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Differential and Temperature Dependent Regulation of ADP–Glucose Pyrophosphorylase by Specific Chromosome in Wheat Grains

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A stock of disomic chromosome substitution (DCS) lines having specific chromosome of wheat variety C591 substituted in the background of rest of Chinese spring chromosomes, were used to analyze grain yield components as a function of enzyme activity of ADP–glucose pyrophosphorylase (AGPase), a starch biosynthesis enzyme in wheat grains. Associations between yield characteristics, grain growth rate (GGR) and AGPase enzyme activity of DCS lines suggested a major involvement of chromosome 3A, 4B, 7D and 2D in a temperature dependent manner. Assessment of AGPase assay at different developmental stages such as 14, 21, 28 days post anthesis (DPA) embodied that gene(s) for this enzyme are present on specific chromosomes and operate at different stages of grain development. The DCS line with 7D chromosome has a major contribution in determining the grain starch content. In this line, AGPase enzyme activity was highest at 21 DPA and was the most crucial determinant in its high GGR. Line 4B performed well at only early stage (14 DPA) suggesting that line 4B AGPase requires a lower temperature range for activation as compared to 7D line. Line 3A had substantially reduced (40%) test weights revealing the presence of few down-regulatory elements on chromosome 3A to reduce the activity of AGPase. The DCS line 2D showed higher test weights and grain number than all other lines ascribed to a consistent AGPase activity along with an efficient mechanism for translocation of photosynthates from source to sink. The chromosome 2D shows positive relation with yield attributes therefore, it can be employed to improve wheat productivity via analytical breeding programme.

Keywords: AGPase enzyme, chromosome, days post anthesis (DPA), days to anthesis (DTA), disomic substitution line (DCS), grain growth rate (GGR), wheat

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Introduction

Wheat (*Triticum aestivum* L.) is one of the most important crops in the world as it is staple food for one third of the population. It is the cheapest source of carbohydrates. Wheat is grown in diverse environments, from temperate rain-fed to tropical dry-land areas around the world. This wide-spread cultivation of the crop is due to the high versatility of its genome.

The seed number and seed weight are critical yield components of wheat. Starch accounting for 65–75% of wheat seed weight, is a major factor of yield (Souci et al. 2008). Among the major starch biosynthetic enzymes, AGPase is a key enzyme controlling the amount of cereal grain starch (Preiss and Sivak 1991; Okita 1992; Hannah 1997). It plays a key role in the modulation of photosynthetic efficiency in source tissues and also governs the level of accumulated starch in sink tissues, thereby influencing the overall crop yield potential. Starch biosynthesis is initiated by AGPase with ADP–glucose synthesis being the first committed precursor of starch biosynthesis. These ADP–glucose moieties are the basic building units of starch. Subsequently, starch synthases, branching and debranching enzymes act upon ADP–glucose to synthesize biologically active starch (Smith et al. 1997). The plant AGPase is allosterically regulated by 3-phosphoglyceric acid (3-PGA) and orthophosphate (PPi), where 3-PGA act as an activator and PPi as an inhibitor (Kleczkowski et al. 1993). Cereals with mutations imparting reduction in grain starch content are accompanied by a substantial decrease in AGPase activity reducing grain yield (Giroux et al. 1996; Tsai and Nelson 1966). Increase in the AGPase activity in endosperm has been associated with increased grain yield in diverse cereals like maize and wheat (Zhang et al. 2011).

Yield is a major concern for wheat cultivators, globally. Despite several years of research, a critical gap still remains in our understanding of the factors that control yield. In light of this fact, cytogenetic approaches and breeding for DCS lines to locate genes on chromosome reports are unambiguous (Kubalakova et al. 2003). Wheat DCS line series, differ only for one duo of chromosomes. Thus DCS lines can provide information about localization of genes for AGPase activity and yield attributes (Hajjar and Hodgkin 2007). This can be immensely helpful in analytical wheat breeding through handling of specific chromosomes to achieve the important target of yield increase as a step in achieving food security. In this report we compared GGR, grain yield and AGPase enzyme activities in developing grains among DCS lines with their parents, to evaluate the role of specific chromosomes to regulate yield attributes with respect to yield increment.

Materials and Methods

Plant material

Selected wheat (*Triticum aestivum* L.) disomic substitution lines from a stock of lines having a pair of C591chromosome in the background of Chinese Spring (C.S.), based upon previous data available for grain size and test weight. For A genome, 6A and 3A

likewise for D genome, 2D and 7D represent bold and small grain, respectively. The B genome is highly variable in wheat so four lines were taken 1B and 4B represent bold and small respectively whereas 2B and 5B represent intermediate size between two diverse lines 1B and 4B.

Selected lines were grown under field condition following recommended package of practices in two seasons one experiencing terminal heat stress while other congenial temperature during grain filling duration. Seeds in each line were sown in 3 replicates; rows were 5 m in length at a distance of 20 cm each. During emergence ears were marked and tagged corresponding to the date of anthesis. At a stage of 14, 21 and 28 DPA, the 1/3rd (middle) ears of each line were collected and brought to the laboratory for the enzymatic studies on developing wheat grains.

Biochemical assay

Ears with developing grains at different developmental stage (14, 21 and 28 DPA) were used for preparation of grain extract. The extraction buffer contained 50 mM-MOPS (3-N-Morpholino propane sulphonic acid) pH 7.4, $MgCl_2$ (2mM), EDTA (1 mM) and 2 mM DTT (Dithiothritol). Nearly 15–20 developing grains amounting to 0.5 g of fresh were removed from the middle portion of ear heads, dehusked and were hand homogenized in a pre-chilled pestle and mortar with cold 2 ml of extraction buffer. The homogenate so obtained was centrifuged at 10,000 g for 10 min at 4 °C in a refrigerated centrifuge. The supernatant was used as grain extract for AGPase enzyme analysis. All enzymatic practices were performed on ice bath.

Enzyme assay

The pyrophosphorolytic activity of AGPase was assayed spectrophotometrically using a modification of published method (Kleczkowski 2000), by monitoring the increase in absorbance (340 nm) due to conversion of NADP to NADPH. The data on activity of *AGPase* was analyzed for each of the lines at 14, 21 and 28 DPA and compared with regard to the respective test weights.

Agro-physiological parameters

Grain and plant attributes like DTA, GGR, Test weight, Spike number per plant were also collected.

Grain growth rate (GGR)

The GGR in terms of dry matter accumulation in grains was analyzed for all the DCS lines and their parents at 14, 21, 28 DPA. The grains from the main spike of randomly selected three plants of each line were harvested. Grains were peeled out, fresh weight of

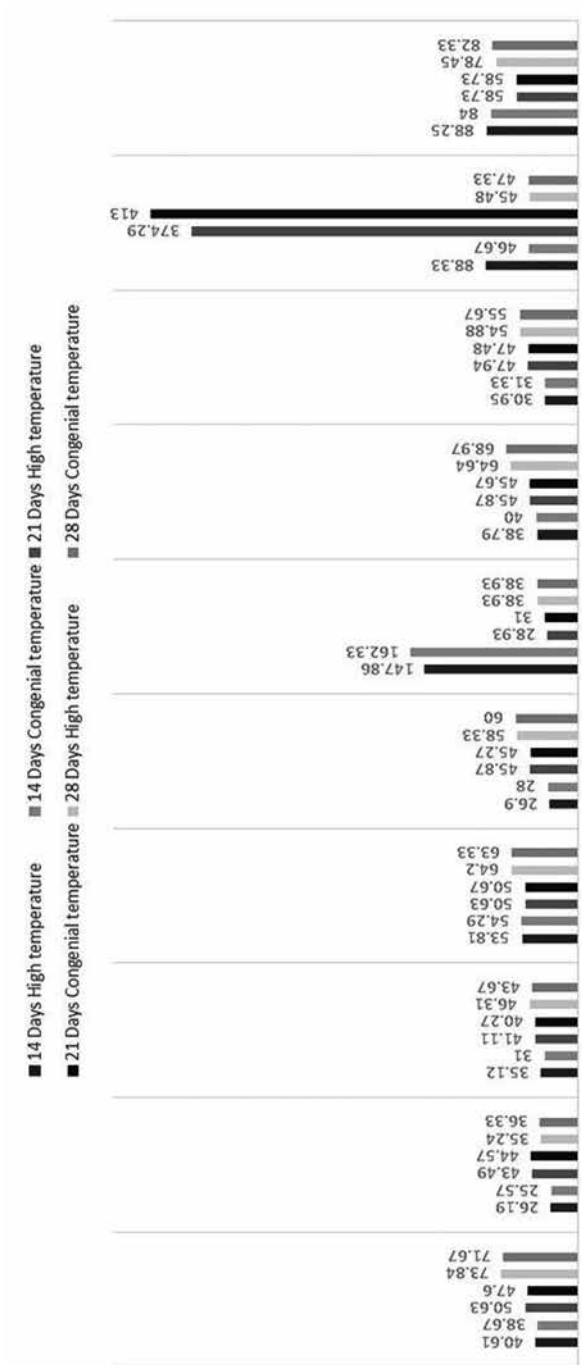

Figure 1. Relative *AGPase* enzyme activity (nM NADPH min^{-1} g^{-1} mL^{-1}) among wheat lines for High temperature terminal heat stress condition and congenial temperature. Results are the averages of three independent experiments for each genotype. Assays for each experiment were performed at 14, 21 and 28 DPA for two constitutive years. The enzyme activities were measured in the direction of ADP-Glucose synthesis in the presence of 3-PGA (10 mM). The spectrophotometeric reverse assay was used

grains (mg) per spike was recorded immediately and these grains were then incubated at 65 °C for 48 hours. After incubation, the dry weight (mg) was recorded for 3 consecutive days until it stabilized. The GGR was estimated for mean grain dry weight at 14, 21, 28 DPA and expressed as μg grain^{-1} day^{-1} (May and Van 1992).

Statistical methods

Data were subjected to correlation coefficient analysis (Dewey and Lu 1959) using suitable online software package. During correlation coefficient calculations, test weight was taken as a dependent trait.

Results

AGPase activity

The AGPase enzyme activity showed considerable variation with mean performance of 54.18, 82.42 and 56.82 at 14, 21 and 28 DPA, respectively, in congenial temperature conditions as against 57.68, 78.74 and 56.03 in terminal heat stress (Fig. 1). The mean AGPase activity in the two crop seasons observed was more by 7% at 14 DPA in stressed conditions, 9% more in congenial temperature at 21 DPA and no significant difference at 28 DPA. Based on these AGPase observations, DCS lines were categorized into three different types: (1) initially low and getting high over subsequent grain growth phases e.g. 6A, 3A, 2B and 5B; (2) initially low and changing over different phases with a specific jump in the activity e.g. 4B and 7D and (3) initially low and consistently increasing towards maturity as in 2D (Fig. 1).

The comparison of AGPase enzyme activities at selected DPA suggest that at 14 DPA stage, the DCS lines 3A, 6A, 1B, 2B, 5B and 2D had insignificant temperature effect, however, 4B performed well at congenial temperature only while line 7D performed well at high temperature with overall mean at stressed condition being 6% higher than the mean value in congenial temperature. At 21 DPA all the lines performed in similar trend except 7D which performed well at congenial temperature with overall mean being 5% higher than the mean of stressed condition. At 28 DPA the effect of temperature was seemingly negligible. The DCS lines 3A, 4B and 7D had higher AGPase activity at 14 DPA whereas these had reduced AGPase activity at 28 DPA as compared to the mean.

Days to anthesis (DTA)

Onset of anthesis in number of days after sowing was monitored. It indicates the duration of vegetative and grain filling period. The maximum DTA recorded were 107.3 and 119 for 3A whereas minimum DTA were 93.67 and 104 for 5B in stressed and congenial conditions respectively (Tables 1, 2).

Table 1. Wheat attributes for test weight, days to anthesis and spikes per plant in terminal heat stress condition crop. Results are the averages of three independent experiments for each genotype to compare the different DCS lines for these traits

Lines	Test Wt.	DTA	Spikes/Plant
C.S.	23.04±1.4	97.67±2.1	6.3±1.0
3A	19.92±3.1	107.33±2.4	6.3±0.7
6A	25.78±3.1	100.67±0.9	5.5±1.2
1B	32.11±1.4	96.33±1.2	5.3±1.5
2B	29.12±0.7	95.67±0.9	5.3±1.5
4B	21.1±0.9	103.67±1.9	5.3±0.9
5B	30.29±1.9	93.67±0.5	6.3±0.7
2D	35.33±0.9	97±1.4	6.3±1.1
7D	23.89±0.9	105±0.8	6.1±0.5
C591	39.27±1.6	104.67±4.2	5.3±1.2

Table 2. Wheat attributes for test weight, days to anthesis and spike per plant in congenial temperature crops. Results are the averages of three independent experiments for each genotype to compare the different DCS lines for these traits

Lines	Test Wt.	DTA	Spikes/Plant
C.S.	24.08±1.2	105.3±1.7	6.3±1.0
3A	17.01±2.5	119±0.0	6.3±0.5
6A	26.69±3.5	116±0.0	5.6±1.1
1B	32.43±1.2	108±0.8	5.3±1.5
2B	28.80±0.2	104.7±1.9	5.3±1.5
4B	21.60±1.0	115.3±6.6	5.4±0.9
5B	30.05±2.2	104.3±2.4	6.3±0.7
2D	35.83±1.0	109±0.8	6.3±1.2
7D	24.59±0.6	118.8±0.0	6.0±0.5
C591	39.97±1.7	105.7±2.1	5.3±1.3

Temperature impacts AGPase activity

Based on mean temperature during grain filling, wheat genotypes experienced maximum temperature of 23.5–26.2 °C while minimum temperature ranged between 8.8–10.1 °C during the congenial condition crop, whereas during stressed conditions these faced maximum temperature of 30.4 °C and minimum of 12.1–12.6 °C at 14 and 21 DPA, respec-

tively. There was 6 °C difference in maximum temperature during the two crop seasons (Table S1*).

The maximum DTA in 3A DCS line was 107.33 (stress condition) and 119 (congenial condition) revealing a difference of 11 days whereas 5B DCS line showed minimum DTA (93 and 104) during stressed and congenial conditions, respectively with a difference of 12 days among all DCS lines.

Test weight

It is one of the primary contributors of grain yield and was determined by weighing the 1000 grains. The maximum test weights shown by 2D line were 35.33 and 35.83 g (25% and 27% higher than that of mean crop weight. In contrast, minimum test weights, 19.92 and 17.01 were shown by 3A line, 29% and 40% lower than the mean value in crops of stressed and congenial conditions respectively (Tables 1, 2).

Grain growth rate (GGR)

It depicts the growth of grains per day which is a major parameter for determining the grain yield. Substantial variability was observed for GGR rate at 14 DPA among different wheat lines. The maximum GGR at 14 DPA was recorded in DCS line 1B which was 40 µg (stress) and 52.9 µg (congenial) whereas minimum was in DCS line 2B, 21.4 µg (stress) and 19 µg (congenial). The mean value for GGR at 14 DPA was 37.25 µg in stress while in congenial it was 38.79 µg. Large capriciousness existed between the GDR at 21 DPA compared to GGR at 14 DPA. The maximum GGR at 21 DPA was recorded in DCS line 4B: that is, 385.7 µg in stress and 420 µg in congenial whereas minimum in DCS line 3A: 27.9 µg (stress) and 25 µg (congenial). The mean value for GGR at 21 DPA was 82.86 µg in stress and 80.60 µg in congenial condition. Considerable variability was also depicted for 28 DPA. The maximum GGR was recorded in DCS line 7D: 171.4 µg and 143 µg in stress and congenial condition respectively whereas minimum in DCS line 2B were 20 µg and 25 µg for stress and congenial respectively. The mean value for 28 DPA was 52.93 µg and 53.17 µg for stress and congenial respectively (Fig. 2).

Spike number per plant

The spikes on five plants were counted and their average number were taken as number of spike/plant. The spike number per plant was recorded in the range of 5.3 to 6.3 for both the crop seasons (Tables 1 and 2).

Correlation matrix

Coefficients of correlation between different quantitative characters under study were computed. In stressed crop season, estimates of AGPase activity at 28 DPA, GGR at

*Further details about the Electronic Supplementary Material (ESM) can be found at the end of the article.

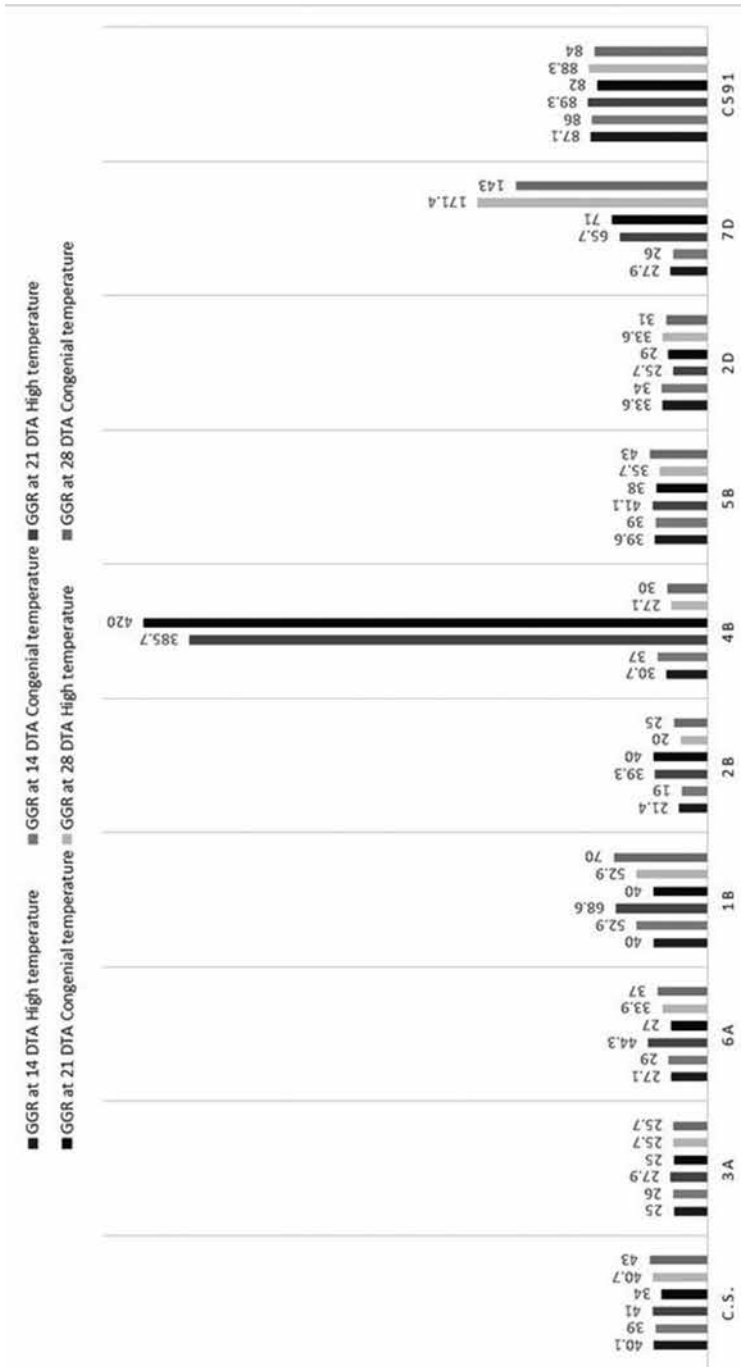

Figure 2. The grain growth rate (GGR) of different wheat lines during high temperature terminal heat stress condition and congenial temperature. Results are the averages of three independent experiments for each genotype which were recorded for two constitutive years at 14, 21, 28 DPA. A total 5 Grains at specific stage were weighed immediately after ripping from plant and after incubation at 65 °C for 48 hours, the dry weight (µg) was recorded and their difference is given in table

Table 3. Quantitative estimation and evaluation of different traits using correlation matrix during terminal heat stress experiencing crop

S.No.		Test Wt.	AGP14	AGP21	AGP28	GGR14	GGR21	GGR28	DTA	Spike/Plant
1	Test Wt.									
2	AGP 14	0.334^{NS}								
3	AGP 21	0.266^{NS}	0.508^{NS}							
4	AGP 28	0.094^{NS}	0.788^{**}	0.277^{NS}						
5	GGR 14	0.144^{NS}	0.837^{**}	0.540^{NS}	0.942^{**}					
6	GGR 21	-0.149^{NS}	0.663^{*}	0.327^{NS}	0.864^{**}	0.871^{**}				
7	GGR 28	0.063^{NS}	0.348^{NS}	0.467^{NS}	0.500^{NS}	0.536^{NS}	0.730^{*}			
8	DTA	0.432^{NS}	0.337^{NS}	0.633^{*}	0.302^{NS}	0.393^{NS}	0.395^{NS}	0.841^{*}		
9	Spike/Plant	0.067^{NS}	0.149^{NS}	0.914^{**}	0.009^{NS}	0.281^{NS}	0.159^{NS}	0.435^{NS}	0.568^{NS}	
10	Wt./spike	-0.037^{NS}	0.134^{NS}	0.633^{**}	0.171^{NS}	0.265^{NS}	0.375^{NS}	0.828^{**}	0.859^{**}	

Table 4. Quantitative estimation and evaluation of different traits using correlation matrix during congenial temperature crop season.

S.No.		Test Wt.	AGP14	AGP21	AGP28	GGR14	GGR21	GGR28	DTA	Spike/Plant
1	Test Wt.									
2	AGP 14	0.990^{**}								
3	AGP 21	0.976^{**}	0.968^{**}							
4	AGP 28	0.867^{**}	0.843^{**}	0.870^{**}						
5	GGR 14	0.571^{NS}	0.481^{NS}	0.576^{NS}	0.780^{**}					
6	GGR 21	0.800^{**}	0.736^{*}	0.818^{**}	0.682^{*}	0.659^{*}				
7	GGR 28	0.841^{**}	0.784^{*}	0.844^{**}	0.782^{**}	0.750^{*}	0.975^{**}			
8	DTA	0.510^{NS}	0.450^{NS}	0.428^{NS}	0.351^{NS}	0.314^{NS}	0.659^{*}	0.614^{NS}		
9	Spike/Plant	0.634^{*}	0.561^{NS}	0.578^{NS}	0.491^{NS}	0.463^{NS}	0.793^{**}	0.743^{*}	0.971^{**}	
10	Wt./spike	0.829^{**}	0.776^{**}	0.833^{**}	0.781^{**}	0.711^{*}	0.951^{**}	0.972^{**}	0.523^{NS}	0.665^{*}

14 DPA and spike/plant were positively correlated while DTA was negatively correlated. In crop season with congenial conditions, AGPase at 21 DPA, GGR at 14 DPA and DTA were positively correlated with respect to test weight while Spike/plant showed no correlation (Tables 3 and 4).

Discussion

Days to anthesis (DTA) differs for different DSC lines. DTA between stressed and congenial temperature shows a variance of 10 days. This observation concurs with the findings of Yin et al. (2009), that an increase in 5 °C temperature increases the rate of grain filling and reduces the grain filling duration by 12 days in wheat. DCS line 3A, 4B and 7D have prolonged vegetative phase (longer DTA period) than their contrasting lines (Tables 1, 2), that explains a longer vegetative stage which ultimately reduces their grain filling duration and faces high temperature during reproductive stages. Hurkman et al. (2003) also reported the effect of temperature on expression of genes encoding enzymes for starch biosynthesis in developing wheat endosperm. Similarly Dias and Lidon (2009) reported that heat stress accelerates the rate of grain filling thus reducing the grain-filling duration. Under these conditions, the supply of photoassimilates may be limited to storage organs (Calderini et al. 2006).

At 14 DPA (Days post anthesis), AGPase activity of DCS lines 3A and 7D was higher in stressed condition whereas in 4B DCS line was higher in congenial conditions. But at 28 DPA 3A and 7D had higher AGPase in congenial condition than stressed (Fig. 1), these can be ascribed to a prerequisite of a specific temperature regime for optimum AGPase activity. At 14 DPA the grains experienced activation temperature whereas, at 28 DPA period, these face high terminal heat, consequently their performance was adversely affected compared to contrasting lines. The same results were reported by Sheikh et al. (2010) that AGPase activity is highly affected by temperature.

Since DCS line 7D had higher mean AGPase activity than other DCSs, its variation was partitioned further into principal determinants. In stress, AGPase showed higher activity at 21 DPA whereas in congenial conditions it showed higher activity at 28 DPA because these lines got the requisite temperature regime at these stages which can be arrived at a mean day temperature 15 °C. This observation correlates with a previous report (Tewolde et al. 2006), wherein the optimum temperature for wheat anthesis and grain filling were concluded as 12 to 22 °C and exposure to higher temperatures would significantly reduce grain yield. These results were further substantiated by partitioning the effects by correlation matrix (Tables 3 and 4).

AGPase activity has a direct impact on GGR and a significant positive correlation with test weights (Ahlawat et al. 2009). But the DCS lines 4B and 7D performed better for AGPase activity than their contrasting lines at 14 and 21 DPA, respectively (Fig. 2). At these particular stages they show distinct increase in AGPase activity, but it did not contribute to GGR, this can be seen by a comparative analysis of test weights of 2D and 1B lines. They have relatively higher test weights than comparative DCS lines, symbolizing

in-efficient photosynthetic translocation mechanism of former (4B and 7D) DCS lines (Verma et al. 2004; Calderieni et al. 2006).

In both stressed and congenial conditions, 3A chromosome line has a low AGPase activity and corresponding reduced test weights, which were 29% and 40%, respectively, less than the means. This suggests some inhibitory elements/gene(s) on 3A chromosome from C591, which down regulate the AGPase gene expression or transport of starch from source to sink organs. Additionally 3A line had higher tiller number (a wider sink) than 6A DCS line indicating a limit on translocates available. Invariably test weights and grain number per spike are negatively correlated, we also found similar results in case of 3A line.

The DCS line 2D showed higher test weights and grain number than all other lines. This may be ascribed to a consistent *AGPase* activity along with an efficient mechanism for translocation of photosynthates from source to sink. These observations (Tables 1, 2) concur with an earlier report (Yin 2009).

Observations about contribution of specific C591 chromosome in the background of 20 other chromosomes of Chinese Spring towards distinguishing behaviour imparted to the plants of these DCS lines have enabled us to dissect the role of specific chromosome elements on AGPase expression and grain attributes affected by high temperature. Precisely chromosome 3A, 4B, 7D and 2D can be inferred to markedly effect grain starch formation by their typical AGPase expression behaviour. Comparing with their parents, DCS lines 3A and 7D showed significantly negative and positive contribution to mean test weights, reflecting on their genes or regulatory elements which down-regulate and up-regulate AGPase enzyme activity, respectively. AGPase activity and GGR analysis suggest a steady trend in all the lines except 4B and 7D. These lines showed a discrete trend in low and high activity at 14 and 21 DPA respectively. This altered trend of 4B and 7D can be utilized at breeding platform to develop higher grain size in winter and summer wheat crop respectively. These results suggest an apparent under-utilization of photosynthates in 7D line, since its higher AGPase activity was not efficiently used towards grain yield. Determinants of grain weight and grain filling have been attributed to 7D by QTL analysis of its translocated lines (Röder et al. 2008) which may be due to its inferior sink capacity. Test weight data substantiates that 2D line was superior over all other DCS lines due to its higher test weights with consistently higher AGPase activity during grain filling which suggests that it probably has an efficient mechanism for translocation of photosynthates from the source to sink. Correlation coefficient as well has provided an insight into the contribution of these traits on grain yield/weight and suggests that selection for traits like high AGPase and GGR during 21 DPA with requisite temperature regime will directly influence grain yield.

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Electronic Supplementary Material (ESM)

Electronic Supplementary Material (ESM) associated with this article can be found at the website of CRC at http://www.akademiai.com/content/120427/

Electronic Supplementary *Table S1.* Temperature variability in February last week to March during different crop seasons in relation to 21 days Post anthesis (DPA)