

ADP-glucose Pyrophosphorylase Activity in Relation to Yield Potential of Wheat: Response to Independent and Combined High Temperature and Drought Stress

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ADP-glucose pyrophosphorylase (AGPase) activity in the developing grains of two contrasting wheat cultivars WH730 (thermo-tolerant) and UP2565 (thermo-sensitive) was determined in relation to their allosteric effectors and grain growth. The developing grains (35 days after anthesis) were excised from the middle portion of spikes of wheat genotypes subjected to high temperature, drought and their combination at booting, post-anthesis and booting+post-anthesis. The impact of stress treatments was studied by measuring starch content and yield attributes in relation to AGPase activity. AGPase, a key enzyme for starch synthesis, is allosterically activated by 3-phosphoglyceric acid (3-PGA) and inhibited by inorganic phosphate (Pi). Sensitivity of AGPase towards individual and combined high temperature and drought has not been adequately investigated, therefore the present study analyzed AGPase activity, its sensitivity to allosteric effectors under influence of high temperature, drought in order to elucidate the relationship of AGPase with starch accumulation and grain growth. Significant difference in behavior of the enzyme and its allosteric effectors were observed between the two cultivars under high temperature and/or drought. AGPase activity was substantially decreased by high temperature, drought and was found to be positively correlated with the 3-PGA, starch accumulation and yield attributes, while negatively correlated with Pi content. The results showed that effects of high temperature and drought were additive and more severe at booting+post-anthesis stage. Such studies might help in understanding the control mechanisms associated with the pathway of starch biosynthesis and thus provide chemical means to manipulate starch content vis-à-vis grain yield under heat and drought stress.

Keywords: *Triticum aestivum*, stress tolerance, AGPase, allosteric effectors, starch, wheat

Abbreviations: AGPase: ADP-glucose pyrophosphorylase, 3-PGA: 3-phosphoglycerate, Pi: inorganic phosphate, HT: high temperature, D: drought, HT+D: high temperature along with drought, DAA: days after anthesis, DAS: days after sowing, PWP: permanent wilting point, RH: relative humidity

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Introduction

Water scarcity and high temperature ($>30^{\circ}\text{C}$) at the time of grain filling is one of the major constraints in increasing productivity of wheat in tropical countries like India (Rane and Nagarajan 2004; Zhao et al. 2007; Yan et al. 2008; Kaur and Behl 2010). Grain filling is mainly a process of starch biosynthesis and accumulation. Reduced starch content accounts for most of the reduction in grain dry matter and thus yield (Yang et al. 2004; Sakulsingharoj et al. 2004; Sheikh et al. 2010). Carbon flux into starch is controlled at several steps. Most conspicuous is the reaction catalyzed by ADP-glucose pyrophosphorylase (AGPase) which produces ADP-glucose, the activated glucose form utilized by the polymerizing enzyme starch synthase. AGPase controls the rate-limiting step in starch biosynthesis and is regulated at various levels. Cereal endosperm enzymes, in contrast to other plant AGPases, are particularly heat labile and transgenic studies highlight the importance of temperature for cereal yield (Boehlein et al. 2014). In cereals, manipulation of this enzyme is a prime target to increase starch production during seed development as a means to increase sink strength and, in turn, crop yields. In photosynthetic tissues, the chloroplast-localized AGPase regulates one of the key control points in carbon partitioning between starch and sucrose (Heldt et al. 1977; Preiss et al. 1991; Stitt 1996) as its catalytic activity is governed by the ratio of two small effector molecules; it is activated by 3-phosphoglyceric acid (3-PGA) and inhibited by inorganic phosphate (Pi) (Preiss et al. 1991); as also reported in developing rice seeds (Sikka et al. 2001; Sakulsingharoj et al. 2004) and potato tubers (Sowokinos and Preiss 1982; Sumesh et al. 2008).

However, the AGPase from developing barley (Kleczkowski et al. 1993; Doan et al. 1999) and wheat (Gómez-Casati and Iglesias 2002) is not reported to be activated by 3-PGA and show moderate Pi inhibition which is reversible. Therefore, activation of AGPase by an increasing ratio of 3-PGA to Pi allows the rate of starch synthesis to be adjusted in response to changes in the balance between photosynthesis and sucrose synthesis in leaves (Smidansky et al. 2003) and to changes in the balance between sucrose breakdown and respiration. Allosteric regulation of AGPase by changes in the plastidial concentrations of 3-PGA as activator and Pi as inhibitor provides a further mechanism for light/dark modulation of starch biosynthesis. Studies in recent past (Wang et al. 2007; Sumesh et al. 2008; Lohot et al. 2010) provide evidence that changes in the allosteric properties of AGPase mediates increased starch production, resulting in increased yields. Accordingly, the thermal stability and regulatory properties of AGPase have received considerable attention. More insight into research was needed to achieve a better understanding of these important aspects of the regulation of starch synthesis as sensitivity of AGPase towards high temperature and drought has not been adequately investigated. The present study therefore analyzed AGPase activity, its sensitivity to allosteric effectors under influence of high temperature, drought and their combination in order to elucidate the relationship of AGPase with starch accumulation and grain growth.

Materials and Methods

Plant materials and growing conditions

Two contrasting spring wheat (*Triticum aestivum* L.) cultivars WH730 (thermo tolerant) and UP2565 (thermo sensitive) were tested for differential response to high temperature and drought tolerance at 35 days after anthesis (DAA) for AGPase activity, starch accumulation and yield parameters. Plants were raised, without any vernalization treatment, in earthen pots (30 cm in diameter) lined with polythene bags and each containing 5 kg of dune sand (*Typic Torripsamments*) (93.3% sand + 3.0% silt + 3.7% clay, pH 8.2, EC_{e2} 0.8 $dS\ m^{-1}$ at 25°C, 10.3 mg (N) kg^{-1} , 2.5 mg (P) kg^{-1} , 180 mg (K) kg^{-1}) from 26 November to 15 April (wheat growing season in northern India), under natural conditions of a screen house. After thinning four healthy plants were kept in each bag.

High temperature and water deficit treatments

The experiment was resolved into $2 \times 4 \times 3$ [(two genotypes, four treatment levels, viz. control, high temperature (HT) stress, drought (D) and combined (HT + D) stress, three stages of treatment imposition (booting, post-anthesis and booting + post-anthesis)] complete randomized design (CRD). Control conditions comprised of growing plants at field capacity (20–22%) and ambient temperature (10–26°C) during crop growth. High temperature stress was given to plants by shifting the pots to polyhouse for one week with maximum temperature 5–8°C > than the ambient. Drought was imposed by withholding water supply till permanent wilting point (PWP, gravimetric soil moisture 6–7%) was attained. The plants were re-irrigated (600 ml water per pot to attain field capacity) after PWP. Combined stress was given to plants by shifting pots to polyhouse for one week and simultaneously drought conditions were maintained by withholding water supply. The plants of both varieties of wheat were exposed to high temperature, drought and the combination of both stresses at booting stage (70 DAS); post-anthesis stage (10 DAA) and booting + post-anthesis stage. The soil moisture was almost similar in both varieties under all treatments. Temperature in general was higher in polyhouse (5–8°C) than the screen house. The light intensity, though not measured, was adequate under screen house and polyhouse conditions to permit sufficient photo-synthetically active radiation.

Sampling

Mother shoots in all the bags were tagged during spike emergence and labeled ears of each treatment were sampled at 35 DAA and stored in liquid nitrogen for estimation of AGPase, 3-PGA and Pi. The control and stressed plants were harvested for yield components at maturity. Number of grains 5 spike⁻¹, grain weight 5 spike⁻¹ (g), grain yield plant⁻¹ (g) and 1000-grain weight (g) was recorded for each of the three replicates in both varieties.

Extraction and estimation of AGPase

AGPase (EC 2.7.7.23, mmol NADP reduced $\text{min}^{-1} \text{g}^{-1}$ grain FW) was extracted following method of Savitch et al. (2000) by grinding two basal grains from the middle spikelets of the liquid nitrogen frozen wheat spikes (35 DAA) in pestle and mortar using 2 ml buffer having 50 mM MOPS (pH 7.5), 5 mM MgCl_2 , 1 mM EDTA, 0.02% (w/v) BSA, 2% (w/v) polyvinylpyrrolidone (PVP), 2.5 mM DTT and 0.05% (w/v) Triton X-100. After centrifugation of homogenate for 1 min at $15,000 \times g$ (Remi refrigerated centrifuge; model C-30), supernatant was separated and used for enzyme assay.

AGPase was assayed in reverse direction by modified method of Kleczkowski et al. (1993). One ml of reaction mixture containing 50 mM MOPS (pH 7.4), 7.5 μmol of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 3 μmol of 3-PGA, 0.5 μmol of NADP⁺, 0.5 μmol of ADP-glucose, 2 units of phosphoglucomutase and 2 units of glucose-6-phosphate dehydrogenase was incubated with 100 μl of enzyme extract. The reaction was started by the addition of 200 μl of sodium pyrophosphate (2.5 μmol). The pyrophosphorolytic activity of AGPase was assayed spectrophotometrically (Systronic India Spectrophotometer-117) by monitoring the increase in absorbance due to conversion of NADP to NADPH at 340 nm.

Extraction and estimation of allosteric effectors

3-PGA and Pi were extracted by the method of Stitt (1990). Basal grains from the middle spikelets of liquid nitrogen frozen wheat spikes were thoroughly washed and grinded in a chilled mortar and pestle with 5.0 ml extraction medium. For preparation of 252 ml extraction medium, 72 ml chloroform, 144 ml methanol and 36 ml (50 mM NaF, 20.0 mM EDTA, 50 mM HEPES) and a pinch PVP was added during grinding of samples. The extract was centrifuged at $7000 \times g$ for 10 min at 0–4 °C. The supernatant was thoroughly mixed with 4.0 ml distilled water on a cyclomixer and allowed to stand for 10 min to have phase separation. After proper separation, upper layer (methanol) containing metabolites were dried at $35 \pm 1^\circ\text{C}$ in vacuum with rotatory evaporator and the residue was dissolved in 4.0 ml distilled water.

3-PGA was estimated following method of Lutzko and Gibbs (1972) by coupling two reactions of glycolytic pathway to reduce 3-PGA to glyceraldehyde 3-P consuming stoichiometric amount of ATP and NADH and oxidation of NADH is followed at 340 nm. To the standard assay mixture in a final volume of 1.0 ml containing 0.1 ml of tris-EDTA buffer (0.4 M in 0.4 M EDTA, pH 7.6), test extract, 20 μl of 5 μM NADH, 0.1 ml of 50 μM ATP, 20 μl of 0.5 M MgCl_2 , 1 unit of glyceraldehyde-3-PDH and 1 unit of 3-PGA kinase, 50 μl glycerate 3-P dehydrogenase (0.05 U) was added and change in absorbance recorded at 340 nm with time interval of 1 second (time scan). Changes in absorbance during linear phase were determined. Inorganic phosphate was estimated by method of Ozbun et al. (1973). Concentration of Pi ($\mu\text{mol g}^{-1}$ grain FW) was calculated using standard curve prepared with 1–10 mg ml^{-1} KH_2PO_4 .

Starch content (mg g^{-1} DW) was estimated by the method of Clegg (1956). Protein content (%) in grain was estimated by Infratec 1241 Grain Analyser, Foss (USA). Also,

seedling stage peptide profile in response to high temperature (HT), drought (D) and dual stress (HT+D) outlined via SDS electrophoresis of leaf extracts was done. These findings have been reported earlier by Kaur et al. (2014).

Statistical analysis

Data were analyzed statistically using complete randomized design (CRD) with three replicates performing analysis of variance (ANOVA) using Statistical Analysis Package (SAS 2011; version 9.2) for each stress imposition stage (booting, post-anthesis, booting + post-anthesis) separately. Means were tested by LSD at $P_{0.05}$ level ($LSD_{0.05}$) and significance of differences among treatments and genotypes were determined.

Results

All the stress treatments either individually applied HT, D or combined HT + D led to a decrease in the mean AGPase activity in both the genotypes, however decline was more in UP2565 compared to WH730. Significant decline in the activity of AGPase was observed in UP2565 when the stress treatments were imposed at post-anthesis as well as booting + post-anthesis stage indicating the severity of stress and sensitivity of the genotype (Table 1). Similar trend was observed in starch content, thus correlating it well to AGPase activity. Few other reports also confirmed these results earlier (Geigenkerger et al. 1998; Ahmadi and Baker 2001; Smidansky et al. 2002; Ballicora et al. 2004; Sumesh et al. 2008). Lower mean value of 3-PGA was observed in grains of UP2565 (4.56) relative to WH730 (5.60). Stress treatments reduced 3-PGA content (exception in UP2565 at booting stage, where the data showed little increase compared to the control) and the trend of increase/decrease in AGPase activity in both the varieties was similar to that of 3-PGA. Higher value of Pi in grain was observed in WH730 (112.13 $\mu\text{mol g}^{-1}$ grain FW) than UP2565 (81.01 $\mu\text{mol g}^{-1}$ grain FW) at all the three sampling stages. All the stress treatments increased Pi concentration with interaction of HT + D leading to the maximum increase. More increase in Pi content was observed in UP2565 (more than 1.5-fold) than WH730 due to combined stress imposed at booting + post-anthesis stage which can be attributed to the corresponding decrease in the starch content and hence grain yield in var. UP2565 over WH730. Results supporting the negative effect of Pi concentration on starch synthesis were also presented by Geigenkerger et al. (1998), Slattery et al. (2004) and Lohot et al. (2010).

Water scarcity combined with high temperature decreased yield components substantially in tested wheat genotypes (Table 2). Overall the effects of high temperature and drought were additive and more detrimental effects were observed with the combined stress imposed at booting + post-anthesis stage. Interaction of HT + D resulted in more decline in yield and yield related parameters in UP2565 than WH730 compared to individually applied stress treatments. Tolerant genotype WH730 had higher grain yield, grain number 5 spike⁻¹, grain weight 5 spike⁻¹ and 1000-grain weight under control conditions and it maintained yield potential under stress conditions as indicated by less

Table 1. Effect of individually applied high temperature (HT), drought (D) and combined (HT+D) stress imposed at different stages on the activities of ADP-glucose pyrophosphorylase (AGPase; mmol NADP reduced min⁻¹ g⁻¹ grain FW), 3-phosphoglyceric acid (3-PGA; μ mol g⁻¹ grain FW), inorganic phosphate (Pi; μ mol g⁻¹ grain FW) and starch content (mg g⁻¹ DW) in the grains of wheat genotypes at 35 DAA

Enzyme/metabolite	Genotype	Booting stage			
		C	HT	D	HT+D
AGPase	WH730(T)	9.87 ^A	9.22 ^{AB}	8.8 ^{ABC}	8.53 ^{ABC}
	UP2565(S)	8.42 ^{BC}	8.25 ^{CD}	8.21 ^{CD}	8.15 ^{CD}
3-PGA	WH730(T)	5.60 ^A	5.52 ^A	5.41 ^{AB}	5.15 ^{ABC}
	UP2565(S)	4.56 ^B	4.59 ^B	4.73 ^{BC}	4.58 ^B
Pi	WH730(T)	112.13 ^A	121.11 ^{AB}	119.75 ^{AB}	123.94 ^{AB}
	UP2565(S)	81.01 ^E	91.05 ^{DE}	87.63 ^E	106.35 ^{CD}
Starch	WH730(T)	881.22 ^A	859.57 ^{AB}	856.35 ^{AB}	810.52 ^C
	UP2565(S)	851.48 ^B	832.52 ^{BC}	822.00 ^{BC}	795.13 ^E
Enzyme/metabolite	Genotype	Post-anthesis stage			
		C	HT	D	HT+D
AGPase	WH730(T)	9.87 ^A	9.46 ^{AB}	8.82 ^{ABC}	7.94 ^{CD}
	UP2565(S)	8.42 ^{BC}	7.69 ^{CD}	7.56 ^{CD}	6.40 ^D
3-PGA	WH730(T)	5.60 ^A	5.09 ^{AB}	5.14 ^{AB}	5.04 ^{AB}
	UP2565(S)	4.56 ^B	4.54 ^B	4.39 ^{BC}	4.36 ^{BC}
Pi	WH730(T)	112.13 ^A	128.34 ^{AB}	125.58 ^{AB}	142.16 ^B
	UP2565(S)	81.01 ^E	105.66 ^{CD}	100.93 ^D	127.73 ^B
Starch	WH730(T)	881.22 ^A	782.61 ^C	768.99 ^C	706.00 ^D
	UP2565(S)	851.48 ^B	779.22 ^C	677.57 ^E	660.78 ^F
Enzyme/metabolite	Genotype	Booting + Post-anthesis stage			
		C	HT	D	HT+D
AGPase	WH730(T)	9.87 ^A	9.10 ^{AB}	8.50 ^{AB}	7.95 ^{BC}
	UP2565(S)	8.42 ^{BC}	6.99 ^D	7.12 ^D	6.09 ^E
3-PGA	WH730(T)	5.60 ^A	5.04 ^{AB}	5.08 ^{AB}	4.92 ^C
	UP2565(S)	4.56 ^B	4.18 ^C	4.06 ^C	3.80 ^D
Pi	WH730(T)	112.13 ^A	143.16 ^B	138.57 ^B	158.34 ^C
	UP2565(S)	81.01 ^E	117.57 ^B	129.88 ^C	138.93 ^C
Starch	WH730(T)	881.22 ^A	730.17 ^C	745.25 ^C	690.24 ^D
	UP2565(S)	851.48 ^B	704.87 ^D	688.96 ^D	603.83 ^E

Statistical comparison was among wheat genotypes within each treatment imposition stage separately. Means with at least one letter common are not statistically significant at P_{0.05} level using Fisher's Least Significant Difference.

C – Control; HT – High temperature; D – Drought; HT+D – High temperature combined with drought; T – Tolerant; S – Susceptible.

Table 2. Effect of individually applied high temperature (HT), drought (D) and combined (HT+D) stress on yield (g) and yield attributes in the grains of wheat genotypes at different stages of treatment imposition

Yield and yield attributes	Genotype	Booting stage			
		C	HT	D	HT+D
Grain weight 5 spike ⁻¹	WH730(T)	7.13 ^A	6.77 ^{AB}	6.56 ^{AB}	6.13 ^B
	UP2565(S)	6.03 ^B	5.86 ^B	6.03 ^B	5.09 ^C
Grain number 5 spike ⁻¹	WH730(T)	170.00 ^A	173.33 ^A	139.67 ^{BC}	153.00 ^B
	UP2565(S)	150.67 ^B	132.67 ^{CD}	122.33 ^D	125.33 ^{CD}
Grain yield plant ⁻¹	WH730(T)	3.53 ^A	3.28 ^A	3.34 ^A	3.21 ^A
	UP2565(S)	3.08 ^{AB}	2.92 ^{BC}	2.85 ^{BC}	2.71 ^{BC}
1000-grain weight	WH730(T)	41.94 ^A	39.16 ^{ABC}	39.06 ^{ABC}	37.32 ^{BCD}
	UP2565(S)	40.58 ^{AB}	39.72 ^{BC}	38.81 ^{BC}	36.84 ^C
Yield and yield attributes	Genotype	Post-anthesis stage			
		C	HT	D	HT+D
Grain weight 5 spike ⁻¹	WH730(T)	7.13 ^A	6.55 ^{AB}	6.66 ^{AB}	6.04 ^{BC}
	UP2565(S)	6.03 ^B	5.67 ^{BC}	5.27 ^{CD}	4.79 ^D
Grain number 5 spike ⁻¹	WH730(T)	170.00 ^B	186.33 ^A	156.67 ^C	189.00 ^A
	UP2565(S)	150.67 ^C	147.67 ^C	149.00 ^C	100.00 ^D
Grain yield plant ⁻¹	WH730(T)	3.53 ^A	2.94 ^B	2.75 ^{BC}	2.68 ^{BC}
	UP2565(S)	3.08 ^{AB}	2.35 ^{CD}	2.37 ^{CD}	2.20 ^D
1000-grain weight	WH730(T)	41.94 ^A	38.78 ^B	38.47 ^B	36.86 ^{CD}
	UP2565(S)	40.58 ^{AB}	38.47 ^{BC}	37.94 ^{BC}	35.30 ^D
Yield and yield attributes	Genotype	Booting + Post-anthesis stage			
		C	HT	D	HT+D
Grain weight 5 spike ⁻¹	WH730(T)	7.13 ^A	5.70 ^B	5.50 ^{BC}	5.36 ^{BC}
	UP2565(S)	6.03 ^B	5.53 ^{BC}	4.64 ^D	3.96 ^D
Grain number 5 spike ⁻¹	WH730(T)	170.00 ^A	145.67 ^{BC}	154.33 ^B	140.33 ^C
	UP2565(S)	150.67 ^{BC}	109.33 ^D	103.33 ^D	102.00 ^D
Grain yield plant ⁻¹	WH730(T)	3.53 ^A	2.71 ^{BC}	2.69 ^{BC}	2.47 ^{CD}
	UP2565(S)	3.08 ^{AB}	2.40 ^{CD}	1.98 ^{CD}	1.83 ^D
1000-grain weight	WH730(T)	41.94 ^A	37.07 ^{CD}	37.32 ^{CD}	36.68 ^{CD}
	UP2565(S)	40.58 ^{AB}	35.95 ^C	35.16 ^C	33.09 ^D

Statistical comparison was among wheat genotypes within each treatment imposition stage separately. Means with at least one letter common are not statistically significant at $P_{0.05}$ level using Fisher's Least Significant Difference.

C – Control; HT – High temperature; D – Drought; HT+D – high temperature combined with drought; T – tolerant; S – susceptible.

Table 3. Relationship of mean ADP-glucose pyrophosphorylase (AGPase) activity and starch content in wheat grains at 35 DAA with yield parameters and allosteric effectors of AGPase

Correlation with	ADP-glucose pyrophosphorylase (AGPase) activity	Starch content
ADP-glucose pyrophosphorylase (AGPase)	1	0.811**
Grain yield plant ⁻¹	0.861**	0.928**
1000 grain weight	0.798**	0.895**
3-phosphoglyceric acid (3-PGA)	0.888**	0.686**
Inorganic phosphate (Pi)	-0.427*	-0.461*

The * and ** indicate correlation significance at the $P_{0.05}$ and $P_{0.01}$ levels of probability, respectively.

reduction in comparison to susceptible genotype, UP2565. These results suggested that higher thermo-sensitivity of UP2565 was related to decreased starch synthesis in this variety indicating WH730 was better performing variety under heat and drought conditions. Few other reports also confirmed that reduced starch content accounts for most of the reduction in grain dry matter and thus yield (Zahedi et al. 2003; Yang et al. 2004; Sakulsingharoj et al. 2004; Sheikh et al. 2010).

The correlations of activities of enzyme AGPase and starch content examined with grain growth and allosteric effectors of AGPase (3-PGA and Pi) were analyzed (Table 3). A positive and highly significant correlation ($P_{0.01}$) of mean AGPase activity with starch accumulation ($r = 0.811$), 3-PGA ($r = 0.888$), grain yield ($r = 0.861$), 1000-grain weight ($r = 0.798$) was observed in tested wheat cultivars, while it was negatively and significantly correlated with Pi content ($r = -0.427$, $P_{0.05}$). Similar results were obtained for correlations of starch content with grain yield, 3-PGA and Pi content. A significant correlation of AGPase activity with starch accumulation, grain yield and 1000-grain weight indicates that enhancing the activities of the enzymes would lead to increase in starch accumulation and thus grain growth.

Discussion

A study in wheat endosperm by Gómez-Casati and Iglesias (2002) reported that inhibition of the purified AGPase by Pi, ADP and fructose-1, 6-*bis*-phosphate could be reversed by 3-PGA. Sakulsingharoj et al. (2004) reported a mutant AGPase independent of 3-PGA activation and resistant to Pi inhibition may enhance starch synthesis and consequently seed weight in rice. Research studies in potato (Stark et al. 1992; Sumesh et al. 2008), wheat (Smidansky et al. 2002; Lohot et al. 2010); rice (Sikka et al. 2001; Smidansky et al. 2003; Sakulsingharoj et al. 2004) and maize (Wang et al. 2007) provide evidence that changes in the allosteric properties of AGPase mediates increased starch production, resulting in increased yields. Our study also supports these earlier results as more concentration of 3-PGA in var. WH730 could have paved way for more AGPase activity by inhibiting Pi in comparison to thermo-sensitive UP2565 (Table 1). More increase in Pi content was observed in UP2565 (more than 1.5-fold) than WH730 due to combined

(HT + D) stress can be attributed to the corresponding decrease in the starch content and hence grain yield in var. UP2565 over WH730. Results for effect of stress treatments imposed at all mentioned stages for protein content in grains as well as peptide profiles in leaf samples have been reported earlier (Kaur et al. 2014). Due to stress treatments, protein content showed considerably less reduction *per se*, however significant changes in peptide profile outlined via SDS electrophoresis of leaf extracts of seedlings exposed to HT, D and combined HT + D stress were observed which revealed the emergence of several new peptides with persistence of few original ones indicating their chaperoning activity and possible role in stress adaptation (Kaur et al. 2014). In the present study, the effects of high temperature and drought were additive with more severe effects on enzyme activity and yield components when combined stress was imposed at booting + post-anthesis. The enzyme activity, starch content and yield parameters appear to be much less affected at booting stage which may happen due to acclimation. Thus, the differential sensitivity of AGPase to allosteric effectors is of particular significance in finding allosterically insensitive AGPase. Expressing an efficient AGPase insensitive to regulation by 3-PGA and Pi in wheat would possibly lead to faster starch accumulation and early and relatively prolonged filling of grains and thus avoid extreme terminal high temperature experienced during later part of grain development as well as water scarcity. Knowledge of the mechanisms by which sugars regulate grain set under drought and high temperature conditions could be exploited in wheat breeding for abiotic stress tolerance/climate change.

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