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Relative growth rate of wheat seedlings as effected by PEG-stimulated drought and various concentrations of plant growth regulators

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Abstract
Presently effect of different concentrations of trehalose (Tre@1mM and Tre@1.5mM) and Glycine betaine (GB@50 and GB@100mM) along with the cytokinins [Kinetin (Kn) @40mg/l and Benzyl adenine (BA)@50mg/L] on wheat (*Triticum aestivum* L.) seedlings under PEG-6000 caused drought stress was studied. For that four wheat genotypes *viz* HD2967, PBW660, WH1105 and PBW658 were sown in petri-plates with distilled water followed by supply of PEG-6000 on 3rd day to cause the drought of -0.4Mpa. Drought stress significantly reduced the relative growth rate (RGR) and vigour index (VI) of all studied wheat genotypes. Reduction in stability of cell membrane and cell viability was also recorded. Foliar application of trehalose and glycine betaine along with kinetin and benzyl adenine significantly increased the RGR and VI of all genotypes and increase in cell viability and stability of cell membranes was also increased. Among all the tested concentrations Kn@40mg/L along with Tre@1.5mM had more positive impact in amelioration of bad effect of PEG-induced drought stress.

Keywords: PEG-6000, wheat, relative growth rate, vigour index

Introduction
Crops respond to the abiotic stresses with various modifications on morphological, cellular, physiological, biochemical and molecular level [1]. In the last decade, lots of studies focused on the response of crops to a single stress [2]. However, several abiotic stresses usually occur concurrently and crops are always subjected to a combination of different abiotic stresses in the field [3]. Among the abiotic stresses, drought and heat stress are two critical threats to crop growth and sustainable agriculture worldwide [4].

Drought stress as a consequence of insufficient rainfall or deficient soil moisture might induce various biochemical, physiological and genetic responses in plants, which severely restricted crop growth [5]. Polyethylene glycol (PEG) compounds used to prompt osmotic stress in petri dish (*in vitro*) for plants to keep up regular water potential during the exploratory period. PEG has been utilized frequently as abiotic stress inducer in many reviews to screen drought tolerant germplasm [6].

Plants frequently amass distinctive types of good compatible solutes under troublesome conditions [7]. For the most part, they shield plant cells from stress through various courses, including detoxification of reactive oxygen species, commitment to cell osmotic alteration, security membrane stability and adjustment and stabilization of enzymes/proteins [8]. Some of these solutes likewise shield cell segments from drying out damage and are ordinarily refer to a osmoprotectants. These solutes are polyols, trehalose proline, sucrose, and quaternary ammonium mixes, for example, glycine betaine, choline O-sulfate, alaninebetaine, pipecolatebetaine and prolinebetaine [9]. Cytokinins are involved in the structural formation and maintenance of the photosynthetic apparatus, stomatal function, supply of CO_2 to carboxylation sites through the leaf mesophyll, synthesis of pigments and enzyme systems and regulation or photoreduction and carbon metabolism [10]. Therefore, a primary aim of this study is to check out whether the plant growth regulators like trehalose, glycine betaine, benzyl

adenine and kinetin helps to maintain the relative growth rate of wheat from the adverse effect of PEG- stimulated drought under lab conditions.

Material and Methods

To conduct the present experiment the seeds of four wheat genotypes *viz.* HD2967, PBW660, WH1105 and PBW658 was collected from the wheat section of Department of Plant Breeding and Genetics of Punjab Agricultural University, Ludhiana. Prior to conduct experiment the healthy seeds of all genotypes were washed with solution of mercuric chloride (0.01%) followed by three washings with normal distilled water. 20 seeds of each genotypes were sown in each petri-plate with distilled water. On 3rd day after sowing (DAS), all the petri-plates except control were supplemented with PEG-6000 to induce the drought stress of -0.4Mpa. On 5th DAS all the seedlings except control and PEG control, were sprayed with different concentrations of trehalose and glycine betaine along with best selected concentrations of cytokinins i.e Kn@40mg/L and BA@50mg/L (published elsewhere). The whole experiment was conducted in 5 replicates and following parameters was estimated:

Relative growth rate (RGR): RGR were calculated as suggested by [11].

Vigour index (VI): Vigour index of seeds were calculated as suggested by [12].

Membrane stability (MS): MS was calculated as suggested by [13].

Triphenyl tetrazolium chloride test (TTC) or cell viability: TTC Reduction Assay was estimated by method of [14].

Statistical Analysis: Analysis of variance (ANOVA), critical difference at 5% level of significance ($P< 0.05\%$) was used for the data analysis.

Results and Discussion

Relative growth rate: In all the four studied wheat genotypes, RGR decreased significantly under PEG induced drought stress as compared to control conditions (Table 1). PBW660 followed by HD2967 had maximum RGR as compared to other genotypes under control as well as under drought stress conditions. Earlier, Relative growth rate in wheat genotypes declined progressively under stress conditions [15]. The foliar application of cytokinins along with osmoprotectants like trehalose and glycine betaine significantly increased the RGR of all genotypes. With foliar spray of Kn@40mg/L along with the Tre@ 1mM, PBW660 had maximum and PBW658 had minimum RGR. All the genotypes showed maximum (less than control) RGR with application of Kn@40mg/L along with Tre @ 1.5mM as compared to other combinations tested and genotype PBW660 had maximum RGR as compared to other genotypes. Kn along with higher concentration of GB i.e GB @ 100mM had more promotary effect as compared to Kn along with lower concentration of GB i.e GB @ 50mM. BA in combination with GB significantly increased the RGR of all genotypes as compared to drought stress. With foliar application of BA in combination with GB @ 100mM, genotype PBW660 had high and PBW658 had less RGR.

Table 1: Effect of Cytokinins (Kn and BA) and Osmoprotectants (Tre and GB) on Relative growth rate (%) of wheat genotypes under PEG induced drought stress.

Treatments / Genotypes	Relative growth rate (%)			
	HD2967	**PBW660**	**WH1105**	**PBW658**
T1-Control	0.179	0.180	0.149	0.142
T2- Stress (PEG)	0.124	0.130	0.113	0.106
T3-T2+Kn+(tre-1mM)	0.155	0.157	0.140	0.138
T4- T2+Kn+ (tre 1.5mM)	0.169	0.172	0.146	0.141
T5-T2+BA+ (tre-1mM)	0.152	0.156	0.138	0.136
T6-T2 + BA+(tre-1.5mM)	0.156	0.169	0.142	0.139
T7- T2+Kn+(GB-50mM)	0.149	0.152	0.140	0.136
T8- T2+Kn+(GB-100mM)	0.153	0.159	0.142	0.139
T9-T2+BA+(GB-50mM)	0.146	0.152	0.138	0.135
T10-T2+BA+(GB-100mM)	0.150	0.158	0.139	0.136
CD at 5%	V =0.039, T = 0.106, V×T =0.986			

Where V= genotype, T= treatment and V×T= interaction between genotype and treatment.

Vigour index: All genotypes had maximum VI under control and significantly reduced under the PEG induced drought stress (Table 2). Under control conditions HD2967 had more VI as compared to other genotypes. Under PEG induced drought stress conditions genotype PBW660 had more vigour as compared to other genotypes. The different PEG concentrations reduced the daily mean germination, germination index and mean germination time in wheat [16]. Foliar application of Kn along with Trehalose significantly increased the VI of all studied genotypes as compared to drought stress conditions. Genotype HD2967 had more and PBW658 had lesser VI with foliar spray of Kn along with Tre @ 1.5mM. BA @ 50mg/L with Tre @ 1mM, significantly increased the VI of all genotypes, along with that application, genotype HD2967 had more VI as compared to other genotypes. Kn along with GB @ 50mM, significantly increased the VI of all genotypes. With foliar application of BA in combination with GB @ 100mM, genotype HD2967 had more and PBW660 had less VI.

Table 2: Effect of Cytokinins (Kn and BA) and Osmoprotectants (Tre and GB) on Vigour index (%) of wheat genotypes under PEG induced drought stress.

Treatments / Genotypes	Vigour index (%)			
	HD2967	PBW660	WH1105	PBW658
T1-Control	272.9	263.5	266.9	263.9
T2- Stress (PEG)	187.2	189.6	187.9	182.3
T3-T2+Kn+(tre-1mM)	268.9	258.4	261.3	260.5
T4- T2+Kn+ (tre 1.5mM)	272.7	263.1	263.6	261.9
T5-T2+BA+ (tre-1mM)	266.8	256.8	245.3	258.2
T6-T2 + BA+(tre-1.5mM)	271.3	260.5	258.9	260.6
T7- T2+Kn+(GB-50mM)	254.6	244.3	244.2	245.8
T8- T2+Kn+(GB-100mM)	261.7	249.8	249.5	258.3
T9-T2+BA+(GB-50mM)	244.8	238.6	237.8	235.9
T10-T2+BA+(GB-100mM)	253.2	242.6	243.7	245.2
CD at 5%	V =0.421, T = 0.256, V×T = 1.066			

Where V= genotype, T= treatment and V×T= interaction between genotype and treatment.

Membrane stability (MS): Table 3 depicted that MTS of all studied wheat genotypes that significantly decreased under PEG induced drought stress. HD2967 followed by PBW660 had maximum MS under control conditions and HD2967 followed by WH1105 had maximum MS under drought stress. Similar significant reduction (p<0.05) has been recorded in membrane stability index in response to water stress [17]. HD2967 had more and PBW658 had lesser MS with exogenously applied Kn in combination with Tre@ 1.5mM. BA in combination with Tre also tends to increase MS of all studied genotypes. Kn in combination with GB significantly increased the stability of membrane as it lowered the electrolyte leakage. HD2967 (88.7) followed by PBW660 (82.3) had more membrane stability as compared to other genotypes with foliar application of Kn along with GB @ 100mM. Similarly, BA along with GB significantly increased the MS of all studied genotypes [18]. Also found that the application of various concentrations of kinetin and benzyl adenine protects the cell membranes from negative effect of PEG-induced drought stress.

Table 3: Effect of Cytokinins (Kn and BA) and Osmoprotectants (Tre and GB) on Membrane stability (%) of wheat genotypes under PEG induced drought stress.

Treatments / Genotypes	Membrane stability (%)			
	HD2967	PBW660	WH1105	PBW658
T1-Control	90.4	86.4	81.4	78.3
T2- Stress (PEG)	69.7	63.6	67.6	61.8
T3-T2+Kn+(tre-1mM)	87.5	85.2	78.8	73.4
T4- T2+Kn+ (tre 1.5mM)	90.4	86.1	81.0	77.6
T5-T2+BA+ (tre-1mM)	78.5	78.8	78.5	72.1
T6-T2 + BA+(tre-1.5mM)	84.7	82.9	80.7	72.6
T7- T2+Kn+(GB-50mM)	86.2	79.5	78.4	72.9
T8- T2+Kn+(GB-100mM)	88.7	82.3	79.3	77.4
T9-T2+BA+(GB-50mM)	80.3	78.5	76.0	70.4
T10-T2+BA+(GB-100mM)	84.4	82.6	78.0	73.0
CD at 5%	V =1.780, T = 1.236, V×T =2.033			

Where V= genotype, T= treatment and V×T= interaction between genotype and treatment.

Triphenyl tetrazolium chloride (TTC) test or cell viability PEG induced drought stress significantly decreased the viability of cells in all presently studied wheat genotypes (Table 4). HD2967 and WH1105 had more cell viability as compared to other studied genotypes under control as well as under drought stress conditions. [19] observed that drought stress led to the decrease in viability of roots. Application of Kn along with Trehalose significantly increased the viability of cells in all genotypes as compared to drought stress conditions. Genotype WH1105 had more and PBW660 had lesser TTC with foliar spray of Kn along with Tre @ 1.5mM. BA @ 50mg/L with Tre @ 1mM, significantly increased the cell viability of all genotypes, along with that application, genotype WH1105 had more TTC as compared to other genotypes. Significant increase was recorded in cell viability with application of trehalose under the heat stressed wheat seedlings [20]. BA in combination with GB significantly increased the TTC of all genotypes as compared to drought stress. With foliar application of BA in combination with GB @ 100mM, genotype WH1105 had more and PBW660 and PBW658 had less cellular viability.

Table 4: Effect of Cytokinins (Kn and BA) and Osmoprotectants (Tre and GB) on Triphenyl tetrazolium chloride (TTC) test (%) of wheat genotypes under PEG induced drought stress.

Treatments / Genotypes	Triphenyl tetrazolium chloride (TTC) test (%)			
	HD2967	PBW660	WH1105	PBW658
T1-Control	84	79	84	79
T2- Stress (PEG)	66	64	66	58
T3-T2+Kn+(tre-1mM)	79	73	77	74
T4- T2+Kn+ (tre 1.5mM)	82	76	83	78
T5-T2+BA+ (tre-1mM)	72	70	76	74

T6-T2 + BA+(tre-1.5mM)	75	73	79	76
T7- T2+Kn+(GB-50mM)	78	70	72	72
T8- T2+Kn+(GB-100mM)	79	73	76	73
T9-T2+BA+(GB-50mM)	70	69	70	70
T10-T2+BA+(GB-100mM)	73	72	74	72
CD at 5%	V =2.030, T = 1.908, V×T =2.906			

Where V= genotype, T= treatment and V×T= interaction between genotype and treatment.

Conclusion

Drought is one of the most common abiotic stresses that severely affect the crop productivity in agricultural lands. So the aim of the present study was to check out the adverse effect of PEG induced drought stress on relative growth rate of four wheat genotypes. Presently, it was observed that the RGR, VI, membrane stability and cell viability was reduced under PEG- stimulated drought in all the genotypes. Foliar application of various plant growth regulators used in present study was able to ameliorated the negative effect of drought on seedlings and maintain the relative growth rate and vigour of seedlings by protecting the stability of cell membranes and retaining the cellular viability.

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