Original Research Article

Induced Water Deficit Tolerance in Hexaploid Wheat Through Exogenous Application of TrehaloseandCytokinins: Water Relations and Physiological Changes

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ABSTRACT

Motive of this study was to evaluate the different physiological responsesofhexaploidgenotypes of wheat (HD2967, PBW660 and WH1105) under drought affected area and to study the effect of exogenous application of osmoprotectants like trehalose(Tre) along withcytokinins (kinetin [Kn] and Benzyl Adenine [BA]) on cell membrane function and retention of water status of wheat as affected by drought. Presently, drought stress had significant negative effect on membrane stability, cell viability, lipid peroxidation, relative leaf water content and significant increase in water saturation deficit, relative saturation deficit and quaternary ammonium compounds. Exogenous application of trehalose in combination with different concentrations (applications) of Kn and BA helps to retain the membrane stability, cell viability and reducedthemalondialdehyde content that in-turns helped in maintenance of cellular water status of plant.

Key words: drought, cell membrane stability, cell respiration,malondialdehyde content, wheat

INTRODUCTION

Wheat yield is extremelyembarrassed by various abiotic stresses such as deficiency of water and high heataffecting wheat production losses up-to 40 and 60% respectively in field (Zampieri*et al* 2017). Water scarcity is a keywarningissue in enhancing yields. To

protect themselves from drought stress, plants undergoes various modifications in their physiological and biochemical properties and the build-up of osmolytes is unique drought tolerance appliances which permits the cells to cope their dehydration and membrane structural integrity to stretch tolerance against drought and cellular dryness (Loutfy*et al* 2012). Beneath drought, plants create and gather compatible solutes such as sugars, polyols, and amino acids to aid osmotic balance, water absorption and water retention (Hussain*et al* 2018). The high relative watercontent (RWC) is an vitalmeter for water scarcity tolerance, and high RWC is the outcome of extra osmotic regulation or slight elasticity of tissue cell wall(Rad *et al*2013;Dwivedi*et al* 2016).The upsurge in the content of malonicdialdehyde (MDA) has been measured as aappropriate marker for membrane downgrading. Thepreceding study conveyed that the lessening in stability of membrane imitates the amount of lipid peroxidation caused by ROS (Sharma *et al*., 2017).

Few studies have reported exogenous application of countlesstypes of plant growth regulators and their belongings to drought and oxidative stress, drop in relative water content, and stability of membrane (Hameed*et al*., 2015).Cytokinins, both in cooperation and antagonisms with additional hormones sway several plant progresses that result in growth and development. Commonly, response of plants to cytokinins is arbitrated by their exogenous application (Aldesuquy*et al*., 2014).Trehalose, a disaccharide (non-reducing), is noteworthy as a stress protectant in several organisms and is well known for its shielding ability, constancy and little reactivity. It protects membranes and proteins from denaturation by exchanging water as it hydrogen bonds to polar residues. The aptitude of wheat plant to uphold membrane integrity under water deficit conditions regulates the ability to tolerate it. Largely, membrane stability principle is used to assess drought tolerance of the crop (Abdullah *et al*., 2011). The main objective of present study was to recognize the effect of cytokinins (Kn and BA) in combination with different concentrations of osmoprotectants like trehalose on various physiological parameters that contributes to harvest of wheat crop under water deficiency stress.

MATERIAL AND METHODS

Plant material:

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The crop was sown under two different *rabi*growing seasons at Punjab Agricultural University, Ludhiana. The trial 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(Table 1) and three wheat varieties as subplot treatments. In case of treatment sprayed plots, aqueous solution of different cytokinins along with trehalosewith 0.1 % TWEEN 20 was sprayed twice a day at vegetative stage (VS) and flag leaf stages (FLS). Control (normal irrigated) and drought stressed plots were sprayed with only water containing 0.1 % TWEEN 20 at both the stages. After 4 days of different applications, leaf samples were collected from each plot and following parameters were performed.

Membrane thermal stability

MTS was calculated as suggested by Shanahan *et al* (1990).

Triphenyltetrazolium chloride test(cell viability or cellular respiration)

TTC or Cellular viability was determined and calculated byTowill and Mazur 1974.

Lipid peroxidation/ malondialdehyde content

MDA content was estimated and calculated as per suggestion of Dhindsa and Matowe1981**.**

Relative leaf water content (RLWC),**relative saturation deficit (RSD) and water saturation deficit (WSD):**Shoot fragments of identical size were cut and directly weighed to attain fresh weight and then soaking by submerging in distilled water for 6 hour and removed. Extra water was blotted off without situating any burden on leaf and weighed to get saturated weight. Later drying at 70°C for 48 hr dry weight was determinedby according to Weatherley 1950.

Estimation of Quarternary ammonium compounds (QACs)

Total QACs were calculated by adding GB and choline content of respective samples.GB and choline was calculated by the

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method as described by Grieve and Grattan (1983)

Statistical analysis: was performed by using two way ANOVA (Cochran and Cox 1967) by CPCS1 software.

Results and Discussion:

Membrane thermal stability (MTS), Triphenyltetrazolium chloride (TTC) and Malondialdehyde content (MDA)

Membrane stability in leaves of wheat genotypes was significantly lower in stressed (drought) plots as compared to control or irrigated ones (Table 2). The decrease was more pronounced at FLS was in PBW660 (24.83%). than at VS (maximum decline was 18.05% in WH1105). Application of trehalose along with Kn and BA enhanced the stability of membrane in all genotypes both at VS and FLS. The maximum percentage increase in membrane stability over drought was in WH1105 (23.80%) at VS and 44.26% at FLS followed by PBW660 (12.44%) at VS and 43.49% at FLS with the application of Kn@40mg/L along with Tre@ 1.5mM. HD2967 had maximum membrane stability at both the stages under the irrigated or control stage.Responsible mechanisms related with water deficit tolerance and recognizingeffective screening assays associated with these are essential for stress tolerant enhancement programs in wheat (Ristic*et al*2007; Priya*et al* 2019). Water deficit circumstancesinitiated loss of water from plant tissues which extremely impaired structure as well as function of membrane that in-turn cause reduction in cell viability (Buchanan *et al*2000;Kaur and Thind 2018). Cell membrane actuality one of the first targets of plant tissues, the capability to maintain membrane integrity under water scarcity defines tolerance towards drought. The electrolyte seepage is interconnected with scarcity (water) tolerance mechanism (Abdullah *et al*2011).

All the genotypes showed significantly decreased TTC or cellular viability in the drought stressed plots as compared to the control plots at both the stages (Table 2). HD2966 (30.66%) showed maximum decrease in TTC content at VS and showed least decrease at FLS (18.65%) as compared to other genotypes. All the genotypes resulted in significant increase in cellular viability with the application of different concentrations of cytokinins and trehalose.Maximum increase in cellular viability was recorded with all the applications or treatments at the VS as compared to FLS. HD2967 (42.42%) followed by PBW660 (42.22%). The maximum increase

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in cell viability at VS with the application of Kn@40mg/Lin combination with Tre@ 1.5mM.Similarly with same application at FLS the PBW660 (25.68%) followed by WH1105 (22.64%) had more TTC content. Previous, it was perceived that the rise in exogenous cytokininapplication increase the cell viability under drought stress (Xu*et al* 2016) maybe by cytokinins-enhanced ROS scavenging through antioxidant accretion and stimulation of antioxidant enzymes.

MDA content of drought stressed plots indicated a noteworthy increase over control (Table 2). This increase was maximum at the FLS (28.38% in HD2967) as compared to VS (19.36% in HD2967). The actual content of MDA was more at VS. Application of different combinations of Kn, BA and trehalose significantly reduced the MDA content or lipid peroxidation content. Kn along with both concentrations of trehalose performed better as compared to BA along with trehalose. Exogenous,trehalosediminished MDA and electrolyte leakage. The identical results were reported previously as electrolyte leakage and MDA content declined with accumulative concentration of trehalose (Li *et al* 2014).

Relative leaf water content (RLWC), water saturation deficit (WSD) and relative saturation deficit (RSD)

Drought stress caused significant decrease in RLWC of stressed plots over the irrigated ones (Table 3). The declension in levels of RLWC under drought was greater at vegetative stage as compared to FLS in all genotypes. HD2967 (20.90%) has maximum decrease in water content at VS and PBW660 (10.39%) and showed lesser decrease in RLWC at VS whereas HD2967 (7.26%) recorded least decline in RLWC at FLS as compared to other two genotypes. Significant increase in RLWC was observed in all the genotypes with application of different treatments.Munns*et al*(2010) also reported comparableconclusions that the Relative water content diminished under the water deficit conditions in wheat.

All genotypes showed enhanced WSD and RSD under the drought stress conditions as compared to irrigated conditions (Table 3). WH1105 (31.71%) followed by PBW660 (26.05%) had more WSD at VS and HD2967 (28.48%) followed by WH1105 (27.76%) had more as compared to control at FLS. All the genotypes recorded with significant declined in status of WSD and RSD with the application of trehalose along with Kn and BA. There was 52.76% decrease in RSD at VS and 43.15% decrease in RSD at

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FLS observed in WH1105 with the application of Kn@40mg/L along with Tre@ 1.5mM. With the same application PBW660 showed 46.13% decrease in WSD at VS as compared to drought stressed plots and WH1105 observed with 18.22% decline at FLS.

Quaternary ammonium compounds (QACs)

All genotypes had lesser QAC accumulation under control conditions (Fig 1a and b) and FLS had more QAC as compared to VS was recorded. All genotypes showed significant increase in QAC under the drought stress conditions at both the stages. Kn in combination with Tre@ 1mM significantly increased QACs of all studied genotypes as compared to during drought stress at VS and significant reduction in QAC at FLS. With foliar application of Kn along with Tre@ 1.5mM all genotypes showed further increase in QACs. HD2967 showed lesser QACs at VS and WH1105 at FLS. With foliar application of BA in combination with Tre@ 1mM, PBW660 followed by WH1105 had lesser QACs accumulation at VS and WH1105 followed by PBW660 had minimum QACs at FLS. Wang *et al* (2010) &Raza*et al* (2007) recorded the GB prompted modulation of antioxidant enzyme actions in wheat under different stresses that might be due to build-up of different compounds under stress conditions.

Conclusion: Present study concluded that thefoliar application of osmoprotectanttrehalosealong with kinetin and benzyl adenine resulted in maintenance of membrane stability, cell viability and reduced lipid peroxidation under the wheat genotypes facing water deficit. Increased membrane stability resulted in increased water status of plant and adequate amount ofquaternary ammonium compounds protect the plant from drought stress. 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.

Authors' contribution

Conceptualization and designing of the research work (SKT); Execution of field/lab experiments and data collection (AK and GS); Analysis of data and interpretation (AK, SKT and AS); preparation of manuscript (SKT, AK, AS and GS).

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References :

1.Abdullah F, Hareri F, Naesan M, Ammar A and Zaherkabar O 2011. Effect of drought on different physiological characters and yield components in different varieties of Syrian Durum wheat.*J AgricSci***3(3):** 127-33.

2.Aldesuquy H, Haroun S, Abo-Hamed S and El-Saied A W 2014. Involvement of spermine and spermidine in the control of productivity and biochemical aspects of yielded grains of wheat plants irrigated with waste water. *Egypt. J. Basic ApplSci* **1:** 16–28.

3.Buchanan B B, Gruissem W and Jones R L 2000. *Biochemistry and Molecular Biology of Plants.*Amer.Soc. Plant Physiol. Rockville.

4.Cochran W G and Cox G M 1967. Experimental design. John Wiley and Sons Ltd., England.

5.Dhindsa R S and Matowe W 1981. Drought tolerance in two mosses correlated with enzymatic defense against lipid peroxidation. *J Exp Bot***32:** 79-91.

6.Dwivedi S K, Arora A and Kumar S 2016. Paclobutrazol-induced alleviation of water-deficit damage in relation to photosynthetic characteristics and expression of stress markers in contrasting wheat genotypes. — *Photosynthetica* **54:** 1–9.

7.Grieve C M and Grattan S R 1983.Rapid assay for determination of water soluble quaternary ammonium compounds.*Plant Soils* **70**: 303-07.

8.Hameed A, Gulzar S and Aziz I 2015. Effects of salinity and ascorbic acid on growth, water status and antioxidant system in a perennial halophyte.*AoB Plants***19**: 7.

9.Hussain H A, Hussain S, Khaliq A, Ashraf U, Anjum S A, Men S and Wang L 2018. Chilling and Drought Stresses in Crop Plants: Implications, Cross Talk, and Potential Management Opportunities. *Front. Plant Sci*. **9**.

10.Kaur A and Thind S K 2018. Effect of cytokinins on membrane stability and cell viability of wheat crop under PEG- induced drought condition. *J of Environ Biol***39:** 1041-46.

11.Li Z G, Luo L J and Zhu L P 2014.Involvement of trehalose in hydrogen sulphide donor sodium hydrosulfide-induced the acquisition of heat tolerance in maize (*Zea mays* L.) seedlings.*Botanical Studies***55:** 20-31.

12.Loutfy N, El-Tayeb M A, Hassanen A M, Moustafa M F M, Sakuma Y and Inouhe M 2012. Changes in the water status and osmotic solute contents in response to drought and salicylic acid treatments in four different cultivars of wheat (Triticumaestivum) *J Plant Res***125:** 173–84.

13.Munns R, James R A, Sirault X R R, Furbank R T and Jones H G 2010.New phenotyping methods for screening wheat and barley for beneficial responses to water deficit.*JExpt Bot***61**: 3499–3507.

14.Priya M, Dhanker O P, Siddique K H M *et al* 2019. Drought and heat stress-related proteins: an update about their functional relevance in imparting stress tolerance in agricultural crops. *TheorAppl Genet* **132:** 1607–38.

15.Rad M R N, Kadir M A and Rafii M Y 2013.Gene action for physiological parameters and use of relative water content (RWC) for selection of tolerant and high yield genotypes in F2 population of wheat. *Aust. J. Crop Sci*. **7:** 407–17.

16.Raza S H, Athar H R, Ashraf M and Hameed A 2007.Glycinebetaine induced modulation of antioxidant enzyme activities and ion

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accumulation in two wheat cultivars differing in salt tolerance. *EnvExp Bot***60**: 368-76.

17. Ristic Z, Bukovnik U and Prasad P V V2007.Correlation between heat stability of thylakoid membranes and loss of chlorophyll in winter wheat under heat stress.*Crop Sci*. **47:** 2067–73.

18. Shanahan J F, Edwards I B, Quick J S and Fenwick 1990. Membrane thermostability and heat tolerance of spring wheat.*Crop Sci***30:** 247-51.

19.Sharma P, Sareen S, Saini M and Shefali2017. Assessing genetic variation for heat stress tolerance in Indian bread wheat genotypes using morpho-physiological traits and molecular markers.*Plant Genet. Resour***15:** 539–47.

20. Towill L E and Mazur P 1974. Studies on the reduction of 2, 3, 5-triphenyl tetrazolium chloride assay for plant tissue culture.*Can J Bot***53:** 1097-1102.

21.Wang G P, Zhang X Y, Li F, Luo Y and Wang W 2010. Over accumulation of glycine betaine enhances tolerance to drought and heat stress in wheat leaves in the protection of photosynthesis. *Photosynthetica***48**: 117-26.

22.Weatherley P E1950 Studies in water relations of cotton plants I. The field measurement of water deficit in leaves.*New Phytol***49:** 81-87.

23. Xu Y, Burgess P, Zhang X and Huang B 2016 Enhancing cytokinin synthesis by overexpressing *ipt*alleviated drought inhibition of root growth through activatingROS-scavenging systems in *Agrostisstolonifera. J Exptl Bot* **67**: 1979-92.

24. Zampieri M, Ceglar A, Dentener F and Toreti A 2017. Wheat yield loss attributable to heat waves, drought and water excess at the global, national and subnational scales. Environ. Res. Lett. 12, 6.doi: 10.1088/1748-9326/aa723b.

25. Zeid I and Shedeed Z 2006. Response of alfalfa to putrescine treatment under drought stress.*BiologiaPlantarum***50(4):**635-40.

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Table 1. Treatments given to wheat genotypes during field experiment

Treatment No.	Application of Cytokinins in combination with Trehalose
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@1.5mM/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

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Table 2 Effect of cytokinins (Kn and BA) and Trehalose on membrane thermal stability, triphenyltetrazolium chloride and malondialdehyde content of wheat genotypes at vegetative stage and flag leaf stage under drought stress.

Treatments / Genotypes	Membrane thermal stability (%)			Triphenyltetrazolium chloride test (%)			Malondialdehyde content (mg/g FW)		
	Vegetative stage			Vegetative stage			Vegetative stage		
	HD2967	PBW660	WH1105	HD2967	PBW660	WH1105	HD2967	PBW660	WH1105
T1	89.45	84.04	86.44	88.46	86.66	82.33	121.756	120.685	126.746
T2	75.97	75.67	70.83	61.33	60.22	60.66	145.335	138.554	146.289
T3	85.89	83.09	87.09	86.74	84.58	81.47	125.567	124.431	132.235
T4	88.97	85.09	87.69	87.35	85.65	83.76	122.457	121.356	127.763
T5	82.85	82.06	82.06	84.78	82.55	79.78	133.745	126.698	136.567
T6	84.88	83.99	84.45	85.46	83.33	81.64	130.789	122.435	133.045

CD(p=0.05)	T=7.391, G=3.090, G×T=8.321			T=6.092, G=2.001, G×T=5.901			T=3.610, G=1.003, G×T=2.009		
	Flag leaf stage			Flag leaf stage			Flag leaf stage		
T1	81.09	80.66	80.08	73.33	73.56	75.22	143.896	146.876	148.956
T2	62.67	60.63	60.99	59.65	58.33	59.89	184.748	187.584	185.876
T3	86.77	85.88	86.88	71.55	72.64	72.35	162.973	166.357	168.278
T4	88.06	87.00	87.99	72.73	73.31	73.45	157.422	162.845	164.898
T5	84.67	83.83	83.56	70.67	71.57	71.26	164.559	168.711	169.654
T6	87.07	85.99	85.78	71.48	72.44	72.67	160.178	167.254	169.245
CD (p=0.05)	T=8.203, G=1.001, G×T=8.033			T=5.022, G=0.090, G×T=5.329			T=3.603, G=2.336, G×T=3.904		

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

Table 3 Effect of cytokinins (Kn and BA) and Trehalose on Relative leaf water content, water saturation deficit and relative saturation deficit of wheat genotypes at vegetative stage and flag leaf stage under drought stress.

Treatments / Genotypes	Relative leaf water content (%)			Water saturation deficit (%)			Relative saturation deficit (%)		
	Vegetative stage			Vegetative stage			Vegetative stage		
	HD2967	PBW660	WH1105	HD2967	PBW660	WH1105	HD2967	PBW660	WH1105
T1	82.45	80.33	80.13	32.36	34.89	34.87	15.29	16.19	16.87
T2	65.21	71.98	65.96	40.02	43.98	45.93	21.34	22.87	23.19
T3	81.33	80.21	80.78	30.05	31.55	31.99	15.89	16.31	16.84
T4	82.40	80.43	80.88	30.02	31.02	31.43	15.21	15.18	15.18
T5	76.98	79.87	79.99	32.18	31.98	32.97	15.67	16.88	16.72
T6	79.56	79.99	80.18	32.08	31.03	31.98	15.32	16.03	16.34
CD (p=0.05)	T=6.091, G=2.162, G×T=4.331			T=3.660, G=1.603, G×T=3.891			T=4.321, G=3.002, G×T=6.009		

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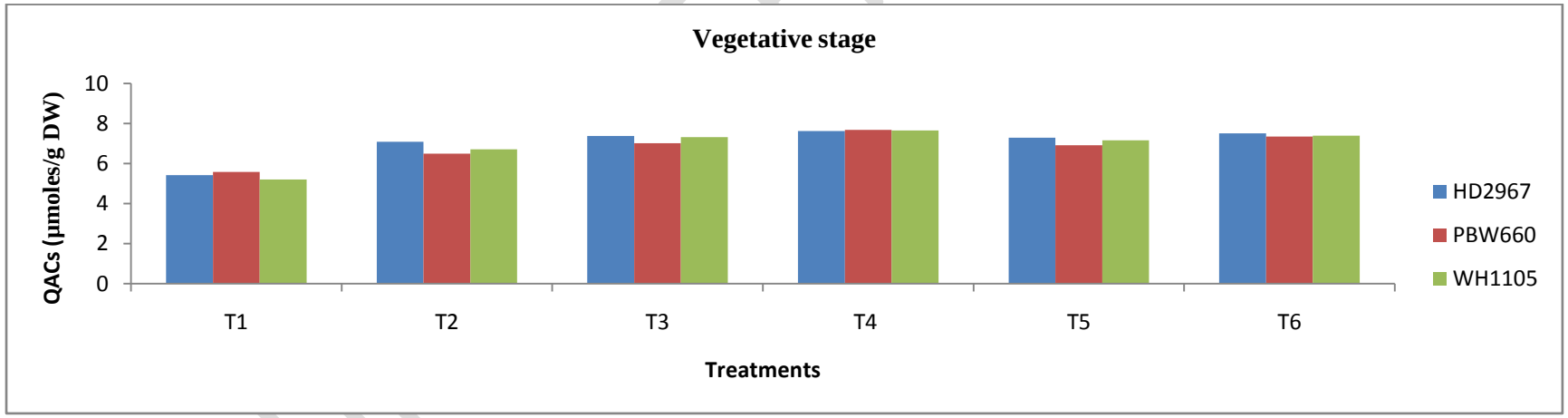
	Flag leaf stage			Flag leaf stage			Flag leaf stage		
T1	82.09	81.67	82.62	33.46	34.78	34.43	16.29	16.98	17.02
T2	76.13	73.78	73.23	42.99	42.79	43.99	23.91	23.99	24.98
T3	82.03	81.55	81.48	38.23	38.21	37.22	19.23	18.25	18.19
T4	82.06	81.65	82.26	37.56	37.86	37.21	18.25	17.96	17.45
T5	81.66	81.33	81.33	38.30	38.97	38.19	20.38	19.25	19.94
T6	81.99	81.57	81.78	37.27	38.95	38.02	19.99	19.02	19.26
CD (p=0.05)	T=4.302, G=0.962, G×T=4.906			T=2.090, G=1.300, G×T=3.360			T=3.408, G=1.406, G×T=4.396		

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

a)



b)

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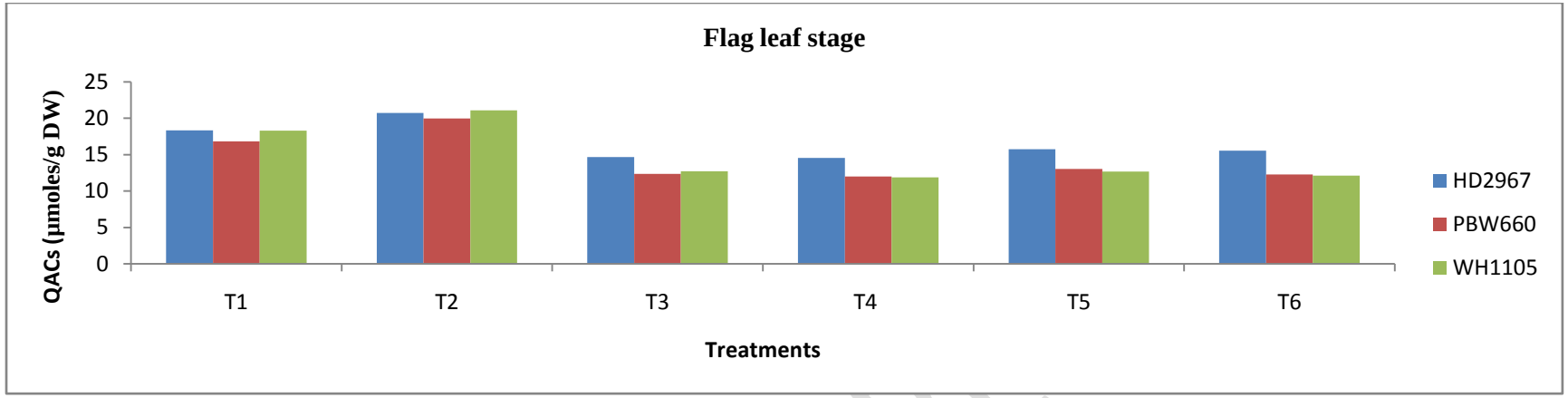

Fig 1 Effect of cytokinins (Knetin and BA) and Trehalose on Quaternary ammonium compounds of wheat genotypes at vegetative and flag leaf stages under field drought stress.

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