Grain growth rate, canopy temperature depression, chlorophyll content and AGPase activity in relation to grain yield in spring wheat genotypes under late sown condition

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Abstract

Twenty four (24) wheat genotypes were grown in 3 meter long two row plots in randomized block design with three replications at CCS Haryana Agricultural University, Hisar, Research Farm under two natural photothermal environments created through alteration of dates of sowing i.e. 13th November 2007(Normal sown, NS) and 3rd January 2008 (Late sown,LS). These genotypes were evaluated for genotypic variation for canopy temperature depression (CTD, ^{0}C), grain growth rate at three intervals (GGR1-3, mg/day/grain) and grain yield (GY, g/plant). Out of these, six genotypes were selected based upon their early (WH 1070 and WH 1021), medium (WH1071 and WH1066) and late (WH1057 and DBW16) flag leaf senescence. These six genotypes were used to study parameters like their AGPase activity (nmol.mg^{-1}.min^{-1}) and chlorophyll content ((µ mol g^{-1}fresh weight) at 28 days after anthesis; in addition to grain growth rate. Significant genotypic variations under different dates of sowing for GGR1-3, GY and CTD were recorded. Significant positive correlation between GY and GGR3 under normal as well as late sown conditions was noticed. Two stay green genotypes WH1057 and DBW16 had higher chlorophyll content and grain growth rate (GGR3) in late sown conditions as compared to other genotypes. The CTD, GGR3, and AGPase at 28 days showed positive correlation with grain yield under late sown conditions. Thus CTD, grain growth rate and AGPase activity could be used as selection criteria for yield improvement under late sown conditions.

Key words: wheat, grain yield, grain growth rate, AGPase activity, canopy temperature depression

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Introduction

Wheat (*Triticum aestivum* L. cm. Thell) is one of the most important cereal crops of the world. Wheat is grown under sub-tropical environment in Indian sub-continent during mild winter that warms up towards the grain-filling stage of the crop. High temperature in the month of February-March is not congenial for grain development, thus leading to reduced yield. Available field responses of wheat to high temperature show that there is a yield penalty of 4 per cent for every one degree rise in ambient temperature above the optimal (Fisher and Maurer 1976).

There is preliminary evidence that some stay-green lines contain a high level of cytokinin than normal (Ambler *et al.*, 1987) which reduce the rate of loss of both chlorophyll and photosynthesis in senescing wheat seedling (Wittenbach, 1997). Photosynthetic rates decline due to acceleration of senescence and heat stresses decreases mean photosynthetic rates and mean total biomass. High temperature mainly affects thylakoid membrane activities and damages the thermolabile photosystem II reaction centre (Nash *et al.*1985).

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There are also some indications that the enzyme AGPase (Adenosine diphosphate glucose pyrophosphorylase), the initiator of starch biosynthesis process directly affect the wheat productivity by synthesizing starch that constitutes 70% of the wheat grain. To determine the role of AGPase, particularly for starch production in wheat, its activity needs to be compared in different genotypes/varieties of wheat with diverse temperature tolerance. Ahlawat *et al.*(2007) observed that chlorophyll 'a' at anthesis had a direct positive effect on grain yield and chlorophyll 'a' at 21 days after anthesis displayed a positive direct effect on days to flag leaf senescence. This was due to the outstanding performance of stay-green genotypes under late sown conditions. Prioul *et al.* (1994) studied the expression of AGPase in different varieties of maize and found correlation between AGPase activity and starch accumulation rate measured in grains from the stage of pollination to grain maturity. Decrease in the activities of AGPase has been observed both in maize kernels and wheat endosperms under heat stress, thus resulting in a reduction of starch synthesis (Keeling *et al.*1994; Wilhelm *et al.*1999). Greene and Hannah (1998) reported the isolation and characterization of heat stable variants having enhanced stability of maize endosperm. AGPase gained through altered subunit interactions. High temperature stress affects starch biosynthesis by decreasing AGPase activity. AGPase was irreversibly inactivated when exposed to temperatures above 40°C wheat. High CTD is an indicator of high demand for photo assimilation caused by rapidly filling kernels (i.e. sink strength) in physiologically well adapted lines. CTD offer the potential to discard genetically inferior lines at early generation selection stages adding efficiency to breeding programme. Amani *et al*, (1995) found that stomatal conductance (g_s), maximum photosynthetic rate (A_{max}) and canopy temperature depression (CTD) were closely and positively related.

Keeping above facts in view, present investigation was conducted to evaluate spring genotypes for grain growth rate and associated physiological (CTD) and biochemical (AGPase) parameters in determining yield under terminal heat stress prone late sown conditions.

Material and methods

Twenty four (24) wheat genotypes differing for stay green character were grown in two rows of 3meters each in 3 replications (RBD design) during 2007-08 under normal (Nov.13, 2007) and late (Jan 3, 2008) sown conditions for evaluation of genotypic variation for chlorophyll stability, grain growth rate and grain yield and its main component traits.

From among 24 varieties included in study, 6 were selected based upon their days to flag leaf senescence *i.e.* 2 genotypes having early flag leaf senescence (WH 1070 and WH 1021), 2 with medium flag leaf senescence (WH1071 and WH1066) and 2 with late flag leaf senescence (WH1057 and DBW16). These six genotypes were used to study parameters like their AGPase activity at 14, 21 and 28 days after anthesis; grain growth rate at these stages and to study CTD.

AGPase enzyme analysis: AGPase (Adenosine diphosphate glucose pyrophosphorylase) activity at 14, 21 and 28 days post anthesis ($nmol.mg^{-1}.min^{-1}$) was determined in six selected wheat genotypes. Nearly 15-20 developing grains amounting to 0.5 g of the grains were removed from middle portion of earheads and were hand homogenized in a prechilled pestle and mortar at 4°C with cold 2 ml of buffer on ice. The extraction buffer employed was having composition as 50 mM 3-N-morpholino propane sulphonic acid (MOPS) pH 7.4, 2 mM $MgCl_2$, 1 mM EDTA and 2 mM Dithiothritol (DTT) .The homogenate so obtained was centrifuged at 10,000 x g for 10 min in a refrigerated centrifuge at 4°C. The supernatant was used as grain extract for enzyme analysis . AGPase was assay in the reverse direction by modified method of Kleczkowski *et al*. (1993). The reaction was started by the addition of 200 µl of sodium pyrophosphate (2.5 µmole). The pyrophosphorolytic activity of AGPase was assayed spectrophotometrically by monitoring the increase in absorbance due to conversion of NADP to NADPH at 340 nm.

Canopy temperature depression: CTD was determined using infra red thermometer (IRT) in field conditions at post anthesis stage (Everest Interscience Inc. model 6110.4ZL). It is the difference between the canopy temperature and the air or ambient temperature (°C.) Measurement were taken when IRT viewed 100 per cent canopy cover and held at an angle of 30 degree, approximately 50 cm above the canopy from horizontal and at 1m distance from the edge of the plot end. Data was recorded between 12:00 hrs to 14:00 hrs at 14 days after anthesis.

Grain growth rate (GGR) (mg/day/grain): The fresh weight of 10 grains collected from central spikelets of spikes of tagged plants was taken and similarly their dry weight was measured after keeping them at 60°C for 24 hrs and expressed as mg/grain/day. It was taken at 0-14, 14-21 and 21-28 days time interval after anthesis.

Chlorophyll (µ mol /g fresh wt.): Chlorophyll 'a' and Chlorophyll 'b' and total Chlorophyll were extracted as per standard procedure of Hiscox and Israesltam (1979) at 28 DAA.

The data collected were subjected to analysis of variance (ANOVA) and mean differences were tested using LSD at 5% and 1% probability level. The statistical analysis was done using OPSTAT.

Results and discussion

Mean performance: Table 1 shows the analysis of mean, phenotypic and genotypic co-efficient of variation, heritability, genetic advance (as % of mean) for different characters under late sown conditions indicating considerable influence of heat stress. The analysis of variance for randomized block design was carried out for individual characters to test the significance of difference among the genotypes following the method as suggested by Panse and Sukhatme (1978).

Canopy temperature depression: It ranged from 3.96-7.80(NS) and 5.73-10.83 (LS) with a population mean 6.09 (NS) and 7.76 (LS), Moderate phenotypic coefficient of variation (PCV) [24.48 (NS) and 26.86 (LS)], genotypic coefficient of variation (GCV) [11.68 (NS) and 17.37 (LS)].

GGR at 14 DAA: Narrow range [0.477-1.597 mg/day/grain (NS) and 0.416-1.656 mg/day/grain (LS)] with population mean of 1.093 (NS) mg/day/grain and 1.026 mg/day/grain (LS).

GGR at 21 DAA: This character with a mean of 1.117 mg/day/grain (NS) and 0.921 mg/day/grain (LS) exhibited a narrow range [0.401-1.661 mg/day/grain (NS) and 0.390-1.560 mg/day/grain (LS)] with high PCV [30.94% (NS) and 34.44% (LS)], GCV [25.53% (NS) and 29.53% (LS)].

GGR at 28 DAA: This character with a mean of 0.923 mg/day/grain (NS) and 0.693 mg/day/grain (LS) exhibited a narrow range [0.406-1.422 mg/day/grain (NS) and 0.313-.900 mg/day/].

Table 1 . Mean, range, co-efficient of variation (phenotypic and genotypic), heritability, genetic advance (as % of mean) for different characters under late sown conditions

Character	Mean±SE	Range	Co-efficient of variations (%)	
			PCV	GCV
Grain yield (g)/plant	26.37±2.053	22.5-30.08	14.74	5.95
CTD (°C)	7.76±0.918	5.73-10.83	26.86	17.37
GGR-1 (mg/day/grain)	1.03±0.086	0.42-1.66	32.27	28.79
GGR- 2 (mg/day/grain)	0.92±0.094	0.39-1.56	34.44	29.53
GGR-3 (mg/day/grain)	0.69±0.042	0.31-0.90	22.20	19.54
1000 grain weight (g)	39.70 ±1.36	32.8-43.3	8.28	5.75

Chlorophyll content

Chlorophyll "a", "b" and total chlorophyll increased in late sown conditions as compared to normal sown conditions (Table 2). Though PCV and GCV were little lower in LS than NS (Table 2).

AGPase activity

AGPase activity was assayed in developing grains of wheat under late sown conditions (Table 3). The AGPase activity in general increased from 14 DAA to 21 DAA and ultimately at 28 DAA. However, the rate of increase in AGPase activity was variable among genotypes. It was 26 (14DAA), 89 (21DAA) and 284 (28DAA) in WH 1070. The AGPase activity was quite high in WH1057 (255) and DBW 16 (249) at 14 DAA. However, in WH1066 AGPase activity was reduced from 165 (21DAA) to 108 (28DAA). Genotypes WH1057 and DBW 16 showed comparative less reduction in AGPase activity as compared to other genotypes in late sown conditions. AGPase activity at 14, 21 and 28 DAA generally corresponded with the potential of grain yield in different wheat genotypes.

The CTD, grain growth rate-3, and AGPase at 28DAA showed positive correlation with grain yield under late sown conditions among six selected genotypes (Table 4). AGPase at 14 days after anthesis showed positive correlation with AGPase at 21days but neither of these showed any correlation with AGPase at 28 days. This indicated that AGPase at 28DAA contributes towards grain yield under late sown conditions. Since, grain growth rate-3 showed positive correlation with grain growth rate-1 and grain growth rate-2, thus grain growth rate at all the three stages contributes towards grain yield under late sown conditions. However chlorophyll content did not show any correlation with any trait.

Table 2. Grain yield, 1000 grain weight and chlorophyll content of the genotypes at 28 DAA

Variety	Chlorophyll 'a' (μ mol g-1fresh weight)		Chlorophyll 'b' (μ mol g-1fresh weight)		Total chlorophyll (μ mol g-1fresh weight)		Grain yield (g)/plant		1000 grain (g) weight	
	NS	LS	NS	LS	NS	LS	NS	LS	NS	LS
WH1070 (E)	14.93	15.82	0.64	0.71	15.57	16.51	24.84	23.34	36.9	40.9
WH1021(E)	13.91	14.23	0.57	0.62	14.47	14.85	26.40	22.52	35.2	41.4
WH1071(M)	15.07	16.93	0.81	0.89	15.88	17.73	22.40	28.58	36.0	39.3
WH1066(M)	9.49	10.87	0.69	0.72	10.18	11.69	26.19	29.75	41.1	39.9
WH1057(L)	9.58	12.88	0.63	0.71	10.20	13.59	24.14	26.11	36.9	34.1
DBW16(L)	11.4	13.61	0.65	0.70	12.05	14.31	33.79	30.09	38.4	49.9
Mean	12.40	14.05	0.66	0.73	13.01	14.78	26.39	26.73	37.4	40.9
PCV	17.88	16.87	18.90	18.67	14.84	15.75	16.18	14.74	9.10	8.28
GCV	13.76	12.45	17.88	16.87	13.83	14.34	10.02	5.95	2.68	5.75
CD at 5%	2.45	1.97	0.12	0.15	1.90	2.25	5.93	3.67	3.25	6.45

E= early senescing, M= moderately senescing and l= late senescing genotypes

This study was carried out particularly to find out relation of AGPase activity, grain growth rate, CTD and grain yield in wheat. Measurements of chlorophyll 'a' and chlorophyll 'b' were carried out in selected wheat genotypes. The increase (from 9.582 to 12.88 in μ mol g^{-1}fresh weight WH 1057 and from 11.42 to 13.61 μ mol g^{-1}fresh weight in DBW16) in chlorophyll 'a' content as well as total cholorophyll content in late sown conditions was shown by two partially stay-green genotypes as compared to the remaining wheat genotypes. Thus these two genotypes could be photosynthetically active at later grain developmental stages (28 DAA), thus indicating their importance for late sown condition.

Table 3. CTD, chlorophyll content, grain growth rates, ADP-glucosepyrophosphorylase activity in developing grains of selected wheat varieties at 14 and 28 days anthesis and grain yield under late sown condition

Genotype	CTD (ºC)	AGPase activity (nmol.mg-1 min-1) 14DAA	AGPase activity (nmol.mg-1 min-1) 21DAA	AGPase activity (nmol.mg-1 min-1) 28 DAA	Chlorophyll (μmol/g fresh weight) at 28 DAA	Grain yield (g) / plant	GGR- 1 (mg/day/grain)	GGR-2 (mg/day/grain)	GGR-3 (mg/day/grain)
WH1070	8.10	26	89	284	6.51	23.34	1.007	0.823	0.770
WH1021	6.97	85	88	150	4.85	22.52	0.890	0.806	0.653
WH1066	9.53	165	165	108	1.69	28.58	1.057	0.933	0.727
WH1071	9.93	49	82	90	7.73	29.75	0.590	0.550	0.470
WH1057	6.60	210	255	259	3.59	26.11	0.793	0.773	0.750
DBW16	8.50	236	249	356	4.31	30.09	0.950	0.870	0.800
CD at 5%	2.43	67	147	157	2.66	3.67	0.225	0.198	0.269

Table 4. Phenotypic correlation coefficient among different characters under late sown condition using selected 6 genotypes

Character	Type of correlation	With characters
GY	+ve**	CTD at 14 DAA, GGR-3, AGPase at 28DAA
CTD	+ve*	GY
AGPase 14DAA	+ve	AGPase at 21DAA
AGPase 21DAA	+ve	AGPase 14DAA
AGPase 28DAA	+ve**	GY
Total Chlorophyll content at 28 DAA	-	-
GGR-1	+ve	GGR-3
GGR-2	+ve	GGR-3
GGR-3	+ve*	GGR-1

** indicate significance at 1% LSD, ** indicate significance at 1% LSD*

These results are encouraging for a wheat breeder who is interested to develop improved genotypes particularly for late sown conditions because most of the relevant traits like chlorophyll 'a', total chlorophyll at 28 days after anthesis and GGR at all the 3 stages particularly GGR3 have considerable variation, moderate to high heritability and appreciable genetic grains. Earlier studies of Barma *et al.*, 2002 and Ahlawat *et al.*, 2007) also revealed almost similar findings.

Higher temperature after anthesis abbreviates the duration of grain growth and grain size is reduced. As a net result the overall plant yield is reduced (Evans and Wardlaw, 1996). GGR-3 in late sown conditions was positively correlated with GGR-1 and GGR-2, and grain yield. Since grain growth rate contributes to grain weight, one of the three major yield components in wheat, it can be used as a selection criterion in late sown conditions.

Geigenberger *et al.* (1998) proposed that short term elevated temperatures lead to increased rates of respiration and resulting decline of 3PGA (3-phosphoglyceric acid) which is positive modulate of AGPase and thus inhibits AGPase *vis-à-vis* starch synthesis in potato.

A few studies have been conducted to observe the activity of AGPase under heat stress conditions (Geigenbergar *et al.,* 1998). They concluded that AGPase activity decreases with the increase of temperature at maturity. None of the studies was conducted in line with the present investigation that aimed to observe the rate of decrease in AGPase activity in wheat genotypes differing for their time of leaf senescence (normal and stay green types) under normal and late sown conditions. So, in order to study the association of AGPase enzyme with starch accumulation and the level of thermotolerance in different wheat varieties, AGPase enzyme assays were carried out at 14, 21 and 28 DAA in wheat varieties different for their yield potential, under normal and late sown condition.

Six genotypes were selected from among the 24 genotypes, 2 each with early flag leaf senescence (WH1070, WH 1021), medium flag leaf senescence (WH 1071, WH 1066) and late flag leaf senescence (DBW16, WH1057).

AGPase enzyme activity was found to be positively correlated with grain growth rate (GGR) in all the six genotypes, both in normal and late sown conditions. AGPase activity *per se* was correlated with GGR-3. As the AGPase enzyme activity was high, GGR was also high. In the two stay green genotypes, the AGPase activity in late sown conditions was comparable to that of normal sown. While in other 4 genotypes, the AGPase activity decreased in the late sown conditions.

AGPase was correlated with yield in both the environments. AGPase revealed three patterns of activity- (1) initially low and getting high over subsequent GGR phases. (2) initially high, remaining high over subsequent GGR phases (3) initially low or high and changing over different phases. However in some genotypes (WH1070) AGPase activity was low while GGR was high. This may be due to presence of different isoenzymes of AGPase which would have contributed towards the higher grain growth rate and high yield. Although, AGPase activity was low but a comparative increase was higher under late sown rather than under normal sown condition. This was because of their staygreen character due to which, starch synthesis was still going on even at 28 DAA. Therefore, stay green genotypes; WH1057 and DBW16 should be involved in crossing programme aimed at wheat improvement for late sown conditions to harness photosynthesis for longer duration and hence overcome the loss of yield under late sown conditions to a considerable extent. It is expected that incorporation of stay-green trait in a genotype could help in increasing the ultimate grain yield. However, the strength and direction of the association between stay-green and grain yield varied with both environment and genetic background. Therefore, further research is required to determine if the negative association between stay-green and yield at higher yield levels is a direct effect or the result of an interaction with agronomic practice, in particular sowing date or it is only a case specific finding.

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