	4.42	39.45**	53.18	0.85	63.92**
2005	Timely	4.51	2.47	23.83**	72.47	9.43	49.05**
	Late	6.27	3.46	31.14**	63.32	3.01	54.61**
	Very late	7.88	4.51	41.14**	54.18	1.85	63.92**
2006	Timely	4.74	2.57	22.67**	71.37	8.38	48.48**
	Late	6.31	3.42	26.41**	64.25	3.98	53.33**
	Very late	8.89	4.50	32.83**	53.30	1.26	62.83**

** Indicates significant *t* values at $P < 0.01$

[a] Timely (normal) sowing was done on 15 November, late sowing on 15 December and very late sowing on 10 January

CTD is also very simple to record, and a single reading integrates the temperature of scores of leaves, thus minimizing experimental error (Reynolds et al. 1994). In the studied germplasm, CTD values were significantly higher for staygreen genotypes. This could be due to the cooling effects of transpiration in staygreen genotypes resulting in a lowering of canopy temperature (Reynolds et al. 1994; Fischer et al. 1998). A low CTD value can also be ascribed to delayed senescence in staygreen lines. A strong correlation was found between LAUG and CTD under all sowing conditions. Under terminal heat stress, staygreen genotypes gave a yield advantage over NSG genotypes. Although there was a significant effect of heat stress on the expression of staygreen, the class under which the germplasms were grouped remained the same. There was a decline in the expression of LAUG, yield, biomass, GFD and TGW, but it was much less for genotypes possessing the staygreen ability. These observations indicate that the staygreen/LAUG combination could be used as effective criteria for identifying heat-tolerant genotypes in wheat. The absence of a genotype × year interaction for LAUG and CTD in the second experiment involving 100

Fig. 1 **a** Effect of heat stress on leaf area under greenness (*LAUG*), grain filling duration (*GFD*) and 1,000 grain weight (*TGW*) of staygreen (*SG*) and non-staygreen (*Non-SG*) wheat genotypes under different sowing conditions during the 3 study years (2004–2006) at Varanasi, India. **b** Effect of heat stress on yield and biomass of the staygreen and NSG genotypes under different sowing conditions in the 3 study years (2004–2006) of testing at Varanasi, India

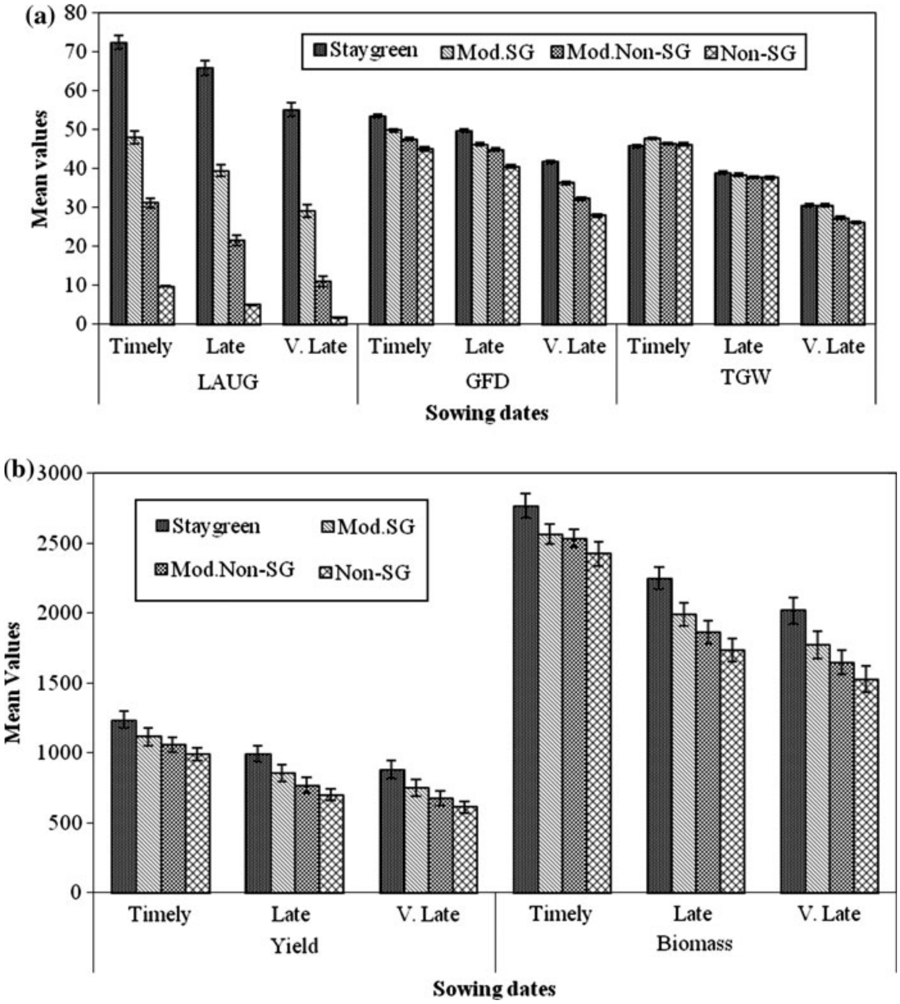

Fig. 2 Percentage decline for yield and yield traits in wheat lines varying for the staygreen trait under late and very late conditions over timely sown

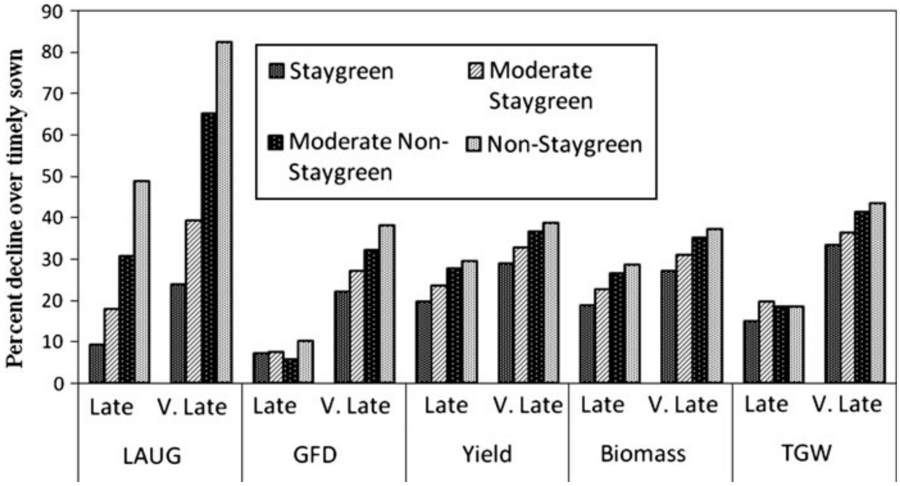

Table 5 Comparison of means of yield and yield traits of 100 staygreen and NSG genotypes of wheat for the 3 study years and three sowing dates

Years	Sowing time	GFD (days)			TGW (g)			Biomass (Kg ha)			Grain yield (Kg ha)		
		SG	NSG	*t* calculated	SG	NSG	*t* calculated	SG	NSG	*t* calculated	SG	NSG	*t* calculated
2004	Timely	52.17	43.51	15.9**	46.61	43.23	2.12	6,300	5,300	22.2**	2,900	2,150	28.2**
	Late	48.11	40.42	14.8**	39.02	37.12	3.14**	5,050	3,350	21.4**	2,050	1,525	86.6**
	Very late	39.22	30.32	30.2**	30.28	26.72	10.8**	4,475	3,100	22.5**	1,850	1,400	18.7**
2005	Timely	50.19	44.71	16.9**	45.81	44.69	2.03	6,625	5,600	20.2**	2,850	2,275	30.2**
	Late	47.00	41.72	15.4**	38.22	37.26	3.12**	5,300	4,100	20.4**	2,225	1,650	16.6**
	Very late	39.56	30.60	31.8**	31.58	27.74	11.44**	4,725	3,600	20.5**	1,975	1,475	16.7**
2006	Timely	53.28	47.92	15.1**	47.82	47.94	0.35	6,450	5,350	18.0**	2,925	2,425	17.8**
	Late	49.00	43.72	15.4**	39.22	38.26	3.16**	5,075	3,875	19.0**	2,325	1,750	18.6**
	Very late	38.56	29.60	31.8**	29.58	25.74	11.42**	4,575	3,425	19.1**	2,025	1,525	18.8**

** Significant at $P < 0.01$

Table 6 Simple linear correlation between LAUG, CTD and yield traits of 100 diverse wheat lines for staygreen tested for three sowing dates and during 3 years

Traits	Sowing time	CTD	GFD	Yield	TGW	Biomass
LAUG	Timely	0.84**	0.74**	0.79**	0.05	0.81**
	Late	0.87**	0.78**	0.82**	0.18**	0.86**
	Very late	0.90**	0.74**	0.83**	0.57**	0.88**
CTD	Timely	–	0.67**	0.71**	0.01	0.72**
	Late	–	0.69**	0.77**	0.22**	0.75**
	Very late	–	0.73**	0.79**	0.50**	0.76**
GFD	Timely	–	–	0.78**	0.28**	0.70**
	Late	–	–	0.63**	0.17**	0.72**
	Very late	–	–	0.69**	0.49**	0.61**
Yield	Timely	–	–	–	0.07*	0.86**
	Late	–	–	–	0.30**	0.97**
	Very late	–	–	–	0.40**	0.86**
TGW	Timely	–	–	–	–	0.07*
	Late	–	–	–	–	0.26**
	Very late	–	–	–	–	0.54*

NS non-significant

*, ** Significant at $P < 0.05$ and 0.01

diverse lines shows the stability of these traits across years. Hence, these traits can be applied effectively as selection criteria in breeding programmes without much difficulty.

The *t* test showed significant differences (Tables 4, 5) between staygreen and NSG genotypes for all traits (CTD, grain yield, biomass and GFD) except TGW under the timely sown condition. The correlation study also revealed a significant positive association of staygreen (LAUG) with CTD, GFD, grain yield and biomass (Table 6). Many other scientists working on different crops have also reported this relationship. For example, Gentinetta et al. (1986) and Thomas and Howarth (2000) found that staygreen was associated with yield increase in sorghum. In the sunflower, grain yield was shown to be positively associated with green leaf area at maturity and negatively correlated with the rate of leaf senescence under post-anthesis drought conditions (Borrel et al. 2000). A positive correlation between the staygreen trait and high grain yield has been found in sorghum (Rosenow et al. 1983; Vietor et al. 1989; Evangelista and Tangonan 1990), soybean (Phillips et al. 1984) and maize (Duvick 1984; Russel 1986; Ceppi et al. 1987). However, Ismail et al. (2000) reported no association between heat tolerance and seed weight in cowpea. Jiang et al. (2004) reported a negative correlation between staygreen and yield traits in rice, whereas Phillips et al. (1984) found a negative association between DLS and seed yield in sorghum. Rehman et al. (2009) screened 442 wheat germplasms for heat tolerance by exposing the germplasms to heat shock (>32 °C) and found that staygreen lines withstood heat shock for a longer period but there was no direct relationship with seed setting. In our study there was no significant correlation between TGW, CTD and LAUG under the timely sowing condition, but there was a significant reduction under the late to very late sowing conditions because of improper grain filling and enforced maturity caused by high temperature stress. Chen et al. (2010) physiologically characterized 'staygreen' by investigating two wheat cultivars, Chuannong 12 (CN12) and Chuannong 18 (CN18), harbouring the wheat–rye 1BL/1RS translocated chromosome after flowering under field conditions. These staygreen cultivars showed higher and longer photosynthetic competence during the grain filling stage. Gong et al. (2005) reported that hybrid vigour in wheat leads to an unfavourably delayed senescence which results in a much unused carbon reserve in its straws. Zhang et al. (2006) compared photosynthetic characteristics between two field-grown spring wheat (*Triticum aestivum* L.) cultivars, Ningmai 8 (NM8) and Ningmai 9 (NM9), and found that the slower photosynthetic decline in NM9 due to delayed flag leaf senescence compared to NM8 may be partly responsible for its higher grain yield.

Fischer et al. (1998) also found significant cultivar × date interaction for CTD, but cultivar × year interaction for this trait was non-significant. The shift from the timely to very late sowing date resulted in a significant reduction in the performance of all the categories of genotypes, but staygreen appeared to offer certain advantages over NSG lines, as the decline in yield was comparatively lesser. CTD values were observed to have a tendency to increase in the late to very late sown conditions, possibly because the higher air temperature under the late and very late sown conditions cause more transpiration and consequently a lower canopy temperature. Although there was a significant decline in yield, biomass, GFD and TGW due to heat stress in plants sown under the late and very late conditions, staygreen lines continued to maintain their superiority over their NSG counterparts. CTD showed high genetic correlation with yield traits (biomass, grain yield and GFD), indicating that the trait is heritable and therefore amenable to early generation selection (Reynolds et al. 1998). Amani et al. (1996) and Fischer et al. (1998) also reported a significant correlation of CTD with grain yield and biomass.

The results of this investigation suggest that there is significant variability for the staygreen trait in wheat and that this trait can be used as an effective selection criterion for tolerance to heat stress in wheat.

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