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TREHALOSE-CYTOKININ INTERPLAY IN WHEAT (*Triticum aestivum* L.) CARBOHYDRATE METABOLISM FOR DROUGHT RESILIENCE

Aparjot Kaur[1], Norah Johal[2*], Mamta Pal[3], Mandeep Kaur[4] and S K Thind[5]

[1]Regional Research Station, Bathinda-151 001, Punjab Agricultural University, Punjab, India
[2]Department of Agronomy, Punjab Agricultural University, Ludhiana-141 004, Punjab, India
[3]Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana-141 004, Punjab, India
[4]Department of Soil Science, Punjab Agricultural University, Ludhiana-141 004, Punjab, India
[5]Ex-Professor, Department of Botany, Punjab Agricultural University, Ludhiana-141 004, Punjab, India

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ABSTRACT

The current study was carried out to study the changes in activities of carbohydrate metabolizing enzymes under drought stressed wheat (*Triticum aestivum* L.) (HD2967, PBW660 and WH1105) genotypes and how their regulation was maintained by exogenously applied cytokinins (kinetin and benzyl adenine) and trehalose: an osmoprotectant. Alterations in contents of various sugars (total soluble sugars, reducing sugars and non-reducing sugars) were recorded under control and drought stressed crop plants on foliar application of cytokinins and trehalose at vegetative and flag leaf stages. Drought stress significantly increased total soluble sugars (1.2, 1.4 folds), reducing sugars (1.1, 1.3 folds), non-reducing sugars (1.2, 1.5 folds) and sucrose content (1.3, 1.2 folds) at both vegetative stage and flag leaf stage respectively. Carbohydrate metabolizing enzymes viz., sucrose phosphate synthase (1.2 folds), acid invertase (1.1 folds) and sucrose synthase (1.4 folds) were significantly upregulated under drought stressed plants at flag leaf stage. However, the osmoprotectant mitigated the effect by lowering the enzymatic activities in treatments (T3-T6), T4 treatment (Drought + Kn(40g)+ Tre (1.5mM) had the most promising mitigating effect against drought stress at both the stages. A significant negative correlation was observed between grain yield and sugar metabolizing enzymes, specifically SPS (r = -0.70), SS (r = -0.67), and sucrose content (r = -0.70), during the vegetative stage. Moreover the acuteness of the grain yield with the starch content in loading plot indicates the regulation of sugar metabolism that underwent modifications in presence of exogenous PGRs (trehalose, kinetin and benzyl adenine) that leads to significant increase in starch content that ultimately enhances grain yield of wheat.

Keywords: Abiotic stress, Carbohydrate metabolizing enzymes, Osmoprotectants, Physiological traits, Wheat genotypes

Wheat (*Triticum aestivum* L.), cultivated on 216 million hectares globally, yields 3.5 t ha^{-1} (at 11% moisture content) with a total production of 765 million tons (FAOSTAT, 2019). Primarily used for human consumption, the remainder supports animal feed and processing industries (FAOSTAT, 2018). With the global population projected to reach 9 billion by 2050, wheat production must increase by 60% to ensure food security, a challenge exacerbated by climate change, rising temperatures, and erratic rainfall patterns (Borisjuk *et al*., 2019; Zhao *et al*., 2017).

Developing countries like India, where 99 million hectares are under wheat cultivation with 32% affected by drought, face significant hurdles due to limited technological access for small and marginal farmers (Shamsi *et al*., 2011). Abiotic stresses, including drought, heat, salinity, and water excess, reduce wheat yield by up to 71%, with drought being a major contributor by limiting water uptake relative to leaf transpiration, leading to decreased water content, reduced leaf water potential, stomatal closure, and impaired cell growth (Rana *et al*., 2013; Naeem *et al*., 2015; Jaleel *et al*., 2009; Jamali *et al*., 2020).

Critical wheat growth stages such as jointing, tillering, and anthesis are highly water-dependent, and water shortages during the transition from vegetative to reproductive stages, particularly at jointing, significantly reduce yield (Thapa *et al*., 2020; Mahpara *et al*., 2014). Drought disrupts carbohydrate metabolism by altering source-sink partitioning, diverting nutrients toward defense mechanisms rather than sugar, sucrose, and starch synthesis. High temperatures, often concurrent with drought,

*Corresponding author: norah-cobsbot@pau.edu
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impair key enzymes (e.g., granule-bound starch synthase, starch synthases, sucrose synthase, and starch branching enzymes) and genetic expressions involved in sucrose-to-starch conversion, further reducing starch content (Lu *et al*., 2020). To cope, wheat produces osmoprotectants like trehalose, a non-reducing disaccharide that stabilizes macromolecules and membranes, enhancing drought tolerance (Jang *et al*., 2003). Cytokinins also improve drought resilience by modifying root architecture and fitness (Hai *et al*., 2020). However, studies on prolonged field stress, where multiple stresses interact, are limited. This experiment investigates how drought alters wheat sugar metabolism and evaluates the effects of exogenously applied trehalose and cytokinins on sugar content and sugar-metabolizing enzyme activities at vegetative and flag leaf stages under field drought conditions.

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MATERIALS AND METHODS

The seeds of three wheat genotypes namely, HD 2967 (timely sown irrigated), PBW660 (improved variety for rainfed) and WH1105 (timely sown irrigated) were procured from Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. Field trials were conducted during *Rabi* seasons 2014-15 and 2015-16 for two consecutive years in a split plot design. The wheat genotypes constituted of three replications each in each treatment. The details of treatment are mentioned in Table 1. The solutions containing 0.1% TWEEN-20 were sprayed on wheat plants twice (Morning between 8 to 9 am and evening between 4 to 5 pm) a day during its application at vegetative and flag leaf stage. Crop was maintained by following typical agronomic practices as per package of practices Punjab Agricultural University, Ludhiana, Punjab. Control plots were provided with all five irrigations throughout the crop growth. Drought stress or dry spell was executed by withholding irrigations except first irrigation. The stressed field plots were protected with the help of rainout shelters during rains and night periods in order to protect the crop from frost conditions. The fully extended leaf at vegetative stage and flag leaf were taken to estimate sugars and enzymatic activities. Five plants from each replication were taken for biochemical estimations that were carried out in laboratories of Department of Botany, Punjab Agricultural University at both stages.

Soluble sugars were predicted as per Dubois *et al.* (1956). The amount of sugars was calculated against the standard curve prepared by using pure glucose (10-100µg/ml) and expressed as mg g^{-1} dry weight. Total reducing sugars were estimated by method as given by Nelson (1944) method. Non reducing sugars were determined by subtracting the reducing sugars from the total soluble sugars.

Table 1. Details of the different treatments

Treatment	Description
T_1	Control
T_2	Drought stress (withholding the 2nd, 3rd and 4th irrigation)
T_3	Drought + Kn(40)+(Tre-1mM)
T_4	Drought +Kn(40)+(Tre-1.5mM)
T_5	Drought + BA(50)+(Tre-1mM)
T_6	Drought + BA(50)+(Tre-1.5mM)

BA- Benzyladenine; Kn-Kinetin; Tre-Trehlaose

Starch quantity was calculated as per Mc Cready (1958) while sucrose was estimated as per Roe (1934) by recording the intensity of pink colour at 490nm. Standard curve was primed by using sucrose as standard for the assessment of concentration of sucrose (10-100µg/mg).

The extraction and assay of sucrose phosphate synthase (SPS) and sucrose synthase (SuSy) were performed following the protocol described by Morell and Copeland (1985), with minor modifications. Fresh leaf tissue (0.5 g) was homogenized thoroughly in an ice-cold mortar and pestle using 10 mL of extraction buffer consisting of 50 mM HEPES-NaOH (pH 7.5), 5 mM $MgCl_2$, 1 mM sodium EDTA, 2.5 mM dithiothreitol (DTT), 0.5 mg/mL bovine serum albumin (BSA), and 0.05% (v/v) Triton X-100. The homogenate was centrifuged at 12,500 rpm for 20 minutes at 4°C. The resulting supernatant was dialyzed overnight at 0-4°C against 3-4 liters of a fourfold-diluted extraction buffer using dialysis membranes. The dialysate obtained was used for enzyme activity assays.

For the SuSy assay, the reaction mixture (0.5 mL) contained 0.1 mL of 150 mM fructose, 0.1 mL of 20 mM UDP-glucose, 0.2 mL of 50 mM HEPES-NaOH buffer (pH 7.5), and 0.1 mL of enzyme extract. The mixture was incubated at 37°C for 30 minutes. Subsequently, 0.1 mL of 30% KOH was added to terminate the reaction, and the tubes were heated in a boiling water bath for 30 minutes to eliminate free fructose. After cooling to room temperature, 1 mL of resorcinol solution and 3 mL of 30% HCl were added. The mixture was vortexed thoroughly and incubated at 80°C for 10 minutes. Absorbance was measured at 490 nm against a reagent blank, in which UDP-glucose was added only after the boiling step. Sucrose concentration was determined

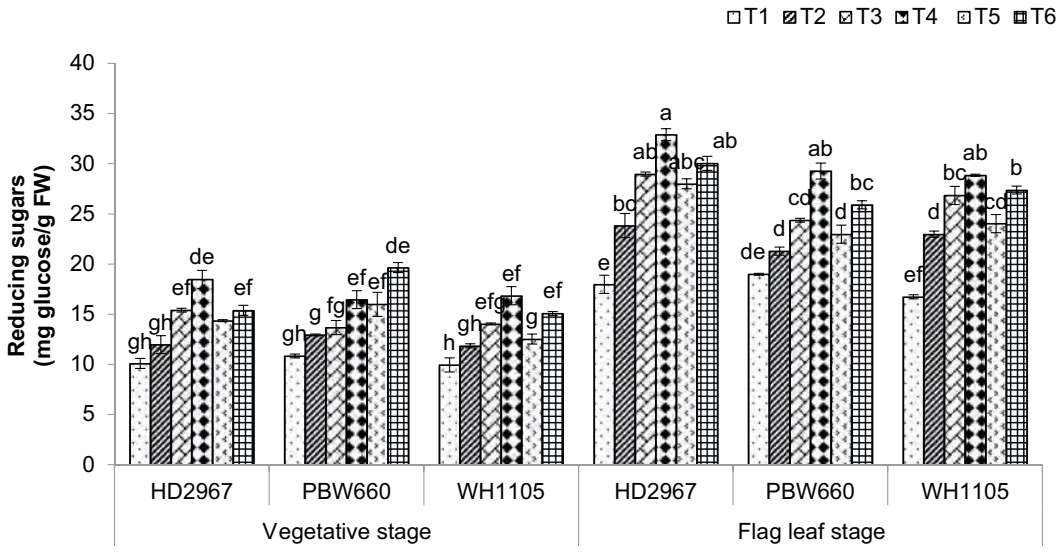


Fig. 1. Impact of cytokinins (kinetin, Kn; benzyl adenine, BA) and trehalose (Tre) on reducing sugar content (mg glucose/g fresh weight, FW) in three wheat genotypes (HD2967, PBW660, WH1105) under drought stress, measured at vegetative stage (VS) and flag leaf stage (FLS)

T_1 (control, irrigated), T_2 (drought stress, no irrigation except first), T_3 (drought + Kn 40 mg), T_4 (drought + Kn 40 mg + Tre 1.5 mM), T_5 (drought + BA 40 mg), T_6 (drought + BA 40 mg + Tre 1.5 mM).
Within each genotype and stage, bars with different alphabets differ significantly at $p<0.05$ after Tukey's post hoc test. Each bar represents the mean value ± standard error (S.E.) from five replications.

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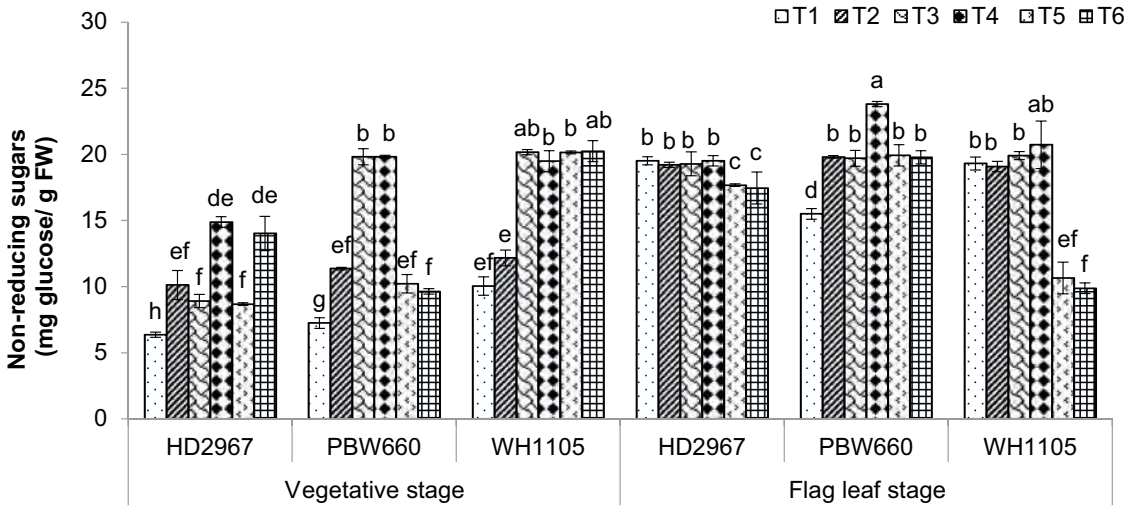


Fig. 2. Impact of cytokinins (kinetin, Kn; benzyl adenine, BA) and trehalose (Tre) on non-reducing sugar content (mg glucose/g fresh weight, FW) in three wheat genotypes (HD2967, PBW660, WH1105) under drought stress, measured at vegetative stage (VS) and flag leaf stage (FLS)

T1 (control, irrigated), T2 (drought stress, no irrigation except first), T3 (drought + Kn 40 mg), T4 (drought + Kn 40 mg + Tre 1.5 mM), T5 (drought + BA 40 mg), T6 (drought + BA 40 mg + Tre 1.5 mM).
Within each genotype and stage, bars with different alphabets differ significantly at $p<0.05$ after Tukey's post hoc test. Each bar represents the mean value ± standard error (S.E.) from five replications.

using a standard curve generated from known concentrations of sucrose (10-60 µg). SPS activity was measured using the same protocol as for SuSy, with the exception that 150 mM fructose was replaced with fructose-6-phosphate.

Invertase activity was determined following the method described by Singh *et al.* (1978). Leaf tissue was homogenized in 0.1 M acetate buffer (pH 5.0) containing 1% (w/v) polyvinylpyrrolidone (PVP) and 1 mM EDTA. The homogenate was centrifuged at

Table 2. Correlation between carbohydrate content, carbohydrate metabolizing enzymes and grain yield under drought stress, measured at vegetative stage (VS)

Parameter	RS	NRS	TSS	Starch	Sucrose	SPS	SS	AI	GY
RS	1.00								
NRS	0.33	1.00							
TSS	**0.66***	**0.87***	1.00						
Starch content	0.13	0.22	0.18	1.00					
Sucrose content	0.35	0.18	0.28	-0.33	1.00				
SPS	0.29	0.19	0.28	-0.49	0.59	1.00			
SS	-0.25	0.25	0.07	-0.04	0.29	0.38	1.00		
AIA	-0.01	-0.07	-0.03	-0.12	0.17	0.17	0.12	1.00	
GY	0.14	0.13	0.12	**0.58***	**-0.70***	**-0.77***	**-0.67***	-0.19	1.00

RS: Reducing sugars, NRS: Non reducing sugars, TSS: Total soluble sugars, SPS: Sucrose phosphate synthase, SS: Sucrose synthase, AIA: Acid invertase activity, GY: Grain yield
*Significant at *p<0.05*

Table 3. Correlation between carbohydrate content, carbohydrate metabolizing enzymes and grain yield under drought stress, measured at flag leaf stage (FLS)

Parameter	RS	NRS	TSS	Starch	Sucrose	SPS	SS	AI	GY
RS	1.00								
NRS	0.33	1.00							
TSS	0.12	-0.14	1.00						
Starch content	0.09	0.31	**0.58***	1.00					
Sucrose content	0.44	0.02	0.22	0.31	1.00				
SPS	0.44	0.17	-0.02	0.11	**0.63***	1.00			
SS	-0.57	-0.19	-0.16	-0.45	0.26	0.19	1.00		
AIA	-0.44	-0.28	-0.09	-0.43	0.38	0.39	**0.82***	1.00	
GY	-0.21	0.07	0.11	0.03	0.06	0.11	0.15	0.19	1.00

RS: Reducing sugars, NRS: Non reducing sugars, TSS: Total soluble sugars, SPS: Sucrose phosphate synthase, SS: Sucrose synthase, AIA: Acid invertase activity, GY: Grain yield
*Significant at *p<0.05*

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9,000 rpm for 10-15 minutes at 4°C, and the resulting supernatant was collected as the crude enzyme extract. Extracts were stored at -10°C and used within 48 hours. The reaction mixture for invertase activity contained 0.6 mL of 0.2 M acetate buffer (pH 4.8), 0.3 mL of 0.4 M sucrose, and 0.1 mL of enzyme extract. In control tubes, the enzyme was inactivated by boiling for 5 minutes before sucrose addition. Reaction mixtures were incubated at 30°C for 30 minutes, after which 1 mL of 3,5-dinitrosalicylic acid (DNS) reagent was added. Tubes were then placed in a boiling water bath for 10 minutes, cooled to room temperature, and the volume was adjusted to 5 mL with distilled water. Absorbance was recorded at 560 nm. Reducing sugars released were quantified using a glucose standard curve (100 µg/mL). Enzyme activity was expressed as µmol of sucrose hydrolyzed per minute per milligram of protein.

Grain yield was recorded by harvesting biomass from a 1 m² area in each subplot at physiological maturity. Samples from each treatment were weighed separately, and the mean yield was calculated. Each treatment consisted of five replicates, and each replicate comprised five individual plants.

Data were analyzed using analysis of variance (ANOVA), followed by Tukey's multiple range test ($p<0.05$) using SPSS software to determine statistically significant differences among treatments and genotypes. Groups showing significant differences were denoted by different alphabetic labels. Pearson's correlation coefficients were calculated using Microsoft Excel to explore relationships among

Table 4. Rotated component matrix of carbohydrate content and carbohydrate metabolizing enzymes at vegetative stage and flag leaf stage in wheat genotypes

Variable	Vegetative stage		Flag leaf stage	
	PC_1	PC_2	PC_1	PC_2
RS	0.043	-0.44	0.28	0.45
NRS	-0.09	-0.54	0.16	0.26
TSS	0.01	-0.62	0.31	0.50
Starch content	-0.43	-0.14	0.38	-0.27
Sucrose content	0.39	-0.21	-0.26	0.50
SPS	0.43	-0.17	-0.40	-0.14
SS	0.23	-0.02	-0.44	-0.04
AI	0.45	0.16	-0.32	0.19
Grain yield	-0.46	-0.09	0.32	-0.31
Eigen value	**4.24**	**2.61**	**4.14**	**2.01**
Proportion (%)	**47.1**	**29.0**	**46.1**	**22.3**
Cumulative (%)	**47.1**	**76.1**	**46.1**	**68.4**

RS: Reducing sugars, NRS: Non reducing sugars, TSS: Total soluble sugars, SPS: Sucrose phosphate synthase, SS: Sucrose synthase, AIA: Acid invertase activity, GY: Grain yield

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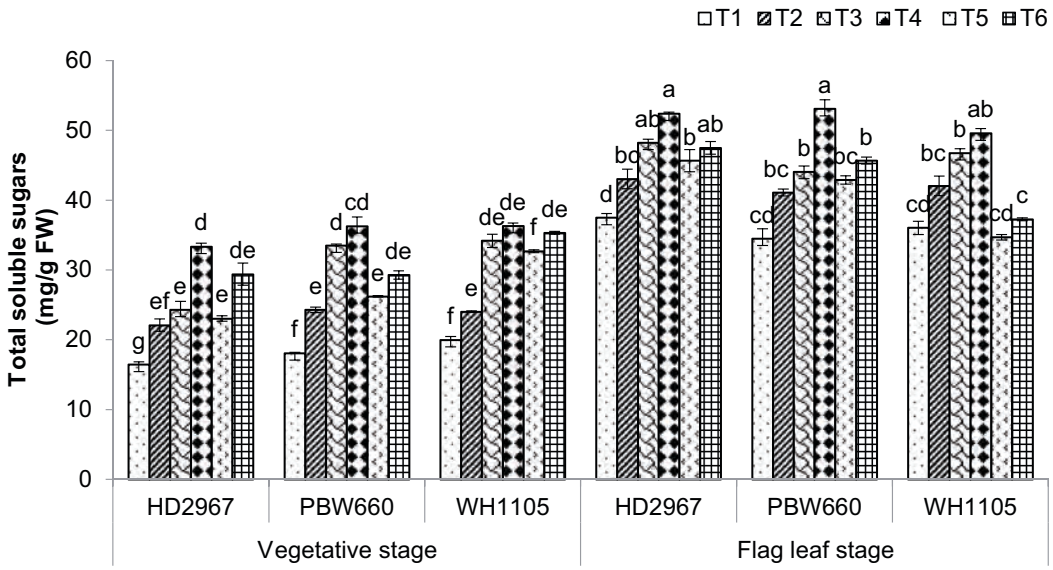


Fig. 3. Impact of cytokinins (kinetin, Kn; benzyl adenine, BA) and trehalose (Tre) on total soluble sugar content (mg /g fresh weight, FW) in three wheat genotypes (HD2967, PBW660, WH1105) under drought stress, measured at vegetative stage (VS) and flag leaf stage (FLS)

T_1 (control, irrigated), T_2 (drought stress, no irrigation except first), T_3 (drought + Kn 40 mg), T_4 (drought + Kn 40 mg + Tre 1.5 mM), T_5 (drought + BA 40 mg), T_6 (drought + BA 40 mg + Tre 1.5 mM).
Within each genotype and stage, bars with different alphabets differ significantly at $p<0.05$ after Tukey's post hoc test. Each bar represents the mean value ± standard error (S.E.) from five replications.

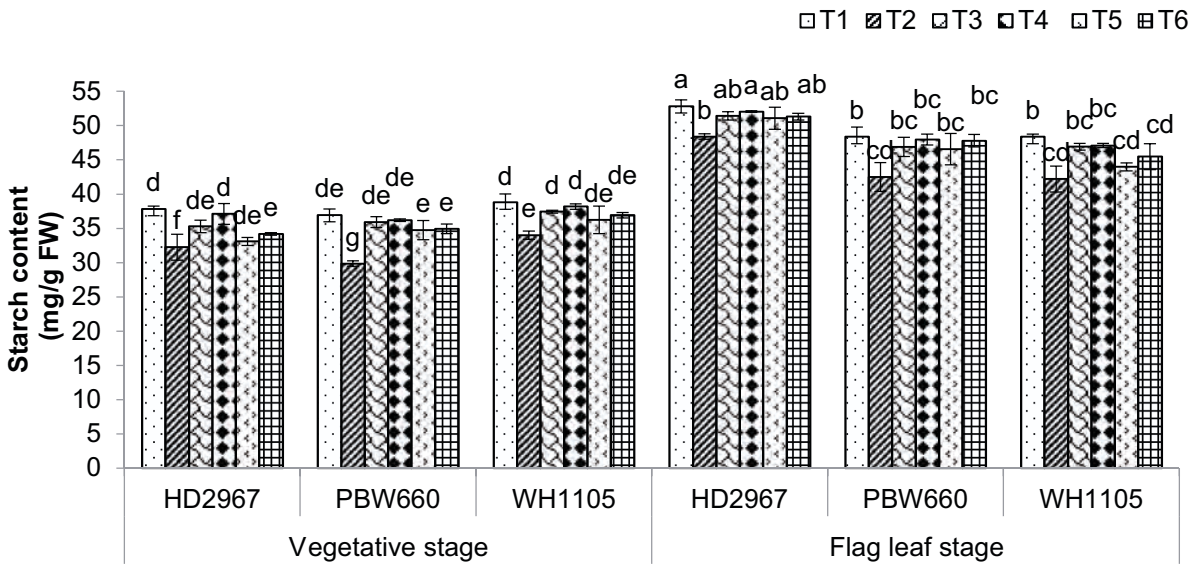


Fig. 4. Impact of cytokinins (kinetin, Kn; benzyl adenine, BA) and trehalose (Tre) on starch content (mg /g fresh weight, FW) in three wheat genotypes (HD2967, PBW660, WH1105) under drought stress, measured at vegetative stage (VS) and flag leaf stage (FLS)

T1 (control, irrigated), T2 (drought stress, no irrigation except first), T3 (drought + Kn 40 mg), T4 (drought + Kn 40 mg + Tre 1.5 mM), T5 (drought + BA 40 mg), T6 (drought + BA 40 mg + Tre 1.5 mM).
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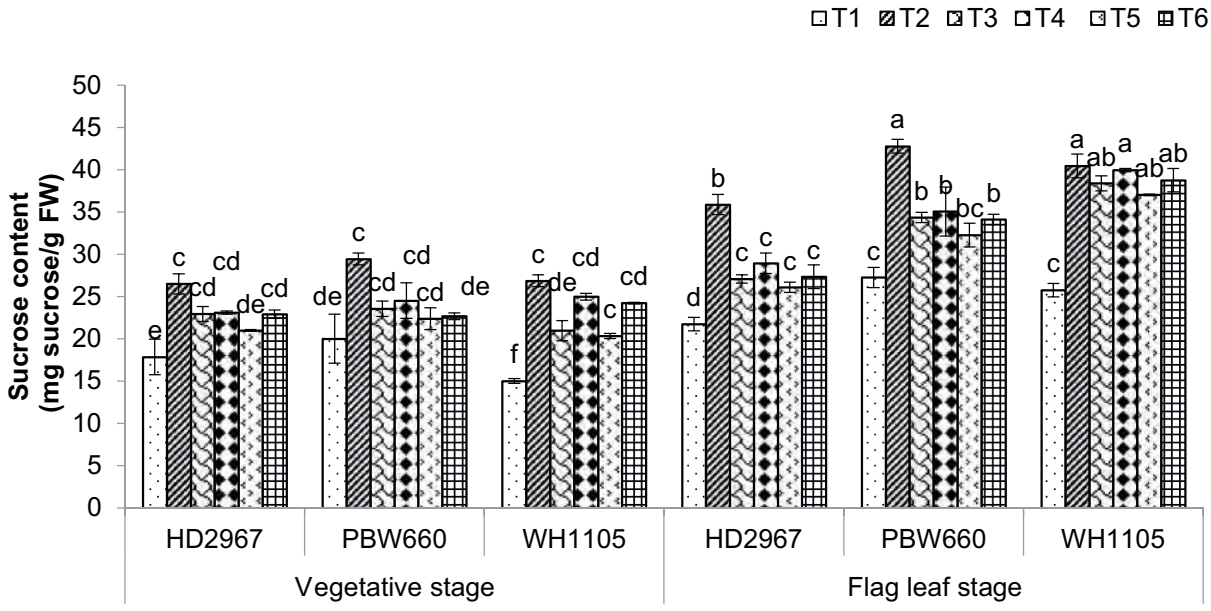


Fig. 5. Impact of cytokinins (kinetin, Kn; benzyl adenine, BA) and trehalose (Tre) on reducing sugar content (mg sucrose/g fresh weight, FW) in three wheat genotypes (HD2967, PBW660, WH1105) under drought stress, measured at vegetative stage (VS) and flag leaf stage (FLS)

T_1 (control, irrigated), T_2 (drought stress, no irrigation except first), T_3 (drought + Kn 40 mg), T_4 (drought + Kn 40 mg + Tre 1.5 mM), T_5 (drought + BA 40 mg), T_6 (drought + BA 40 mg + Tre 1.5 mM).
Within each genotype and stage, bars with different alphabets differ significantly at *p<0.05* after Tukey's post hoc test. Each bar represents the mean value ± standard error (S.E.) from five replications.

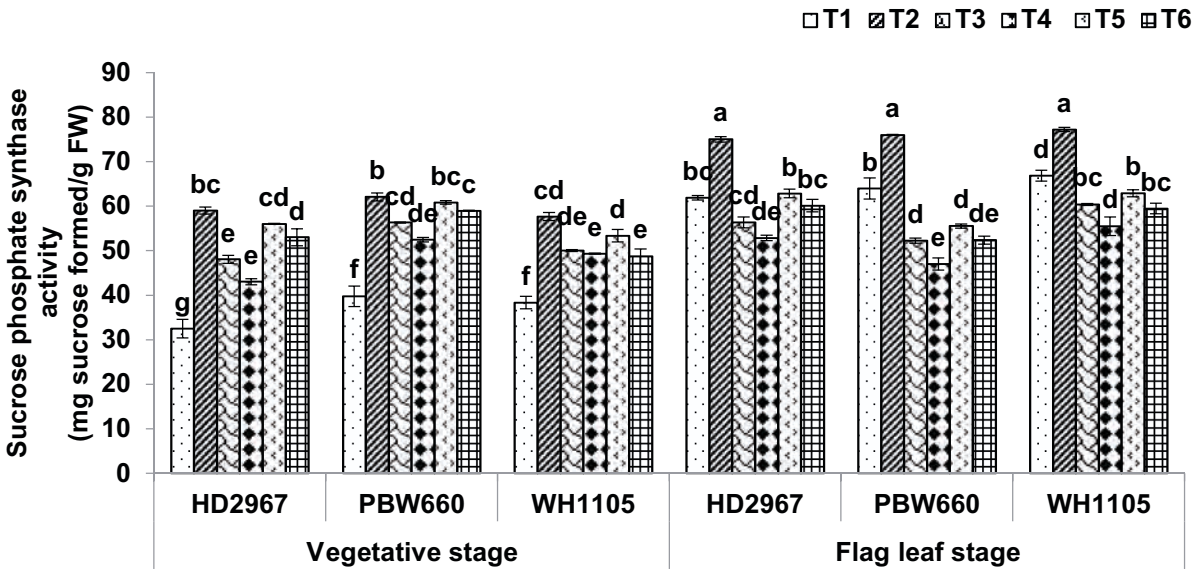


Fig. 6. **Impact of cytokinins (kinetin, Kn; benzyl adenine, BA) and trehalose (Tre) on sucrose phosphate synthase activity (mg sucrose formed/g FW) in three wheat genotypes (HD2967, PBW660, WH1105) under drought stress, measured at vegetative stage (VS) and flag leaf stage (FLS)**

T_1 (control, irrigated), T_2 (drought stress, no irrigation except first), T_3 (drought + Kn 40 mg), T_4 (drought + Kn 40 mg + Tre 1.5 mM), T_5 (drought + BA 40 mg), T_6 (drought + BA 40 mg + Tre 1.5 mM).
Within each genotype and stage, bars with different alphabets differ significantly at $p<0.05$ after Tukey's post hoc test. Each bar represents the mean value ± standard error (S.E.) from five replications.

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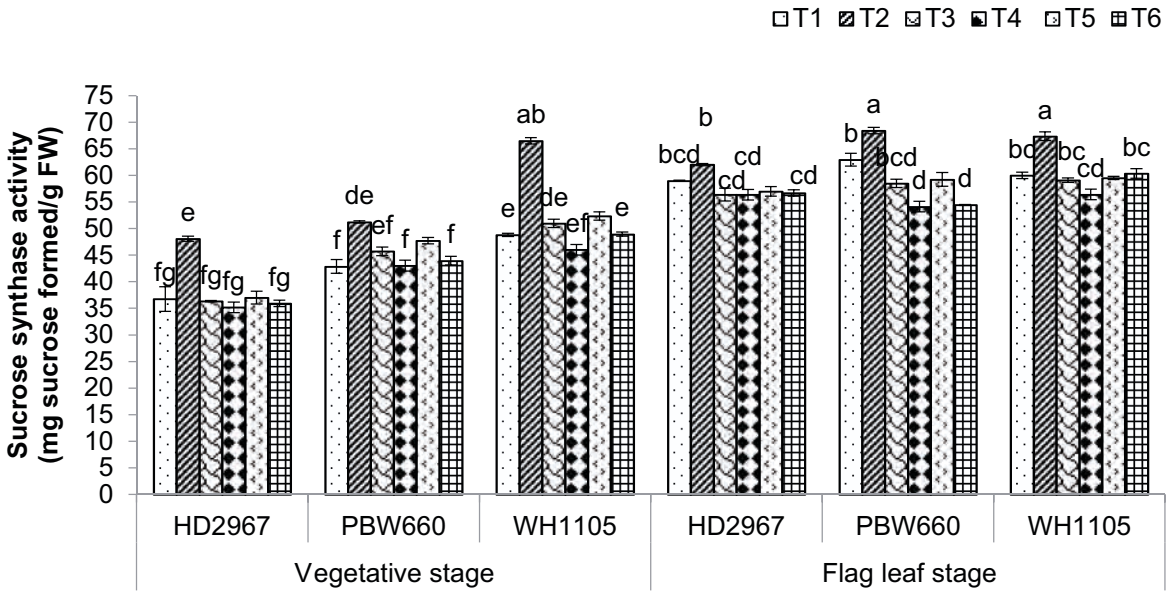


Fig. 7. **Impact of cytokinins (kinetin, Kn; benzyl adenine, BA) and trehalose (Tre) on sucrose synthase activity (mg sucrose formed/g FW) in three wheat genotypes (HD2967, PBW660, WH1105) under drought stress, measured at vegetative stage (VS) and flag leaf stage (FLS)**

T_1 (control, irrigated), T_2 (drought stress, no irrigation except first), T_3 (drought + Kn 40 mg), T_4 (drought + Kn 40 mg + Tre 1.5 mM), T_5 (drought + BA 40 mg), T_6 (drought + BA 40 mg + Tre 1.5 mM).
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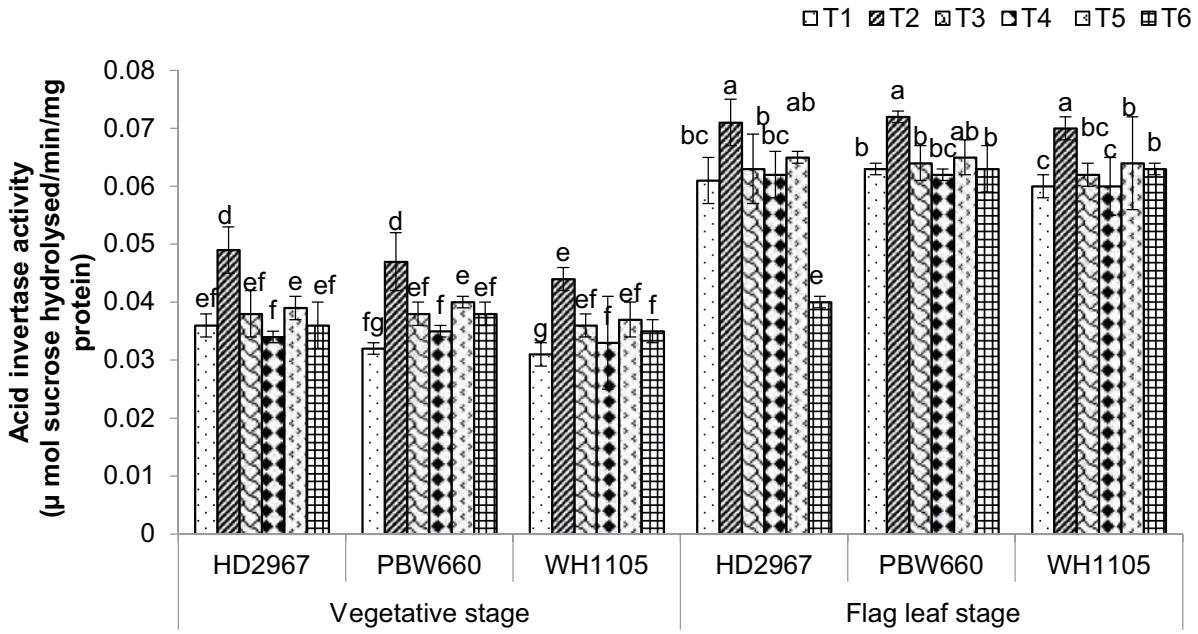


Fig. 8. **Impact of cytokinins (kinetin, Kn; benzyl adenine, BA) and trehalose (Tre) on acid invertase activity (µmol sucrose hydrolysed/min/mg protein) in three wheat genotypes (HD2967, PBW660, WH1105) under drought stress, measured at vegetative stage (VS) and flag leaf stage (FLS)**

T_1 (control, irrigated), T_2 (drought stress, no irrigation except first), T_3 (drought + Kn 40 mg), T_4 (drought + Kn 40 mg + Tre 1.5 mM), T_5 (drought + BA 40 mg), T_6 (drought + BA 40 mg + Tre 1.5 mM).
Within each genotype and stage, bars with different alphabets differ significantly at *p<0.05* after Tukey's post hoc test. Each bar represents the mean value ± standard error (S.E.) from five replications.

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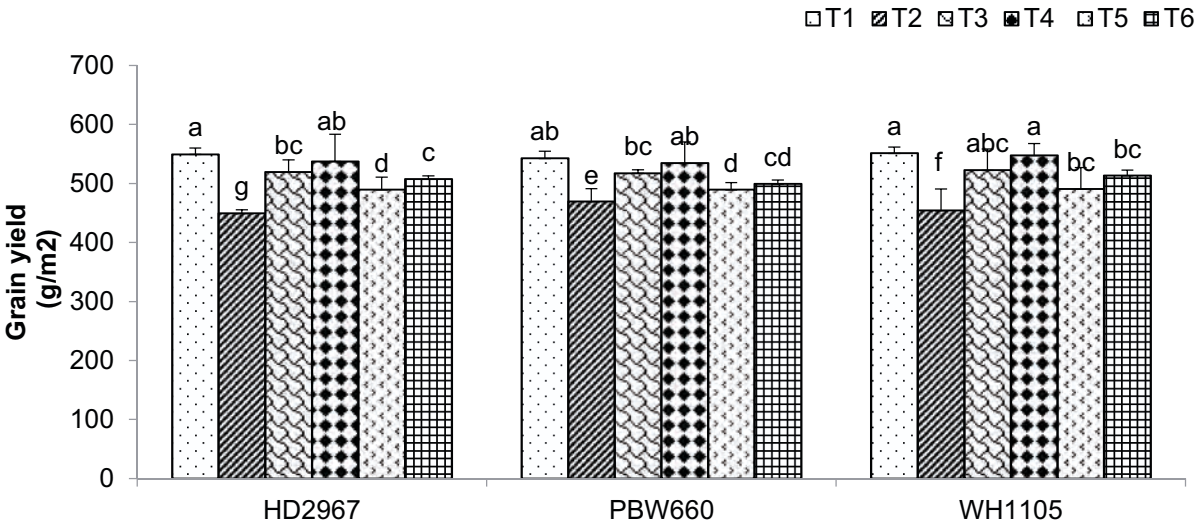


Fig. 9. **Impact of cytokinins (kinetin, Kn; benzyl adenine, BA) and trehalose (Tre) on grain yield (g/m²) in three wheat genotypes (HD2967, PBW660, WH1105) under drought stress, measured at vegetative stage (VS) and flag leaf stage (FLS)**

T_1 (control, irrigated), T_2 (drought stress, no irrigation except first), T_3 (drought + Kn 40 mg), T_4 (drought + Kn 40 mg + Tre 1.5 mM), T_5 (drought + BA 40 mg), T_6 (drought + BA 40 mg + Tre 1.5 mM).
Within each genotype and stage, bars with different alphabets differ significantly at *p<0.05* after Tukey's post hoc test. Each bar represents the mean value ± standard error (S.E.) from five replications.

physiological traits, carbohydrate contents, enzyme activities, and grain yield. Correlations significant at the 0.01 and 0.05 levels were reported. Additionally, rotated component matrices, score plots, and loading plots were generated using Minitab software to visualize associations among traits and their relationship to grain yield.

RESULTS AND DISCUSSION

Drought stress significantly influenced carbohydrate metabolism in wheat across all genotypes. Reducing sugars increased by 1.1-fold at the vegetative stage (VS) and by 1.3-fold at the flag leaf stage (FLS) in cultivars HD2967 and WH1105 (Fig. 1). Non-reducing sugars also showed a 1.2- to 1.5-fold increase at VS (Fig. 2). Total soluble sugars were higher at FLS (34-37 mg glucose/g fresh weight (FW)) than at VS (16-19 mg glucose/g FW), with drought inducing significant increase (Fig. 3). Among genotypes, PBW660 recorded the greatest total soluble sugar increase (1.3-fold) at VS, whereas WH1105 showed the least (1.2-fold). Under control conditions, starch content was greater at FLS but declined by 1.0 to 1.2-fold under drought at both stages (Fig. 4). Sucrose content, initially low under control, increased significantly under drought, with PBW660 showing the highest accumulation at VS and HD2967 exhibiting a 1.6-fold increase at FLS (Fig. 5).

Foliar application of kinetin (Kn, 40 mg) combined with trehalose (Tre) at 1 mM and 1.5 mM further enhanced carbohydrate accumulation. Reducing sugars increased by 1 to 1.2-fold (Kn 40 mg + Tre 1 mM) and up to 1.5-fold with benzyl adenine (BA) plus Tre at 1.5 mM, depending on genotype and growth stage. Similar trends were observed for non-reducing and total soluble sugars, with Kn + Tre 1.5 mM (T_4 treatment) inducing the greatest enhancement (Fig. 1-3). Kn and BA treatments also increased starch content relative to drought alone, particularly at VS (Fig. 4). On the other hand, sucrose content decreased with both cytokinin + Tre treatments at both concentrations (Fig. 5).

Drought stress significantly upregulated activities of sucrose phosphate synthase (SPS), sucrose synthase (SS), and acid invertase (AI) enzymes (Fig. 6-8). SPS activity increased the most in HD2967 by 1.8-fold at VS and 1.2-fold at FLS. SS activity showed a sharp rise across genotypes under drought. Acid invertase activity was higher at FLS than VS under control and increased further with drought stress.

Cytokinin + Tre treatments (Kn or BA combined with trehalose at 1 mM and 1.5 mM) consistently reduced the activities of SPS, SS, and AI compared to drought stress alone. The reductions ranged from 1.1- to 1.6-fold depending on enzyme, treatment, stage, and genotype.

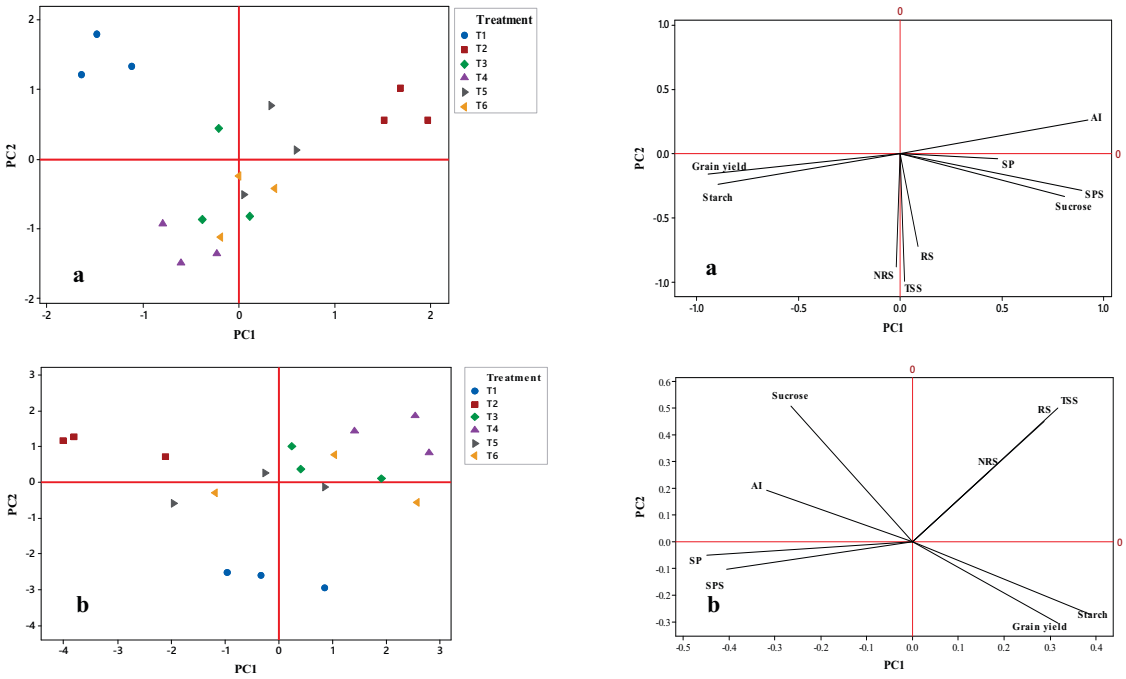


Fig. 10. Score plot and loading plot indicating correlations between carbohydrate content and carbohydrate metabolizing enzymes under drought stress, measured at vegetative stage (VS) and flag leaf stage (FLS)

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Drought stress decreased grain yield by 10-20% in comparison to control. WH1105 gave the highest yield under normal irrigation (552 g/m²), whereas PBW660 performed best under drought (470 g/m²) (Fig. 9). Foliar treatments with Kn + Tre (both 1 mM and 1.5 mM) enhanced grain yield by 10-20%, with BA + Tre increasing yield by up to 10%.

Pearson's correlation analysis at VS revealed significant positive correlations between total soluble sugars and both reducing (r = 0.66) and non-reducing sugars (r = 0.87). Grain yield correlated positively with starch content (r = 0.58) but negatively with SPS (r = -0.70), SS (r = -0.67), and sucrose content (r = -0.70) (Table 2). At FLS, starch showed positive correlation with total soluble sugars (r = 0.58), sucrose correlated positively with SPS activity (r = 0.63), and acid invertase correlated with SS (r = 0.82) (Table 3). The principal component analysis (PCA) indicated that the first two components explained 68.5% and 61.4% of variance at VS and FLS, respectively. Total soluble sugars and starch loaded positively on PC1, highlighting their role in source-sink dynamics. Grain yield was associated with starch at VS, while SPS, SS, and AI clustered with sucrose content (Fig. 10).

Drought stress resulted in significant alteration in carbohydrate metabolism in wheat, which is consistent with earlier studies (Abd-Elgawad, 2020; Gujjar *et al.*, 2021). The accumulation of reducing and non-reducing sugars under drought may be due to osmotic adjustment mechanisms, where soluble sugars function as osmolytes and signalling molecules to maintain cellular homeostasis (Thalmann and Santelia, 2017). The observed decrease in starch content under drought is attributable to reduced starch biosynthesis and enhanced mobilization of starch reserves to meet energy demands during stress (Homayouni and Khazarian, 2014). The increase in sucrose content suggests that there is impaired sucrose utilization or transport, which is further supported by elevated SPS and SS activities under drought.

Application of cytokinins (Kn, BA) combined with trehalose mitigated drought effects by modulating carbohydrate metabolism. The enhanced accumulation of soluble sugars and starch and the concomitant reduction in sucrose levels suggest improved carbon partitioning and source-sink balance, which support stress tolerance and maintain growth (Gujjar and Supaibulwatana, 2019; Niakan and Ahmadi, 2014). The downregulation of SPS, SS, and acid invertase activities by cytokinin + trehalose treatments indicates a protective mechanism where osmoprotectants are regulating enzymatic activities to optimize sugar metabolism without compromising protein function (Aldesuquy *et al.*, 2018; Moradi *et al.*, 2012).

Grain yield reduction under drought is associated with disrupted photosynthesis, stomatal closure, and impaired assimilate translocation (Abid *et al.*, 2016; Qaseem *et al.*, 2019). The yield improvement with cytokinin + trehalose treatments may result from enhanced photosynthate partitioning, improved sink capacity, and better ROS scavenging (Yang *et al.*, 2016; Khater *et al.*, 2018). Correlation and PCA analyses emphasize the critical role of carbohydrate metabolism enzymes and sugar accumulation in determining drought tolerance and yield stability. The prominent effect of the T_4 treatment (Kn 40 mg + Tre 1.5 mM) suggests an optimal combination for modulating photosynthate partitioning and enhancing drought resilience.

CONCLUSION

Drought stress disrupts carbohydrate metabolism in wheat, leading to altered sugar and starch dynamics which have negative impact on the grain yield. Foliar application of cytokinins combined with trehalose effectively modulates these metabolic changes by enhancing soluble sugar and starch accumulation while regulating key enzyme activities. This dual treatment improves source-sink balance and osmotic adjustment, resulting in enhanced drought tolerance and grain yield across diverse wheat genotypes. Among the tested treatments, kinetin (40 mg) with trehalose (1.5 mM) showed the most pronounced benefits. Thus, it can be concluded that cytokinin and trehalose foliar applications can be used as practical strategies for mitigating drought effects in wheat cultivation. Further long-term and field-based studies are needed to optimize application protocols and explore broader stress resilience mechanisms.

Authors' contribution

Planning and development of the research framework (AK, SKT); Conducting field and laboratory experiments and gathering data (AK, MP); Data evaluation and interpretation (AK, SKT); Drafting and revising the manuscript (NJ, MK, MP).

Conflicts of interests

Authors declare that there are no conflicts of interests.

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