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Induced Water Deficit Tolerance in Hexaploid Wheat through Exogenous Application of Trehalose and Cytokinins: Water Relations and Physiological Changes

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Authors' contributions

This work was carried out in collaboration among all authors. Author SKT conceptualized and designed the research work, Authors AK and GS executed the field/lab experiments and collected the data, Authors AK, SKT and AS analyzed and interpreted the data, Authors SKT, AK, AS and GS prepared the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The purpose of this study was to evaluate the different physiological responses of hexaploid genotypes of wheat (HD2967, PBW660 and WH1105) in drought-affected areas and to study the effect of exogenous application of osmoprotectants like trehalose (Tre) along with cytokinins (Kinetin [Kn] and Benzyl Adenine [BA]) on cell membrane function and retention of water status of

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wheat as affected by drought. Presently, drought stress has a significant negative effect on membrane stability, cell viability, lipid peroxidation, relative leaf water content, and a 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 membrane stability, cell viability, and reduced malondialdehyde content, which in turn helps in the maintenance of the cellular water status of plants.

Keywords: Drought; cell membrane stability; cell respiration; malondialdehyde content; wheat.

1. INTRODUCTION

Wheat yield is extremely embarrassed by various abiotic stresses such as deficiency of water and high heat, affecting wheat production losses up to 40 and 60%, respectively, in the field [1]. Water scarcity is a key issue in enhancing yields. To protect themselves from drought stress, plants undergo various modifications in their physiological and biochemical properties, and the build-up of osmolytes is a unique drought tolerance appliance that permits the cells to cope with their dehydration and membrane structural integrity to stretch tolerance against drought and cellular dryness [2]. Under drought, plants create and gather compatible solutes such as sugars, polyols, and amino acids to aid osmotic balance, water absorption, and water retention [3]. The high relative water content (RWC) is a vital metric for water scarcity tolerance, and a high RWC is the outcome of extra osmotic regulation or slight elasticity of the tissue cell wall [4,5]. The upsurge in the content of malonicdialdehyde (MDA) has been measured as an appropriate marker for membrane degradation. The preceding study conveyed that the decrease in membrane stability imitates the amount of lipid peroxidation caused by ROS [6].

Few studies have reported exogenous application of countless types of plant growth regulators and their belongings to drought and oxidative stress, drop in relative water content, and stability of membrane [7]. 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 [8]. 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 [9]. 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.

2. MATERIALS AND METHODS

Plant material: 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 the application of cytokinins in combination with different concentrations of trehalose (Table 1) and three wheat varieties as subplot treatments. In the case of treatment-sprayed plots, an aqueous solution of different cytokinins along with trehalose with 0.1% TWEEN 20 was sprayed twice a day at the vegetative stage (VS) and flag leaf stages (FLS). Control (normally irrigated) and drought-stressed plots were sprayed with only water containing 0.1% TWEEN 20 at both stages. After 4 days of different applications, leaf samples were collected from each plot, and the following parameters were performed.

Membrane thermal stability: MTS was calculated as suggested by Shanahan et al*.,* [10].

Triphenyltetrazolium chloride test (cell viability or cellular respiration): TTC or Cellular viability was determined and calculated by Towill and Mazur [11].

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

Lipid peroxidation/ malondialdehyde content: MDA content was estimated and calculated as per suggestion of Dhindsa and Matowe [12]**.**

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 determined by according to Weatherley [13].

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 method as described by Grieve and Grattan [14].

Statistical analysis: was performed by using two way ANOVA Cochran and Cox [15] by CPCS1 software.

3. RESULTS AND DISCUSSION

3.1 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 recognizing effective screening assays associated with these are essential for stress tolerant enhancement programs in wheat [16,17]. Water deficit circumstances initiated loss of water from plant tissues which extremely impaired structure as well as function of membrane that in-turn cause reduction in cell viability [18,19]. 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 [9].

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 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 cytokinin application increase the cell viability under drought stress [20] 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, trehalose diminished MDA and electrolyte leakage. The identical results were reported previously as electrolyte leakage and MDA content declined with accumulative concentration of trehalose [21].

3.2 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*.,* [22] also reported comparable conclusions that the Relative water content diminished under the water deficit conditions in wheat. Exogenous trehalose reduced the water stress and wilt disease in drought effected tomato [23].

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 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.

3.3 Quaternary Ammonium Compounds (QACs)

All genotypes had lesser QAC accumulation under control conditions (Figs. 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*.,* [24], Raza et al*.,* [25] 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. Under drought stress conditions plants accumulate metabolites such as glycine, betaine, proline, mannitol, quaternary ammonium compounds and soluble sugars that help to protect the plant against drought [26,27].

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

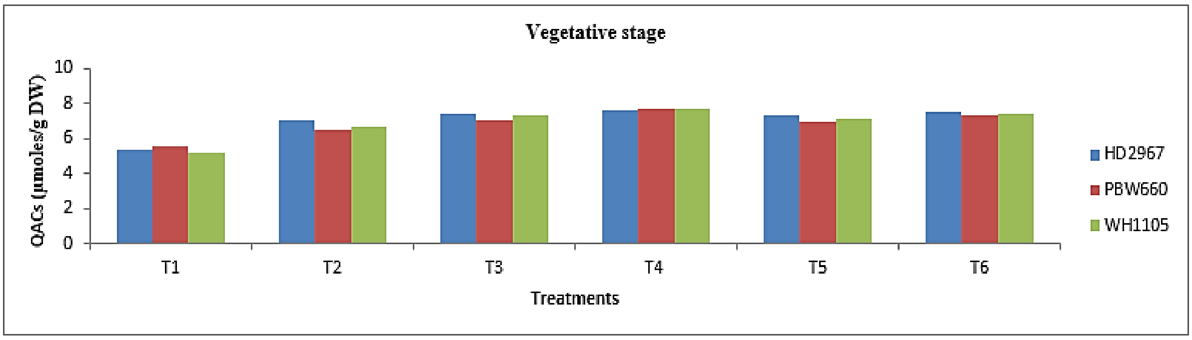
Treatments / Genotypes	Membrane thermal stability (%)			Triphenyltetrazolium chloride test (%)			Malondialdehyde content (mg/g FW)		
	Vegetative stage			**Vegetative stage**			**Vegetative stage**		
	HD2967	**PBW660**	**WH1105**	**HD2967**	**PBW660**	**WH1105**	**HD2967**	**PBW660**	**WH1105**
T1	89.45	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		
	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)

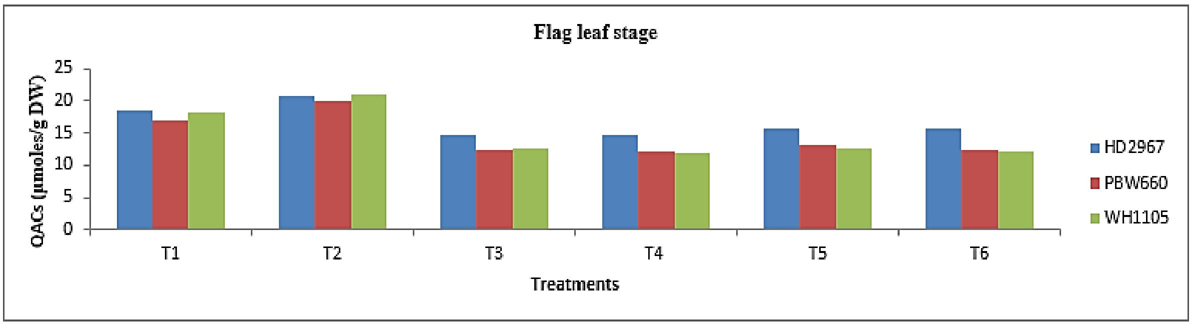


Fig. 1. Effect of cytokinins (Knetin and BA) and Trehalose on Quaternary ammonium compounds of wheat genotypes at vegetative and flag leaf stages under field drought stress

4. CONCLUSION

Present study concluded that the foliar application of osmoprotectant trehalose along 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 of quaternary 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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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