International Journal of Advanced Biochemistry Research 2025; SP-9(12): 1791-1796

International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; SP-9(12): 1791-1796
www.biochemjournal.com
Received: 01-09-2025
Accepted: 05-10-2025

Priyanka Parihar
Department of Genetics and Plant Breeding, G.B. Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India

Jai Prakash Jaiswal
Department of Genetics and Plant Breeding, G.B. Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India

Mitali Tiwari
Department of Genetics and Plant Breeding, G.B. Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India

Vartika Phakaliyal
Department of Genetics and Plant Breeding, G.B. Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India

Nimish Kala
Department of Genetics and Plant Breeding, G.B. Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India

Ashok Kumar Verma
Department of Biochemistry, G.B. Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India

Corresponding Author:
Jai Prakash Jaiswal
Department of Genetics and Plant Breeding, G.B. Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India

Sucrose synthase activity and its association with phenological and yield-related traits under heat stress in wheat

Priyanka Parihar, Jai Prakash Jaiswal, Mitali Tiwari, Vartika Phakaliyal, Nimish Kala and Ashok Kumar Verma

DOI: https://www.doi.org/10.33545/26174693.2025.v9.i12Sv.6839

Abstract
Rising temperatures associated with climate change pose a serious threat to wheat productivity, particularly through terminal heat stress during reproductive and grain-filling stages. Understanding the physiological and biochemical mechanisms underlying heat tolerance is therefore critical for sustaining yield stability. The present study evaluated the response of sucrose synthase (SuSy) activity and its association with phenological and yield traits under non-stress (timely sown) and heat-stress (late sown) conditions in a doubled haploid (DH) wheat population. A total of 23 DH lines representing extremes of maturity, along with their heat-tolerant and heat-susceptible parents, were evaluated over two thermal regimes using a randomized block design. Phenological traits, grain filling duration, thousand grain weight, grain yield, and SuSy activity at the soft dough stage were recorded. Heat stress significantly accelerated phenological development, shortened grain filling duration, and reduced grain yield and thousand grain weight, accompanied by an overall decline in SuSy activity. Substantial genotypic variation was observed for SuSy activity under both conditions. Several DH lines, notably DH 182, DH 154, DH 42, and DH 33, maintained higher SuSy activity under heat stress than the tolerant parent, indicating superior biochemical resilience. Correlation analysis revealed that under non-stress conditions, SuSy activity was positively associated with grain yield and thousand grain weight, whereas under heat stress, SuSy activity remained positively correlated with thousand grain weight but not with grain yield. These results highlight the importance of sucrose metabolism in sustaining grain filling under elevated temperatures and suggest that SuSy activity may serve as a useful biochemical indicator for selecting heat-tolerant wheat genotypes.

Keywords: Heat-stress, phenology, grain filling duration, enzyme, correlation

Introduction
Wheat (*Triticum aestivum* L.) serves as the primary staple food for a large proportion of the global population. However, its productivity is highly vulnerable to rising temperatures, making heat stress (HS) a major threat under current climate-change scenarios. In recent years, global warming has increasingly contributed to substantial yield penalties in wheat-growing regions. Projections suggest that HS will continue to reduce the productivity of major cereal crops, raising critical concerns about long-term food security (Ahmad *et al.*, 2024) [2]. Studies indicate that wheat may lose approximately 3-5% of its grain yield for every 1 °C increase above optimal temperatures during sensitive stages such as grain filling. Consequently, HS is recognized as one of the most significant abiotic constraints limiting wheat production worldwide (Jagadish *et al.*, 2021; Fan *et al.*, 2025) [6, 4]. Exposure to high temperature during reproductive phases particularly anthesis and grain filling, adversely affects fertilization, grain development, and ultimately yield. Wheat plants rely on two primary adaptive strategies: escaping terminal heat by adjusting phenology, or activating stress-responsive mechanisms that enhance thermotolerance. Agronomic practices, especially the adjustment of sowing time, strongly influence temperature exposure. Late-sown wheat often encounters terminal HS, which accelerates phenological transitions, shortens grain-filling duration, and reduces yield potential (Ullah *et al.*, 2022) [15].
Heat stress disrupts the source-sink balance by altering photosynthesis, respiration, and assimilate partitioning.

Additional heat-stress induced injuries, including leaf scorching, impaired growth, and reduced net assimilation rate, contribute to declined kernel weight and overall productivity (Abdelrahman *et al.*, 2020) [1]. A major component of heat-mediated yield loss is the reduction in starch deposition within the endosperm. Starch biosynthesis depends on enzymes such as sucrose synthase, sucrose transporters, ADP-glucose pyrophosphorylase (AGPase), and soluble starch synthase (SSS), many of which exhibit thermal sensitivity. High temperatures can cause denaturation or reduced activity of starch-synthesizing enzymes, leading to inadequate starch accumulation and shrivelled grains (Parihar *et al.*, 2025) [10]. Given these challenges, the present study aims to examine the effects of terminal heat stress on yield and phenological traits in wheat. Additionally, the activity of sucrose synthase, a crucial enzyme in sucrose metabolism and starch biosynthesis is assessed to understand its role under heat-stress conditions.

Materials and Methods

Plant Materials and Experimental Design: A doubled haploid (DH) mapping population consisting of 189 wheat lines was generated using the wheat-maize DH technique from a cross between KSG 1222, a derivative of the widely grown yet heat-sensitive Indian cultivar HD 2967, and a heat-tolerant selection line derived from Giza 168. From this population, 23 DH lines representing contrasting maturity classes, along with both parents, were chosen for comprehensive physiological and biochemical analyses. Field evaluation was carried out at the N.E. Borlaug Crop Research Center, Pantnagar, Uttarakhand, following a randomized block design with two replications. Thermal environments were manipulated through staggered sowing, with one set established under timely sowing conditions (non-stress) and another under delayed sowing to induce terminal heat stress. The selected genotypes were further evaluated for key phenological and yield-related traits, including days to heading, days to anthesis, days to maturity, grain filling duration, thousand grain weight, and grain yield.

Sucrose Synthase (SuSy) Enzyme Assay: Spikes were harvested at growth stage 85 (soft dough stage) from both non-stress and heat-stress conditions. Immediately after sampling, the material was snap-frozen in liquid nitrogen and stored at −20 °C until enzymatic analyses were performed. Enzyme extraction was carried out using a modified procedure based on Nakamura *et al.* (1989) [9]. Briefly, 5-10 frozen grains were weighed and finely ground in a pre-chilled mortar with 10 mL of ice-cold extraction buffer containing 50 mM HEPES-NaOH (pH 7.5), 2 mM KCl, 5 mM EDTA, and 1% (w/v) polyvinylpyrrolidone (PVP-30). An aliquot of the homogenate was further diluted with 1.8 mL of the same buffer and centrifuged at 10,000 g for 10 min at 0-4 °C. The supernatant obtained was used as the crude enzyme source for SuSy activity estimation. Prior to analysis, assay conditions were optimized with respect to pH and substrate concentration.

SuSy activity was determined following the method described by Wardlaw and Willenbrink (1994) [17], with slight modifications. The total reaction volume (110 µL) comprised 50 mM HEPES-NaOH (pH 7.5), 100 mM fructose, 100 mM UDP-glucose, and 50 mM $MgCl_2$. The reaction was initiated by the addition of 50 µL of crude enzyme extract and incubated at 30 °C for 30 min. Enzymatic activity was terminated by heating the reaction mixture at 100 °C for 1 min. Sucrose production was detected by adding 200 µL of 2 M NaOH, followed by incubation at 100 °C for 10 min. After cooling, 2 mL of 30% (v/v) HCl and 1 mL of 0.1% (w/v) resorcinol were added, and the mixture was incubated at 80 °C for 10 min. Absorbance of the resulting coloured complex was measured at 480 nm using a UV-VIS spectrophotometer, and SuSy activity was expressed as UDP-glucose-dependent sucrose formation.

Results and Discussion

Here The descriptive statistics of the seven traits evaluated in 25 wheat genotypes revealed substantial variation across phenological, biochemical, and yield-related parameters. Under Non-stress (NS) condition, days to anthesis (DA), days to heading (DH), and days to maturity (DM) exhibited moderate variability, with mean values of 86, 83, and 125.28 days, respectively, suggesting a reasonable spread in developmental timing among genotypes. Grain filling duration (GFD) showed comparatively low diversity (sd = 4.52), indicating a narrow range of grain development phases across the panel. ANOVA (Table 1) supported these observations, with significant genotypic effects for DH, DA, DM, and GFD in both environments. However, the mean sum of squares (MSS) for these traits declined under heat stress (HS), suggesting that genotypic differences in phenology became less pronounced as HS compressed developmental stages across all genotypes. This narrowing of variability was also reflected in lower standard deviations and interquartile ranges in the descriptive statistics. Grain yield (GY) displayed the highest absolute variation (sd = 133.65; range: 234-796 g), followed by thousand grain weight (TGW) (sd = 7.64), indicating substantial genetic differences in yield potential and grain size. The skewness and kurtosis values indicated approximately normal distributions with no extreme outliers, confirming the suitability of the dataset for correlation and multivariate analyses. The ANOVA results reinforced these trends: genotype effects for GY and TGW were highly significant under both conditions, but the MSS values declined sharply under heat. The mean square for grain yield decreased from 35834 to 12283 and for thousand grain weight, it reduced from 115.92 to 25.90. This indicates that heat stress not only reduced absolute yields but also compressed the range of genotypic expression, possibly due to a uniform physiological ceiling imposed by high temperature.

A comparison of trait performance under NS and HS revealed clear and consistent stress induced alterations in phenology and yield. DH and DA decreased noticeably under HS (72.60 and 75.44 days, respectively) compared to NS (83.26 and 86.64 days), indicating accelerated development and a typical heat-escape response. DM was similarly reduced from 125.28 to 108.30 days, confirming that heat stress shortened the total crop duration. GFD declined from 38.66 to 32.86 days, reflecting restricted assimilate deposition under elevated temperatures. These reductions are consistent with rapid senescence and limited physiological time for starch synthesis. Yield-related traits were strongly affected, with GY decreasing from 503.85 g under NS to 363.55 g under HS, while TGW dropped from 38.65 to 29.58 g, highlighting the negative influence of heat on both source-sink dynamics and grain plumpness. Replication effects were generally non-significant under NS, indicating good experimental control. Under HS, replications became significant for DH, DA, and DM, suggesting subtle environmental heterogeneity under stress (e.g., differential heat intensity across blocks). Error mean squares were consistently lower under HS, supporting the observation of phenotypic compression under stress. This pattern is in agreement with the findings of Rani *et al.* (2022) [11], who reported that under stress conditions reduced residual variability can lead to the replication mean square becoming significant.

Sucrose Synthase activity

Among the two parental lines, KSG 1222 exhibited higher sucrose synthase (SuSy) activity under non-stress conditions, recording 1.10 µmol sucrose min^{-1} g^{-1} FW while under heat-stress it was recorded to be 0.68 µmol sucrose min^{-1} g^{-1} FW, respectively. In contrast, KSG 1203 showed comparatively lower activity, with 0.85 µmol sucrose min^{-1} g^{-1} FW under NS but a slightly higher activity of 0.7 µmol sucrose min^{-1} g^{-1} FW under HS. Thus, despite showing a higher SuSy activity in TS, KSG 1222 exhibited a greater reduction in SuSy activity compared to KSG1203 in LS. Among the DH lines, DH 182 displayed the highest SuSy activity, surpassing even the heat-tolerant parent. It recorded 1.283 µmol sucrose min^{-1} g^{-1} FW under NS and 1.066 µmol sucrose min^{-1} g^{-1} FW under HS. Notably, the reduction in activity under HS was minimal, indicating a strong inherent ability to maintain metabolic function under elevated temperatures, and suggesting superior heat-tolerance potential. Similarly, DH 154, DH 42, and DH 33 maintained higher SuSy activity under HS compared with the tolerant parent, further indicating their robust enzymatic resilience during heat stress. Additionally, DH 141 and DH 41 exhibited SuSy activity under HS conditions that exceeded that of the heat-susceptible parent, suggesting moderate tolerance. All remaining DH lines exhibited low SuSy activity in both environments, indicating limited thermotolerance at the biochemical level. Figure 1 illustrates the comparative SuSy activity of all DH lines under NS and HS conditions.

Under non-stress, SuSy activity is typically high during early mid grain filling because it channels sucrose into UDP-glucose for starch biosynthesis; several TaSUS genes are transcribed during grain filling (Dale and Housley, 1986) [3]. Whereas, under heat stress (especially terminal heat during grain filling) many studies show decreased activities of SuSy, and reduced starch accumulation. The genotypes under heat stress are likely to show a larger reduction because its milk/early-dough window coincides with hotter conditions. The timely genotype may maintain relatively higher SuSy (and therefore better starch synthesis) if its milk stage occurs under cooler conditions (Yang *et al.*, 2018) [18]. Because the late sown genotype's grain-filling window likely falls into hotter calendar time, and because heat reduces SuSy and other starch-synthesis enzymes, the late/short-fill genotype will usually suffer greater yield loss and show lower SuSy activity (especially during milk/dough), leading to poorer starch accumulation compared with the timely/longer-fill genotype, unless the late sown genotype has specific heat-tolerance mechanisms (heat-stable enzymes, protective chaperones, or superior canopy cooling) that compensate.

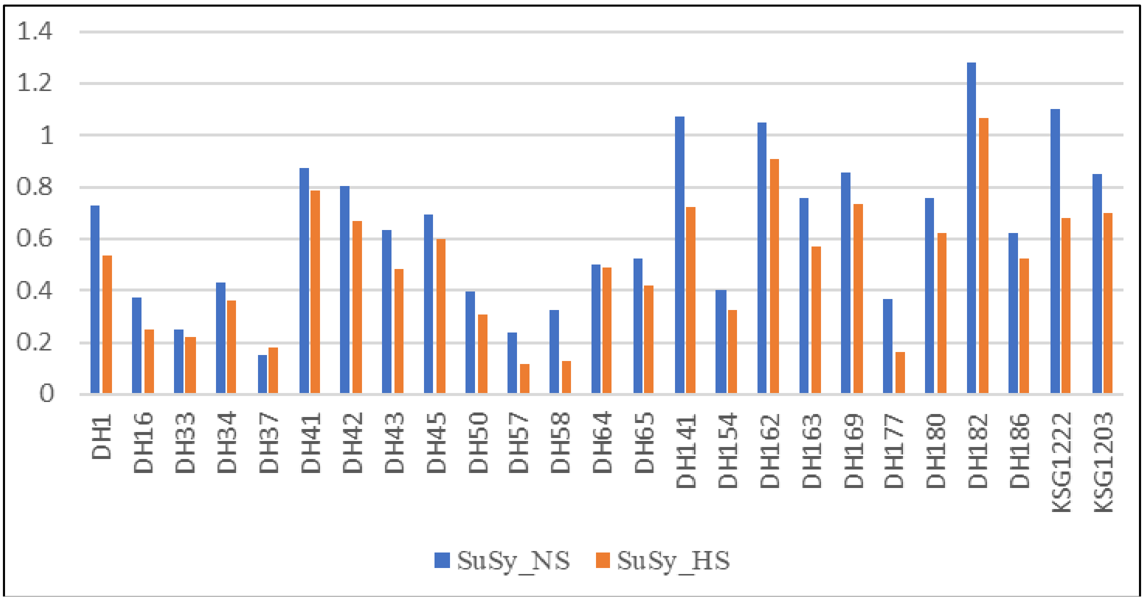

Fig 1: Sucrose Synthase activity in selected DH lines during Non-stress (SuSy_NS) and Heat stress (SuSy_HS) conditions at soft dough stage (GS 85)

Correlation between phenological, yield traits and SuSy:

Under non-stress conditions, days to heading, anthesis, and maturity were strongly and positively interrelated, while showing significant negative associations with thousand grain weight and sucrose synthase (SuSy) activity (Figure 2). Thousand grain weight exhibited a significant positive correlation with both grain yield and SuSy activity but was not associated with other traits. Grain yield was also positively and significantly correlated with SuSy activity. Under heat-stress conditions, phenological traits (days to heading, anthesis, and maturity) remained significantly and positively correlated with each other but were negatively associated with thousand grain weight (Figure 3). Thousand grain weight showed a significant positive correlation with SuSy activity, whereas no other significant trait associations were detected. Notably, under heat stress, SuSy activity did not exhibit a significant correlation with grain yield.

SuSy plays a central role in sucrose cleavage and carbon allocation during grain development. The high variability observed in SuSy activity among the genotypes indicates that this enzyme is a key differentiating factor influencing performance under normal and heat-stress conditions. Genotypes with higher SuSy activity are expected to maintain a more efficient supply of hexoses for starch synthesis, thereby supporting grain filling even when exposed to elevated temperatures. The moderate variability in phenological traits (DA, DH, DM) suggests that differences in yield are not primarily due to variations in crop duration but are more likely driven by physiological efficiency, particularly carbon metabolism. This is supported by the wide variation in GY and TGW, both of which are final indicators of successful assimilate partitioning. Thus, genotypes combining higher SuSy activity with adequate grain filling duration may exhibit superior performance under heat stress due to their ability to sustain metabolic activity and maintain grain weight. Conversely, genotypes with low SuSy activity may experience reduced starch deposition despite similar maturity periods, resulting in poor yield under stress. This relationship positions SuSy as a potential biochemical marker for identifying heat-tolerant genotypes.

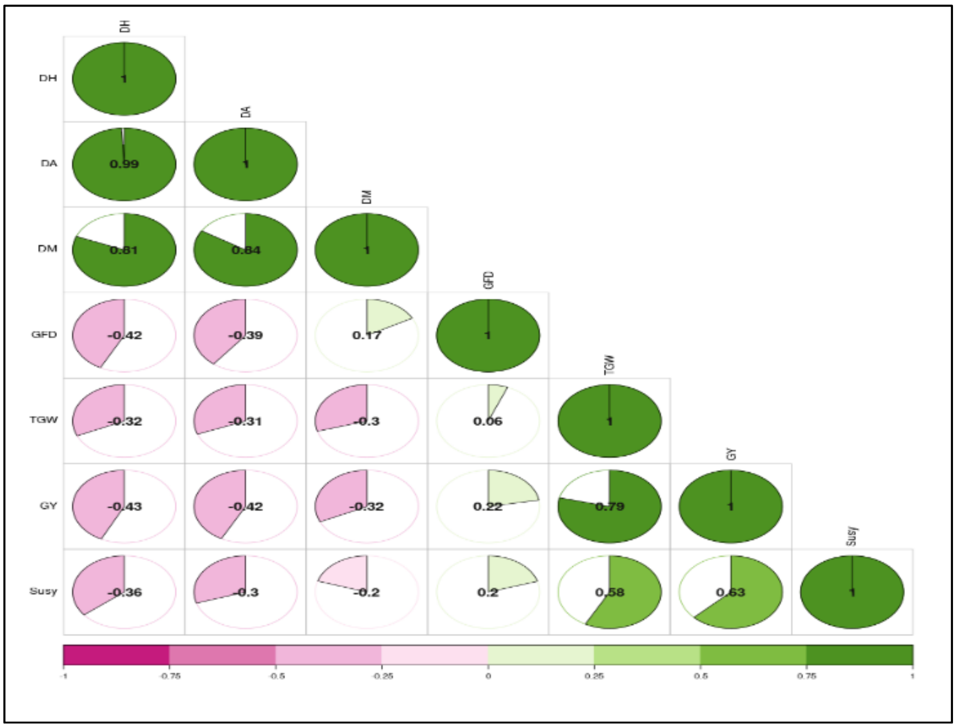

Fig 2: Correlation between phenological, yield related traits and Sucrose synthase activity in DH wheat grains under non-stress condition.

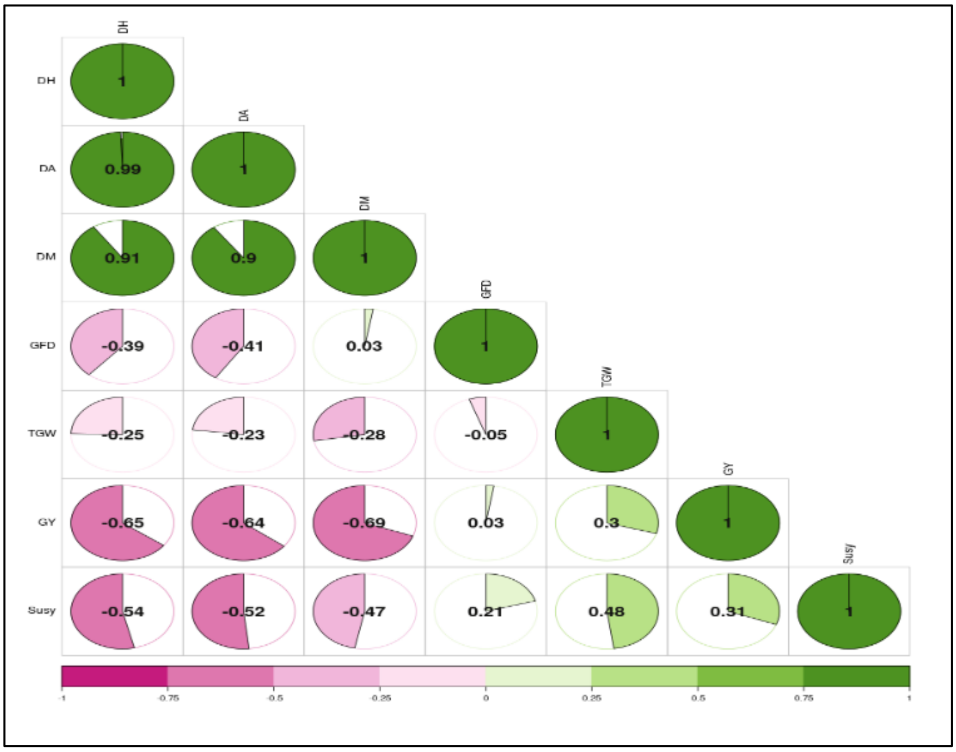

Fig 3: Correlation between phenological, yield related traits and Sucrose synthase activity in DH wheat grains under heat stress condition.

Heat stress imposed during grain filling markedly constrained assimilate accumulation, as reflected by reduced grain weight and shortened grain-filling duration, a response commonly attributed to accelerated developmental rates under elevated temperatures (Mirosavljevi *et al.*, 2024) [7]. Although rapid grain filling may occur under heat stress, the overall reduction in grain-filling duration typically results in lower final dry matter deposition, particularly in genotypes with inherently shorter grain-filling phases (Talukder *et al.*, 2014; Mehdi *et al.*, 2023) [14, 8]. This explains why, despite measurable enzymatic activity, grain yield does not always scale proportionally under stress conditions.

The ability of certain genotypes to maintain higher sucrose synthase activity under heat stress suggests improved metabolic stability during endosperm development. Peak SuSy activity during the early to mid grain-filling period has been reported as critical for starch accumulation (Mukherjee *et al.*, 2015), and genotypes that sustain enzyme activity during this phase are better positioned to buffer the adverse effects of temperature stress. Molecular evidence further supports this interpretation, as thermo-responsive alleles of sucrose synthase 3 (Sus3) have been shown to enhance grain quality and heat tolerance during ripening by stabilizing sucrose metabolism (Takehara *et al.*, 2018) [13]. Consistent with this, SuSy expression has been shown to closely track starch accumulation, indicating that sucrose cleavage during grain filling is predominantly mediated via SuSy-dependent pathways (Gu *et al.*, 2021) [5]. Overall, these findings suggest that heat tolerance in wheat is governed not solely by phenological adjustment but also by the capacity to sustain sucrose metabolism during grain filling. Genotypes that maintain higher SuSy activity under stress are therefore more likely to preserve grain weight, even when yield responses are constrained by broader source-sink limitations.

Table 1: Comparative ANOVA of all traits studied during NS (Non-stress) and HS (Heat Stress), and mean and CV (%); Env: Environment; df: degree of freedom.

Source of Variation	df	Env	Mean Sum of Square					
			DH	DA	DM	GFD	TGW	GY
Genotypes	24	NS	131.422***	132.47**	115.087**	40.863**	115.923**	35834***
		HS	105.542**	91.013**	75.875**	17.022	25.9021**	12283**
Replications	1	NS	1.620	1.62	92.480	69.620	0.845	5445
		HS	87.120*	46.080*	106.580*	12.500	0.8398	4087.9
Error	24	NS	11.162	15.37	39.147	21.453	32.558	7398
		HS	7.328	6.997	23.455	13.292	5.2679	1481.8
CV (%)		NS	9	10	6	12	20	27
		HS	9	10	6	9	12	22
Mean		NS	86.64	83.26	125.28	38.66	38.65	503.85
		HS	75.44	72.60	108.30	32.86	29.58	363.55

Conclusion

Terminal heat stress significantly altered wheat phenology, grain filling dynamics, and yield formation, resulting in accelerated development, shortened grain filling duration, and reduced grain yield and thousand grain weight. Although sucrose synthase activity declined under heat stress, substantial genotypic variation was observed, highlighting differential biochemical adaptability among DH lines. Several DH lines maintained higher SuSy activity under heat stress than both parental lines, indicating enhanced capacity to sustain sucrose metabolism and carbon allocation during grain filling. The consistent positive association between SuSy activity and thousand grain weight under both environments underscores the importance of sucrose metabolism in stabilizing grain weight under elevated temperatures. The weakened relationship between SuSy activity and grain yield under heat stress suggests that yield stability is governed by multiple interacting physiological mechanisms beyond carbon metabolism alone. Overall, genotypes combining stable SuSy activity with adequate grain filling duration demonstrated superior performance under terminal heat stress. These findings identify SuSy activity as a promising biochemical indicator for screening heat-tolerant wheat genotypes and provide valuable insights for breeding programs aimed at improving yield stability under warming climatic conditions.

Acknowledgments

Author would like to acknowledge Council of Scientific & Industrial Research (CSIR), Government of India for fellowship and BIRAC/USAID for the financial assistance to conduct research.

References

1. Abdelrahman M, Burritt DJ, Gupta A, Tsujimoto H, Tran LSP. Heat stress effects on source-sink relationships and metabolome dynamics in wheat. J Exp Bot. 2020;71(2):543-554. doi:10.1093/jxb/erz296.
2. Ahmad W, Bibi N, Sanwal M, Ahmed R, Jamil M, Kalsoom R, *et al.* Cereal crops in the era of climate change: an overview. In: *Environment, Climate, Plant and Vegetation Growth*. 2024. p. 609-630.
3. Dale EM, Housley TL. Sucrose synthase activity in developing wheat endosperms differing in maximum weight. Plant Physiol. 1986;82(1):7-10.
4. Fan YC, Yuan YQ, Yuan YC, Duan WJ, Gao ZQ. Research progress on the impact of climate change on wheat production in China. PeerJ. 2025;13:e18569.
5. Gu Y, Han S, Chen L, Mu J, Duan L, Li Y, *et al.* Expression and regulation of genes involved in the reserve starch biosynthesis pathway in hexaploid wheat (*Triticum aestivum* L.). Crop J. 2021;9(2):440-455.
6. Jagadish SK, Way DA, Sharkey TD. Plant heat stress: concepts directing future research. Plant Cell Environ. 2021;44(7):1992-2005.
7. Mirosavljević M, Mikić S, Čupunski V, Abdelhakim L, Trkulja D, Zhou R, *et al.* Effects of heat stress during anthesis and grain filling stages on physiological and agronomic traits in diverse wheat genotypes. Plants (Basel). 2024;13(15):2083.

8. Mehdi F, Liu X, Riaz Z, Javed U, Aman A, Galani S. Expression of sucrose metabolizing enzymes in different sugarcane varieties under progressive heat stress. Front Plant Sci. 2023;14:1269521. doi:10.3389/fpls.2023.1269521.
9. Nakamura Y, Yuki K, Park SY, Ohya T. Carbohydrate metabolism in the developing endosperm of rice grains. Plant Cell Physiol. 1989;30(6):833-839.
10. Parihar P, Jaiswal JP, Verma AK, Kumar A. Sucrose synthase dynamics and its potential role in heat stress tolerance in cereals. Front Plant Sci. 2025;16:1652076.
11. Rani S, Kumar M, Kamboj N. ANOVA and MANOVA: a comparative study for correlated characters of wheat. [Journal name not provided]. 2022.
12. Shi W, Yin X, Struik PC, Solis C, Xie F, Schmidt RC, *et al.* High day-and night-time temperatures affect grain growth dynamics in contrasting rice genotypes. J Exp Bot. 2017;68(18):5233-5245.
13. Takehara K, Murata K, Yamaguchi T, Yamaguchi K, Chaya G, Kido S, *et al.* Thermo-responsive allele of sucrose synthase 3 (Sus3) provides high-temperature tolerance during the ripening stage in rice (*Oryza sativa* L.). Breed Sci. 2018;68(3):336-342. doi:10.1270/jsbbs.18007.
14. Talukder ASMHM, McDonald GK, Gill GS. Effect of short-term heat stress prior to flowering and early grain set on the grain yield of wheat. Field Crops Res. 2014;160:54-63.
15. Ullah A, Nadeem F, Nawaz A, Siddique KHM, Farooq M. Heat stress effects on the reproductive physiology and yield of wheat. J Agron Crop Sci. 2022;208(1):1-15.
16. Djanaguiraman M, Narayanan S, Erdayani E, Prasad PVV. High temperature effects on kernel weight and assimilate partitioning in wheat. Crop Sci. 2020;60(4):1906-1920.
17. Wardlaw IF, Willenbrink J. Carbohydrate storage and mobilization by the culm of wheat between heading and grain maturity: relation to sucrose synthase and sucrose-phosphate synthase. Aust J Plant Physiol. 1994;21:251-271.
18. Yang H, Gu X, Ding M, Lu W, Lu D. Heat stress during grain filling affects activities of enzymes involved in grain protein and starch synthesis in waxy maize. Sci Rep. 2018;8(1):15665.