

Original Research Article

Cytokinins along with osmoprotectant trehalose overcome the deleterious effect of drought stress on wheat

Abstract

Experiment was conducted to evaluate, how the foliar application of trehalose (tre) along with kinetin (kn) and benzyl adenine (BA) affect the wheat genotypes (HD2967, PBW660 and WH1105) under drought stress conditions. Trehalose, Kn and BA tested in laboratory studies for their best concentrations, were applied in field grown wheat under drought. Presently, significant reduction was observed in leaf area, relative growth, Hill reaction and harvest index and sharp increase was recorded in concentration of non-enzymatic antioxidants constituents at both stages except reduced ascorbic acid content showed decline at flag leaf stage along with the upsurge in the activities of enzymatic antioxidants of wheat under drought as compared to control or irrigated ones. Foliar application of growth regulators altered activities of enzymatic and non-enzymatic antioxidants at both vegetative and flag leaf stage and helps in mitigation of negative impact of drought by regulation of Hill reaction, leaf area and growth rate of wheat that tends to maintain the final harvest of wheat crop.

Key words: drought, relative growth, Hill reaction, leaf area, antioxidants.

Abbreviations:

Hill reaction activity (HRA), Leaf area (LA), Relative growth rate (RGR), Reduced glutathione content (RGC), Reduced ascorbic acid content (RAAC), Superoxide dismutase (SOD), Catalase (CAT), Monodehydroascorbate reductase (MDHAR), Dehydroascorbate reductase (DHAR), Harvest index (HI), Vegetative stage (VS), Flag leaf stage (FLS)

Introduction

World's consumption of wheat is estimated to increase beyond production raising concerns about the food security (FAO, 2018; FAO, 2019). Drought stress during growth period of wheat significantly resulted in high yield losses. Drought antagonistically influences seed germination, seedling development, plant development, chemical movement and macromolecules. Various abiotic stresses including drought, leads to production of reactive oxygen species (ROS) that can be superoxide radicals, singlet oxygen, hydrogen peroxide, and hydroxyl radical and these in turn resulted in oxidative damages to plants. During water deficit conditions, a significant rise in expression pattern of genes encoding CAT, APX, and GPX enzymes was recorded in drought-tolerant wheat genotypes. These genes could play a very important role in controlling drought stress in the wheat genome (Dudziak *et al.*, 2019).

Plant growth regulators are believed to play major roles in lessening the deleterious effects of major abiotic stresses. Few of the important phytohormones which promote accumulation of chlorophyll (Chl) and conversion of etioplasts into chloroplasts and delay leaf senescence (Brault and Maldiney 1999) have been used since long (Dwivedi *et al.*, 2018). Cytokinins, both in synergy and antagonisms with other hormones influence several plant processes that result in growth and development. Generally, response of plants to cytokinins is judged by their exogenous application (Aldesuquy *et al.*, 2014). Trehalose, a non-reducing disaccharide, is notable a stress protectant in several organisms and is well known for its protective ability, stability and low reactivity. Trehalose decreased the deleterious effect of high temperature stress on *P. lactiflora* growth by enhancing antioxidant systems, activating photosynthesis, and protecting cell structure (Zhao 2019). The main objective of present study was to recognize the cumulative effect of cytokinins (Kn and BA) in combination with different concentrations of osmoprotectants like trehalose on various physiological and biochemical parameters that contributes to yield of wheat crop under drought stress.

MATERIAL AND METHODS

Plant material:

The crop was sown under the experimental field area of Department of Botany, Punjab Agricultural University, Ludhiana. The experiment was laid out in a split plot design with six main plot treatments (i.e. control or normal irrigated, drought stress and drought stress with application of cytokinins in combination with different concentrations of trehalose as: T1= Control (with all 5 irrigations), T2= Drought stress (withholding 3rd, 4th and 5th irrigation), T3= Drought stress + Kn@40mg/L in combination with Trehalose@1mM/L, T4= Drought stress + Kn@40mg/L in combination with Trehalose@1mM/L, T5= Drought stress+ BA@50mg/L in combination with Trehalose@1mM/L, T6= Drought stress+ BA@50mg/L in combination with Trehalose@1.5mM/L at both VS and FLS and samples were collected after 4 days of foliar sprays for estimation of physiological and biochemical attributes.

Hill reaction activity

Hill reaction activity was estimated by method as given by Cherry (1973).

Extraction

Leaf samples (100mg) were taken and gently ground in 5ml extraction medium (0.067 M Phosphate buffer, pH 7.5 containing 0.35M sucrose). During extraction the temperature was maintained at 0-4°C.

Estimation

Potassium ferricyanide solution was prepared by dissolving sodium chloride (1.02g) and potassium ferricyanide (13mg) in phosphate buffer (25 ml). The reaction was started by mixing 0.5ml of supernatant from above extract with 2.5ml of ferricyanide solution. The tubes were kept in light (approx.5000 lux) for 10 min. and another similar set of experiment was kept in dark. The reaction was stopped by adding 20% TCA (0.3ml). The absorbance was recorded at 420 nm on spectrometer. Hill reaction activity was expressed as decrease in absorbance $\text{mg}^{-1}\text{Chl h}^{-1}$.

Leaf Area

Leaf area was measured from 3 leaves per replication of each genotype for given treatment by measuring their length and

breadth. Further the mean values were used for calculations.

$$\text{Specific Leaf Area} = L \times B \times 0.81$$

Where L = Length of leaf in centimeters, B = Breadth in Centimeters, 0.81 constant factor for wheat leaf

Relative growth rate: RGR were calculated as suggested by Gardener et al. (1985).

Extraction and assay of enzymatic and non-enzymatic antioxidants

Reduced glutathione content

Reduced glutathione content from the plant leaf was determined according to the method described by Smith (1985). 100mg of leaf sample was homogenized in 2ml of 5% of sulpho-salicylic acid. Then centrifuged the mixture at 10,000 for 15 min and to the supernatant added the 0.15ml of 0.2M phosphate buffer (pH 7.5). For estimation, 0.2ml of extract was mixed with 3ml of 0.1M potassium phosphate buffer (pH 7.5) containing 5mM EDTA, 0.4 ml of 2mM DTNB, 0.2 ml of 2mM NADPH and 0.2ml of glutathione reductase (1U/0.1ml) in test tube. The mixture was kept at 25°C for 5min and then immersed in ice for the termination. Glutathione content was determined spectrophotometrically by following the formation of 2-nitrobenzoic acid at 412nm. Compared the absorbance with a standard curve obtained with known glutathione concentrations (40-200µg).

Reduced ascorbic acid content

Reduced ascorbic acid content was estimated by method described by Law *et al.* (1983) with some modifications. For extraction, leaf tissue (100mg) was homogenized in 2ml of 5% ice cold meta-phosphoric acid solution and centrifuged at 10,000g for 20min. For estimation, 0.2ml aliquot of supernatant and 0.5ml of 150mM potassium phosphate buffer (pH 7.4) containing 5mM EDTA was added to reaction tube. Then in reaction mixture, 0.4ml of 10% TCA, 0.4ml of 44% o-phosphoric acid, 0.4ml of 4% dipyridyl in 70% ethyl alcohol and 0.2ml of FeCl₃ were added. Then mixture was stirred and incubated at 40°C for 40min and colour produced was read at 525nm.

Superoxide dismutase (EC 1.15.1.1)

Activity of SOD was estimated by as the method suggested by Marklund and Marklund (1974).

Catalase (EC 1.11.1.6)

As described by Aebi (1984), Fresh (0.1g) leaf samples were homogenised in 2ml of ice cold 100mM potassium phosphate buffer (pH 7.5) containing 1mM EDTA, 1% polyvinyl pyrrolidone (PVP-40) and 0.1mM TritonX-100 using chilled pestle and mortar. Whole extraction was carried out at 0-4°C. Then centrifuged at 15000g for 30min at 4°C and supernatants was used for enzyme activity assay. A protein concentration was determined according to Lowry *et al.* (1951) using BSA as a standard and was assayed by monitoring the decrease in absorbance due to hydrogen peroxide decomposition at 240nm. The reaction mixture of 3ml consisted of 50mM potassium phosphate buffer (pH 7.5), 12.5mM hydrogen peroxide and 100µl enzyme extract. Addition of H₂O₂ initiated the reaction and decrease in optical density was recorded after an interval of 30 sec upto 3 min. The concentration of reduced hydrogen peroxide was calculated using extinction coefficient ($\epsilon = 39.4\text{mM}^{-1}\text{cm}^{-1}$). Enzyme specific activity is expressed as nmol H₂O₂ reduced min⁻¹ mg⁻¹ protein.

Dehydroascorbatereductase (DHAR, EC 1.15.1.1)

The procedure for extraction of DHAR was similar to catalase and assayed as described by Douliset *al.*(1997).

Monodehydroascorbatereductase (MDHAR, EC 1.6.5.4)

Extraction procedure was similar to catalase and was assayed by the method described by Hossainet *al.*(1984).

Harvest index (HI): was calculated from ratio of grain yield to biomass in each experimental unit.

$$\text{Harvest Index (\%)} = \text{Grain yield (g)} / \text{Biological yield (g)} \times 100$$

Statistical analysis:

Statistical analysis was performed by means of factorial experiment in random block design using two way ANOVA as the method described by Cochran and Cox 1967.

RESULTS AND DISCUSSION

Hill reaction activity (HRA), Leaf area (LA) and Relative growth rate (RGR)

HRA in leaves of wheat genotypes was significantly ($P \leq 0.05$) lower in drought stressed plots as compared to control or irrigated ones (Table 1). HD2967 (43.76%) showed more decline in HRA as compared to control conditions at VS and 24.15% at FLS. Application of trehalose along with Kn and BA enhanced the Hill reaction activity in all studied genotypes both at VS and FLS. The maximum percentage increase in Hill reaction activity over drought was recorded in HD2967 (76.47%) at VS and 31.18% at FLS followed by PBW660 (42.52%) at VS with the application of Kn@40mg/L along with Tre@ 1.5mM although WH1105 had maximum HRA at both the stages under the irrigated or control stage. Hill reaction activity decreased whereas the hydrolytic enzyme activity and total soluble and reducing sugar contents increased under drought stress (Zeid and Shedeed 2006).

All genotypes showed significantly ($P \leq 0.05$) decreased LA under the drought stressed plots as compared to the control plots at both the stages (Table 1). WH1105 (14.17%) showed maximum decrease in LA at VS and showed least decrease at FLS i.e 26.40% as compared to other genotypes. All the genotypes resulted in significant increase in leaf area with the application of different concentrations of cytokinins and trehalose. **Osmoprotectants synthesized by plants play an important role in regulating defense responses to various abiotic stresses, including drought stress (Zulfiqar 2021).** Maximum increase in leaf area was recorded with all the applications or treatments at the FLS as compared to VS. HD2967 (14.85%) followed by PBW660 (11.63%) had maximum increase in LA at VS with the application of Kn@40mg/L along with Tre@ 1.5mM. similarly with same application at FLS the HD2967 (37.44%) followed by PBW660 (31.36%) had more LA as compared under drought conditions. The reduction in flag leaf area under drought stressed bread wheat genotypes has been observed (Allahverdiyev 2015).

RGR in drought stressed plots showed a significant decrease over control (Table 1) in all the genotypes. This decrease was higher in VS as compared to FLS in all genotypes. The actual RGR was although more at FLS. Application of different combinations of Kn, BA and trehalose significantly enhanced the relative growth rate of wheat. Kn along with both concentrations of trehalose performed better as compared to BA along with trehalose. Although all applications had significantly positive effect in enhancement of RGR of

wheat genotypes under drought stress. RGR in wheat genotypes declined progressively under stress conditions (Datta *et al.*, 2011). Kinetin priming was found effective in increasing germination rate and early seedling growth in both salt tolerant and intolerant wheat plants (Iqbal *et al.*, 2006).

Reduced glutathione content (RGC), reduced ascorbic acid content (RAAC)

Genotype PBW660 had maximum content of reduced glutathione at the VS and at FLS. All genotypes showed significant increase in RGC under the drought stress conditions at both the stages (Table 2). WH1105 had maximum and PBW660 had minimum RGC at the VS and at FLS maximum content of reduced glutathione was recorded in WH1105 under drought stress. Kn in combination with Tre@ 1mM significantly reduced the RG content of all studied genotypes as compared to drought stress in all genotypes. With that foliar spray HD2967 had maximum and PBW660 lesser RGC accumulation at VS and PBW660 had lesser RGC at FLS. With foliar application of Kn along with Tre@ 1.5mM all genotypes showed further decrease in RG content. PBW660 showed lesser RGC at VS and as well as at FLS. Application of BA in combination with Tre@ 1mM, PBW660 had lesser RGC at VS and WH1105 followed by HD2967 had minimum RGC accumulation at FLS.

Under control conditions, selected wheat genotypes had lower RAAC accumulation (Table 2). FLS had slight lower values of RAAC as compared to with the content recorded at VS in all genotypes. Significant increase in RAAC under the drought stress conditions at both the stages was recorded. HD2967 had maximum and WH1105 had minimum accumulation of RAAC at the VS and at FLS maximum was recorded in HD2967 under drought stress. With foliar application of Kn along with Tre@ 1.5mM all genotypes showed increase in RAA content. WH1105 had more RAAC at VS and PBW660 had more at FLS. Application of BA in combination with Tre@ 1mM significantly increased the RAAC in all genotypes as compared to control as well as at drought stress, WH1105 had more RAAC at VS and PBW660 had more and WH1105 had lesser amount of reduced ascorbic acid content recorded presently. BA with Tre@ 1.5 mM further increased the RAAC of all genotypes at both stages. PBW660 had maximum and WH1105 had minimum RAAC at the VS and PBW660 had maximum reduced ascorbic acid content at the FLS with application of BA with

Tre@ 1.5 mM.

Superoxide dismutase (SOD), Catalase (CAT)

All genotypes showed significant increase in activity of SOD under the drought stress conditions at both the stages (Fig 1). Kn in combination with Tre@ 1mM significantly reduced the SOD activity of all studied genotypes as compared to drought stress in all genotypes. With that foliar spray PBW660 had maximum SOD activity at VS and WH1105 followed by PBW660 had maximum SOD at FLS. With foliar application of Kn along with Tre@ 1.5mM all genotypes showed further decrease in activity of SOD. HD2967 showed lesser SOD activity at VS and as well as at FLS. Application of BA in combination with Tre@ 1mM, HD2967 had lesser SOD at VS and HD2967 followed by PBW660 had minimum SOD at FLS. BA with Tre@ 1.5 mM further decreased the SOD of all genotypes at both stages. PBW660 had maximum and HD2967 had minimum SOD at the VS as well as at the FLS with application of BA with Tre@ 1.5 mM. In rice, the SOD activity increased in rice under salt stress and was decreased with the application of trehalose (Nounjan and Theerakulpisut, 2012). Water stressed wheat was recorded with enhanced SOD activity and BAP application further regulated the activity of superoxide dismutase (Nawaz *et al.*, 2016).

All genotypes showed significant increase in catalase activity under the drought stress conditions at both the stages (Fig 2) the results were similar with the findings of Zhao et al (2019) as the catalase activity increase under the stress conditions. The activities of the superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) enzymes were enhanced in water-stressed sunflower plants (Kosar 2022). Kn in combination with Tre@ 1mM significantly reduced the activity of catalase. With that foliar spray HD2967 had maximum and PBW660 had lesser catalase activity at VS and HD2967 had lesser catalase activity at FLS. With foliar application of Kn along with Tre@ 1.5mM all genotypes showed further decrease in catalase activity. WH1105 showed lesser catalase at VS and at FLS the activity was measured low in HD2967 and high in WH1105. BA with Tre@ 1.5 mM decreased the enzyme catalase activity in all genotypes at both stages. HD2967 had maximum and PBW660 had minimum catalase activity at the VS and HD2967 was recorded with low catalase activity at the FLS with application of BA with Tre@ 1.5 mM. Present results are in contradictory with findings of Mostofa *et al.*, (2015) and in agreement with findings of Luo *et al.*, (2008) as CAT activity decreased

with trehalose application.

Monodehydroascorbate reductase (MDHAR) and Dehydroascorbate reductase (DHAR)

All genotypes showed significant increase in MDHAR activity under the drought stress conditions at both the stages (Fig 3). WH1105 had maximum and PBW660 had minimum MDHAR activity at the VS and at FLS maximum MDHAR activity was recorded in HD2967 under drought stress. Kn in combination with Tre@ 1mM significantly reduced the MDHAR of all studied genotypes as compared to drought stress at the VS and at FLS there was further significant increase in MDHAR as compared to drought and control. With that foliar spray PBW660 had maximum and HD2967 had lesser MDHAR activity at VS and PBW660 had more MDHAR activity at FLS. With foliar application of Kn along with Tre@ 1.5mM all genotypes showed further decrease in MDHAR at VS and significant increase was recorded in all genotypes at FLS. PBW660 showed lesser MDHAR at VS and WH1105 had lesser activity of MDHAR at FLS. BA with Tre@ 1.5 mM further decrease in MDHAR was recorded in all genotypes at VS and significant increase in activity of MDHAR was observed in all genotypes at FLS with that foliar application. WH1105 had maximum and HD2967 had minimum MDHAR at the VS and WH1105 had maximum MDHAR activity at the FLS with application of BA with Tre@ 1.5 mM. Mostofa *et al.*, (2015), significant increase in MDHAR activity was reported under stress conditions but significant reduction in MDHAR activity with trehalose application, which is agreement with present study only at VS but in contradictory with results at FLS.

Significant increase in DHAR activity was recorded in all genotypes under the drought stress conditions at both the stages (Fig 4). Activity of DHAR was higher at FLS as compared to at VS. PBW660 had maximum and HD2967 had minimum DHAR activity at the VS and at FLS maximum was recorded also in HD2967 under drought stress. Kn in combination with Tre@ 1mM significantly reduced the DHAR of all studied genotypes as compared to drought stress in all genotypes. With that foliar spray PBW660 had maximum and WH1105 had lesser DHAR activity at VS and WH1105 had less activity of DHAR at FLS. With foliar application of Kn along with Tre@ 1.5mM all genotypes showed further decrease in DHAR activity. WH1105 showed lesser DHAR activity at VS and as well as at FLS. Application of BA in combination with Tre@ 1mM, WH1105 and HD2967 had comparable

DHAR activity i.e 145.364 and 145.477 respectively at VS and maximum DHAR activity was recorded in PBW660 at FLS. BA with Tre@ 1.5 mM further decreased the DHAR in all genotypes at both stages. PBW660 had maximum and HD2967 had minimum DHAR at the VS and WH1105 had minimum DHAR activity at the FLS with application of BA with Tre@ 1.5 mM. According to reports of Mostofa *et al.*, (2015) DHAR activity increased under the stress conditions and significant reduction was observed with application of trehalose which is comparable with the results of present study.

Harvest Index (HI)

Harvest index was recorded more under control in all studied genotypes. Drought stress significantly decreased the HI in all the genotypes (Fig 5). WH1105 had maximum HI as compared to other genotypes under control and PBW660 had more harvest index under drought stress. Kn in combination with Tre@ 1mM significantly increased the HI in all studied genotypes. With that application, WH1105 had more harvest index. Kn along with Tre@ 1.5mM significantly further increased HI. WH1105 had maximum and PBW660 had minimum HI with this foliar application. BA in combination with different trehalose concentrations significantly increased the HI in all genotypes. With foliar spray of BA in combination with Tre @ 1mM, all the genotypes showed significant increase in harvest index. WH1105 followed by PBW660 had more HI. WH1105 had maximum HI with foliar application of BA along with Tre@ 1.5mM. The application of cytokinins in combination with osmoprotectants was able to reduce the negative effect of drought on number of kernels/spike and number of productive tillers/m² (Kaur and Thind 2018) that ultimately leads to retention of harvest index in present findings.

Conclusion

Foliar application of trehalose along with kinetin and benzyl adenine resulted in maintenance of relative growth rate, leaf area, Hill reaction activity and harvest index of wheat crop under drought stress by altering the enzymatic and non-enzymatic activities of antioxidants. Among all the studied concentrations the application of Tre@ 1.5mM along with Kn@40mg/L was found more effective in amelioration of bad effect of drought in wheat genotypes.

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Table 1 Effect of cytokinins (Kn and BA) and trehalose on Hill reaction activity, leaf area and Relative growth rate of wheat genotypes at vegetative stage and flag leaf stage under drought stress.

Treatments	Hill reaction activity (mg chlorophyll ⁻¹ hr ⁻¹)	Leaf area (cm ²)	Relative growth rate (%)
	Vegetative stage	Vegetative stage	Vegetative stage

Genotypes	HD2967			PBW660			WH1105		
	HD2967	PBW660	WH1105	HD2967	PBW660	WH1105	HD2967	PBW660	WH1105
T1-Control	0.786	0.701	0.806	28.326	27.352	28.999	0.261	0.256	0.251
T2- Stress	0.442	0.522	0.603	24.368	24.096	24.889	0.221	0.214	0.214
T3-T2+Kn+(tre-1mM)	0.657	0.664	0.756	27.887	26.890	26.789	0.242	0.241	0.232
T4- T2+Kn+ (tre 1.5mM)	0.780	0.744	0.806	27.989	26.899	26.997	0.248	0.246	0.238
T5-T2+BA+ (tre-1mM)	0.609	0.621	0.707	26.847	25.999	26.334	0.232	0.236	0.230
T6-T2+ BA+(tre-1.5mM)	0.704	0.665	0.719	26.909	26.009	26.465	0.235	0.239	0.236
CD5%	T=0.206, G=0.109, G×T=0.330			T=3.666, G=1.320, G×T=3.906			T=0.019, G=0.023, G×T=0.033		
	Flag leaf stage			Flag leaf stage			Flag leaf stage		
T1-Control	0.799	0.751	0.887	30.667	30.438	31.232	0.333	0.319	0.309
T2- Stress	0.606	0.690	0.675	21.099	21.997	22.985	0.299	0.298	0.286
T3-T2+Kn+(tre-1mM)	0.786	0.725	0.785	28.970	28.786	29.786	0.315	0.308	0.301
T4- T2+Kn+ (tre 1.5mM)	0.795	0.748	0.798	28.999	28.897	30.002	0.322	0.316	0.306
T5-T2+BA+ (tre-1mM)	0.696	0.692	0.749	28.342	28.445	29.675	0.311	0.306	0.299
T6-T2 + BA+(tre-1.5mM)	0.698	0.698	0.762	28.668	28.534	29.800	0.314	0.311	0.302
CD5%	T=0.103, G=0.033, G×T=0.120			T=4.321, G=1.001, G×T=2.261			T=0.100, G=0.023, G×T=0.096		

Where, T and G corresponded to treatments and genotypes, T×G were treatments and genotypes interaction.

Table 2 Effect of cytokinins (Kn and BA) and Trehalose on Reduced glutathione content, reduced ascorbic acid content and quaternary ammonium compounds of wheat genotypes at vegetative stage and flag leaf stage under drought stress.

Treatments	Reduced glutathione content (mg of glutathione/g FW)	Reduced Ascorbic acid content (mg ascorbic acid/g FW)
	Genotypes	Vegetative stage

	HD2967	PBW660	WH1105	HD2967	PBW660	WH1105
T1-Control	1.364	1.385	1.287	1.876	1.987	1.876
T2- Stress	1.977	1.787	1.982	2.287	2.276	2.202
T3-T2+Kn+(tre-1mM)	1.676	1.487	1.587	3.876	3.476	3.876
T4- T2+Kn+ (tre 1.5mM)	1.499	1.208	1.343	4.002	3.972	4.265
T5-T2+BA+ (tre-1mM)	1.754	1.587	1.665	3.567	3.464	3.650
T6-T2 + BA+(tre-1.5mM)	1.731	1.445	1.601	3.807	3.987	3.802
CD5%	T=0.231, G=0.331, G×T=0.621			T=1.031, G=0.936, G×T=1.638		
	Flag leaf stage			Flag leaf stage		
T1-Control	1.402	1.487	1.365	1.668	1.765	1.459
T2- Stress	1.990	1.923	2.102	1.202	0.968	1.033
T3-T2+Kn+(tre-1mM)	1.654	0.987	1.587	1.987	2.775	1.753
T4- T2+Kn+ (tre 1.5mM)	1.002	0.765	1.398	2.098	2.965	1.879
T5-T2+BA+ (tre-1mM)	1.732	1.987	1.665	1.745	2.697	1.665
T6-T2 + BA+(tre-1.5mM)	1.702	1.897	1.512	2.003	2.799	1.694
CD5%	T=0.036, G=0.048, G×T=0.636			T=0.331, G=0.206, G×T=0.625		

Where, T and G corresponded to treatments and genotypes, T×G were treatments and genotypes interaction.

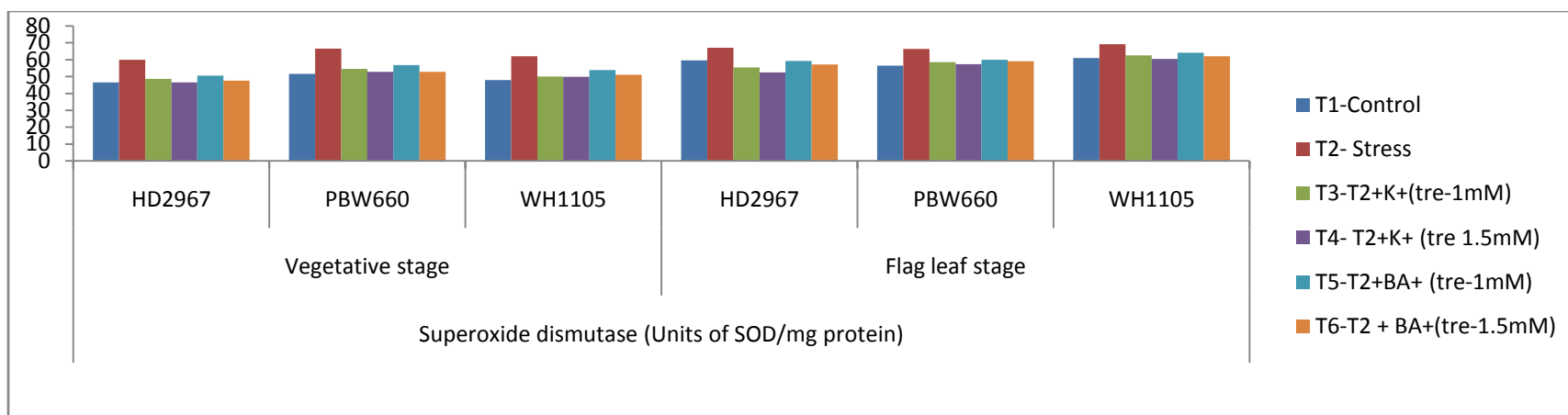


Fig 1 Effect of cytokinins (Knetin and BA) and Trehalose on superoxide dismutase of wheat genotypes at vegetative and flag leaf stages under field drought stress. (A) Vegetative stage: $T = 4.333$, $G = 6.030$, $G \times T = 9.406$. (B) Flag leaf stage: $T = 3.033$, $G = 2.606$, $T \times G = 4.362$. Where, T and G corresponded to treatments and genotypes, $T \times G$ were treatments and genotypes interaction

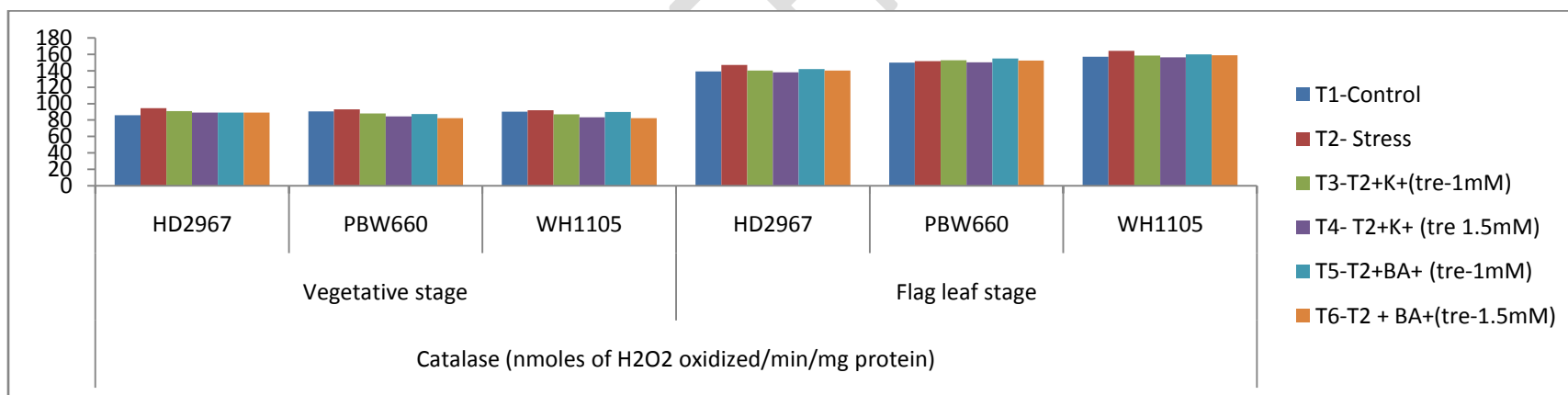


Fig 2 Effect of cytokinins (Knetin and BA) and Trehalose on catalase of wheat genotypes at vegetative and flag leaf stages under field drought stress. (A) Vegetative stage: $T = 2.960$, $G = 3.091$, $G \times T = 4.902$. (B) Flag leaf stage: $T = 3.616$, $G = 1.610$, $T \times G = 2.622$. Where, T and G corresponded to treatments and genotypes, $T \times G$ were treatments and genotypes interaction

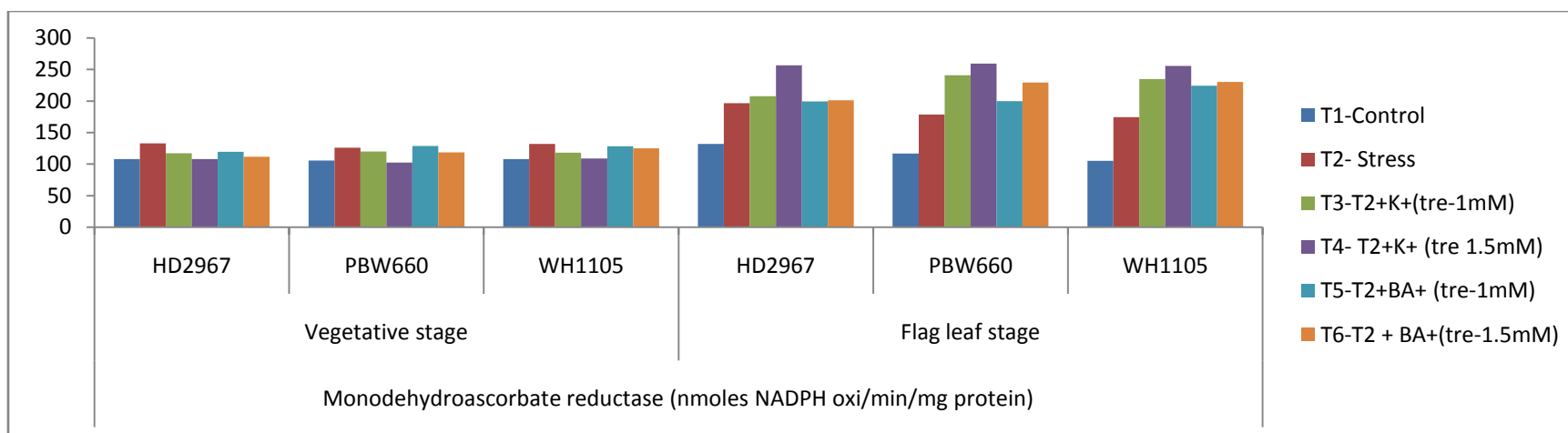


Fig 3 Effect of cytokinins (Knetin and BA) and Trehalose on monodehydroascorbatereductase of wheat genotypes at vegetative and flag leaf stages under field drought stress. (A) Vegetative stage: $T = 2.301$, $G = 1.369$, $G \times T = 2.909$. (B) Flag leaf stage: $T = 2.006$, $G = 2.901$, $T \times G = 3.989$. Where, T and G corresponded to treatments and genotypes, $T \times G$ were treatments and genotypes interaction

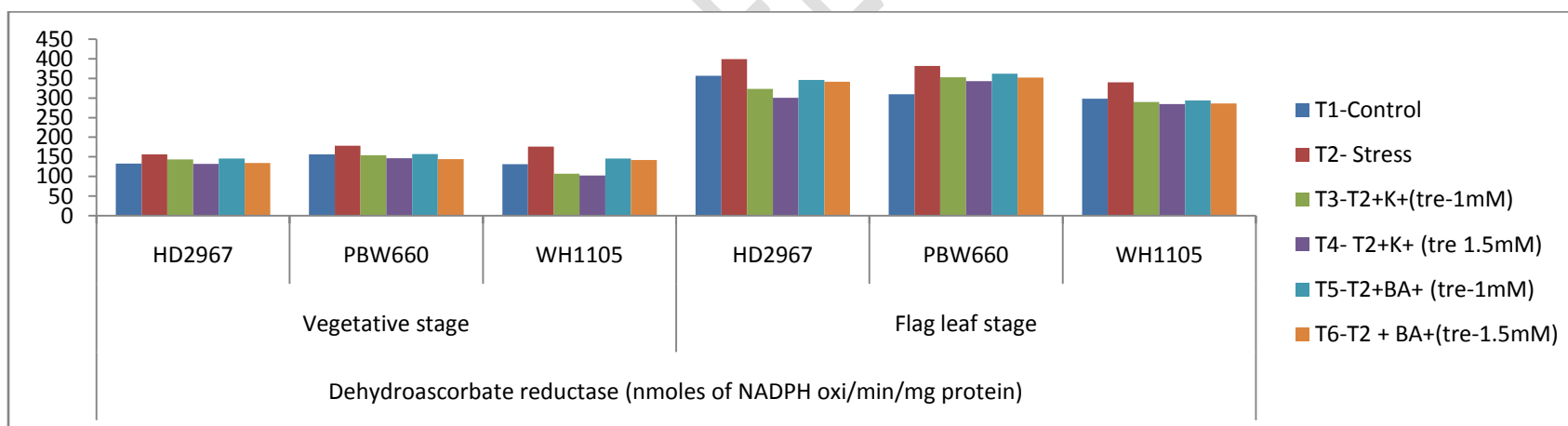


Fig 4 Effect of cytokinins (Knetin and BA) and Trehalose on dehydroascorbatereductase of wheat genotypes at vegetative and flag leaf stages under field drought stress. (A) Vegetative stage: $T = 4.321$, $G = 0.209$, $G \times T = 2.991$. (B) Flag leaf stage: $T = 3.696$, $G = 4.302$, $T \times G =$

6.606. Where, T and G corresponded to treatments and genotypes, T×G were treatments and genotypes interaction

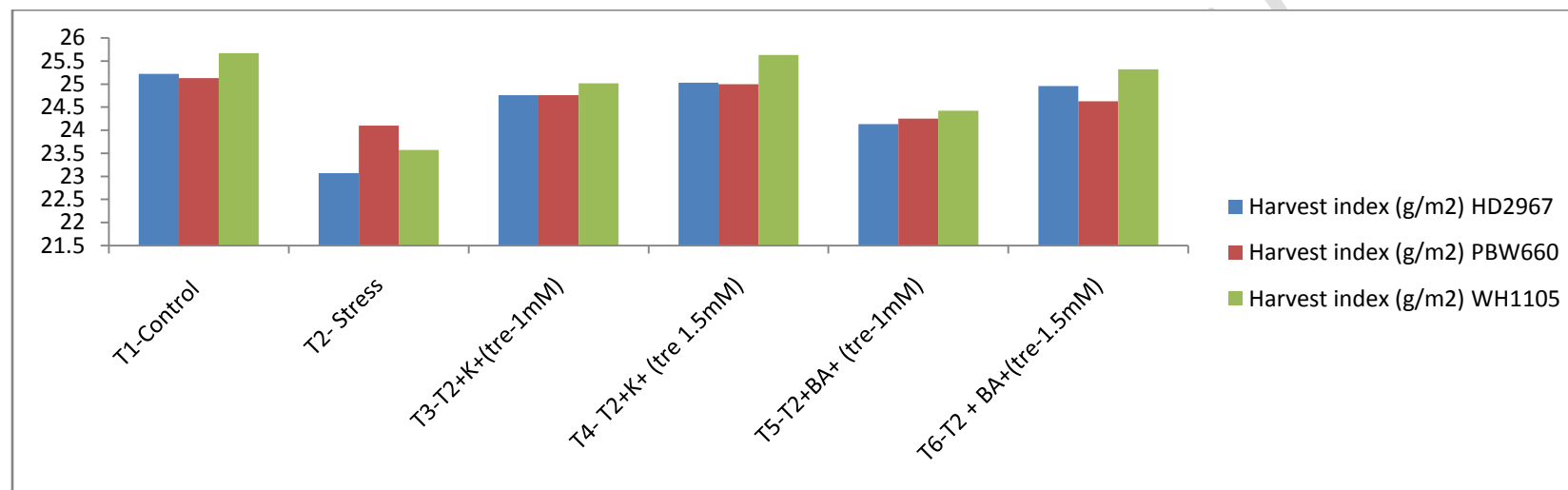


Fig 5 Effect of cytokinins (Kn and BA) and Trehalose on harvest index (g) of wheat genotypes under drought stress. $T = 1.021$, $G = 0.330$, $G \times T = 1.131$. Where, T and G corresponded to treatments and genotypes, T×G were treatments and genotypes interaction.

UNDER PEER REVIEW