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Exogenous application of cytokinin (6-BAP) ameliorates the adverse effect of combined drought and high temperature stress in wheat seedling

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Abstract
Drought and high temperature stress often occur simultaneously especially in rainfed grown wheat crop causing severe yield loss in most of the wheat growing areas of the world. The simultaneous effects of these two stresses on crop performance in terms of growth and development may be quite different than the individual stress, but there are limited studies on this topic. Drought as well high temperature stress inhibits CK synthesis and accelerates CK degradation, reducing CK levels in roots and shoots. Experiments were performed in 10 days old wheat seedling under independent and combined drought and high temperature stress conditions with two contrasting set of wheat genotypes (C-306, tolerant and Ko-307, susceptible). Combined stress significantly reduced the membrane stability, RWC, CSI and photosynthetic pigments contents and increased the lipid peroxidation (TBARS contents). While exogenous application of optimize dose of 10 ppm BAP, (identified in preliminary experiments based on changes in growth and physiological traits) significant increased membrane stability index (MSI), photosynthetic pigments contents, chlorophyll stability index and RWC and other growth parameters in wheat seedlings grown under independent and combined stress conditions. However, genotypes subjected to drought, high temperature & combined drought and high temperature stress in 10 days old wheat seedlings. Overall it is concluded that, combined effect of drought + high temperature stress was more detrimental than the individual stress however, the interaction effect was hypo-additive in nature, which may be due to cross adaptation effect and further exogenous application of cytokinin (6-BAP) ameliorates the adverse effect of combined stress as well individual stress.

Keywords: Wheat, cytokinin, combined stress, Drought stress, Heat stress, Seedling

Introduction

Water-deficit often combined with high temperature stress is the main abiotic factor limiting crop-plants productivity and food-security worldwide. Drought and high temperature stress often occur simultaneously especially in rainfed grown wheat crop affecting crop growth and development and causing severe yield loss in most of the wheat growing areas of the world (Pradhan *et al*., 2012). The simultaneous effects of these two stresses on physiology and crop performance in terms of growth, development, biomass accumulation, and yield may be quite different than the individual stress, but there are limited studies on this topic (Rizhsky *et al*., 2002; Mittler *et al*., 2006) [15, 9]. These aspects are even more important when we consider future climate change scenarios where seasonal changes in temperature and drought and occurrences of extreme weather events are highly expected. Therefore, stress combinations instead of individual stresses have been recognized as realistic threats faced by plants (Suzuki *et al*., 2014; Ramegowda *et al*., 2015) [17, 13]. A common consequence of most abiotic stresses is an increased production of reactive oxygen species. Reactive oxygen species (ROS), viz., superoxide radical, hydrogen peroxide and hydroxyl radical originates from one, two or three electron transfers to dioxygen. These toxic ROS causes damage to DNA, proteins, lipids, chlorophyll and almost every other organic constituent of the living cell.

Heat stress as well as drought stress inhibits synthesis and causes degradation of CKs, and reducing CKs levels in roots and shoots, CKs known to regulate various growth and development processes, including cell division, leaf senescence, and root growth (Nishiyama *et al.*, 2011) [11]. Over expression of a gene isolated from *Agrobacterium tumefaciens* that encodes the enzyme adenine isopentenyl transferase (*ipt*), which catalyses the rate limiting step in CK biosynthesis, has demonstrated positive effects of elevated levels of CK in delaying leaf senescence and improving stress tolerances in various plant species (Rivero *et al*., 2007) [14], including heat tolerance in perennial grass species (Xu *et al*., 2009) [19]. CK have been shown to modulate leaf enzymatic antioxidant activities (POD, SOD, and CAT), activating leaf defenses to abiotic stresses (Zavaleta-Mancera *et al.*, 2007*)* [20]. CKs may retard senescence directly by scavenging or interfering with free radicals, which are proposed to be involved in this process (Miller 1992) [8]. An Attempt has been made to study the physiology of wheat seedling, subjected to independent and combined drought and high temperature stress condition and also test the possible effect of exogenous applied CK (6-BAP) in protection of physiological traits in wheat genotypes under the drought and high temperature stress conditions.

Materials and methods

The present study was performed in the Department of Botany & Plant Physiology, Faculty of Basic Sciences and Humanities, Dr. Rajendra Prasad Central agricultural University, during 2016-2017 to understand the protective role of cytokinin in wheat (*Triticum aestivum* L.) under combined drought and high temperature stress condition. Experiments were done in laboratory conditions in pertiplate with contrasting sets of wheat genotypes (C-306, relatively tolerant to combined stress and KO-307, relatively susceptible to combined stress, identified in separate experiment) and parameters measured in 10 days old wheat seedlings. Optimum dose of cytokinin (10 ppm, 6-BAP identified in separate experiment) were foliar applied. For drought/moisture stress treatments, 20 % PEG 6000 (equivalent to -0.5MPa) were used, for high temperature treatment (37±2°C), kept in incubator and combined drought and high temperature, drought stressed plant (induced by 20 % PEG 6000 application) was shifted to elevated temperature (37±2°C) in incubator. Control plants were grown under ambient/ unstressed conditions (25±2 °C). The sample size per treatment was 30 seeds in four replication and all physiological and biochemical and growth parameters were measured in 10 days old seedling. Leaf relative water content (RWC) was estimated by recording the turgid weight of fresh leaf samples by keeping in water for 4 h, followed by drying in hot air oven till constant weight was achieved (Weatherley 1950) [18]. Membrane stability index (MSI) was estimated as per Sairam *et al*., (1997) [16]. Lipid peroxidation was estimated as the thiobarbituric acid reactive substances, according to the method of Heath and Packer (1968) [5]. Chlorophyll and carotenoid content were estimated by extracting 0.05 g of the leaf material in 10 ml dimethyl sulfoxide (DMSO) (Hiscox and Israelstam, 1979) [6]. Chlorophyll stability index (CSI) was estimated using the method of Murthy and Majumder (1962) [10].

Results and discussion

The Drought, high temperature and combined stress of drought + high temperature significantly decreased leaf RWC by 11.47, 10.13 and 25.07 % in C-306 and 16.73, 12.21 & 26.67 % in KO-307, respectively, compared with the control plant (Table 1). The exogenous application of optimum dose of 6-BAP (10 ppm) significantly improved the leaf RWC in both genotypes compared to respective control. However, both genotypes (C-306 and KO-307) behaved differentially to exogenous application of 6-BAP (Table 1). The BAP (10 ppm) treated plant maintained significantly higher leaf RWC by 7.3, 6.06 & 13.12 % in C-306 and 14.08,12.31& 27.84 % in KO-307 compared to respective control under drought, high temperature and combined stress of drought + high temperature conditions. The Drought, high temperature and combined stress of drought + high temperature significantly decreased MSI by 9.24, 11.7 and 13.41 % in C-306 and 14.74, 9.92 & 25.8 % in KO-307, respectively, compared with the control plant (Table 2). The exogenous application of optimum dose of 6-BAP (10 ppm) significantly improved the MSI in both genotypes compared to respective control. The BAP (10 ppm) treated plant maintained significantly higher MSI by 4.42, 3.91 & 9.05 % in C-306 and 10.06, 7.92 & 10.98 % in KO-307 compared to respective control under drought, high temperature and combined stress of drought + high temperature conditions. The Drought, high temperature and combined stress of drought + high temperature significantly decreased total chlorophyll by 32.3, 18.79 and 39.28 % in C-306 and 51.87, 32.70 &53.48 % in KO-307, respectively, compared with the control plant (Table 3). The BAP (10 ppm) treated plant maintained significantly higher total chlorophyll by 16.11, 12.12 &20.55 % in C-306 and 21.11, 14.21&26.44 % in KO-307 compared to respective control under drought, high temperature and combined stress of drought + high temperature conditions. The Drought, high temperature and combined stress of drought + high temperature significantly decreased chlorophyll stability index by 10.64, 0.3 and 11.3% in C-306 and 12.86, 9.13&14.48 % in KO-307, respectively, compared with the control plant (Table 4). The BAP (10 ppm) treated plant maintained significantly higher chlorophyll stability index by 10.74, 8.29 & 10.57 % higher in C-306 and 16.19, 14.61 & 17.90 % in KO-307 compared to respective control under drought, high temperature and combined stress of drought + high temperature conditions. The Drought, high temperature and combined stress of drought + high temperature significantly increased lipid peroxidation by 21.77, 18.61 and 36.53% in C-306 and 45.11, 29.79 & 53.19 % in KO-307, respectively, compared with the control plant (Table 5). The BAP (10 ppm) treated plant maintained significantly decreased lipid peroxidation by 3.46, 8.89 &6.56 % higher in C-306 and 15.54, 24.3 & 16.08 % in KO-307 compared to respective control under drought, high temperature and combined stress of drought + high temperature conditions.

Water status in plants is one of the most important variables under changing ambient temperatures (Mazorra *et al.* 2002) [7]. Change in water status of leaf adversely affects physiological processes of plant (Tsukaguchi *et al.* 2003). The combined stress of drought + high temperature was more detrimental than independent stress in both genotypes, however reduction in leaf RWC was more in KO-306 compared to C-306. Tolerant cultivars had higher values of RWC indicating their greater ability to water uptake from the soil as compared to susceptible ones. Thus, an ability to maintain high RWC under stress conditions could be an adaptive feature. Our observations are in agreement with the earlier ones reporting reduction in RWC due to water stress in wheat (Agarwal *et al*., 2005) [1] and combined stress in citrus plant (Zandalinas *et al*., 2016). The BAP (10 ppm) treated plant maintained significantly higher leaf RWC both in C-306 and KO-307 compared to respective control under drought, high temperature and combined stress of drought + high temperature conditions. Similar to our result, Gupta *et al*. (2012) [4] also reported that under control and water stress condition benzyladenine (BA) played pivotal role in maintaining higher water status. It is suggested that this increase in leaf RWC as a result of in 6-BAP treatment might be due to accumulation of osmolytes (proline) which enhanced water absorption. Similar to our result, Gupta *et al*. (2012) [4] also reported that under control and water stress condition benzyladenine (BA) played pivotal role in reduction of membrane injury. It is suggested that this increase in MSI and CSI, as a result of in 6-BAP treatment might be due to increase in activity of ROS scavenging enzyme, which protected the membrane from ROS (responsible for membrane damage), which generated during stress condition the ROS (details of activity of antioxidant enzymes. The combined stress of drought + high temperature was more detrimental than independent stress in both genotypes, however increase in TBARS contents were more in KO-306 compared to C-306. Tolerant cultivars able to maintain significantly lower TBARS content compared to susceptible one. Our observations are in agreement with the earlier ones reporting increase in TBARS contents to heat stress in mungbean (Chand *et al.*, 2015) [2], water stress in wheat (Agarwal *et al*., 2005) [1] and combined stress in citrus plant (Zandalines *et al*., 2016). Our results indicated, exogenous application of optimum dose of 6-BAP (10 ppm) significantly reduced the TBARS contents (lipid peroxidation) in both genotypes. The BAP (10 ppm) treated plant maintained significantly lower TBARS contents both in C-306 and KO-307 compared to respective control, however, decrease was more in KO-307 compared to C-306. Sukumar (2014) also reported that exogenous application of CK helped in minimizing the lipid peroxidation in chickpea under high temperature stress condition. The BAP (10 ppm) treated plant maintained significantly higher pigment contents both in C-306 and KO-307 compared to respective control, however, increase was more in KO-307 comared to C-306. Similar to our result, Dwivedi *et al*. (2014) [3] reported also that exogenous application of CKs *via* foliar application helped plants maintaining the chlorophyll pigments both in tolerant and susceptible genotypes and percent increase was higher in susceptible genotypes compared to tolerant under control and water stress condition. Therefore, It is suggested that retention of pigment contents as a result of 6-BAP treatment might be due to reduction in lipid peroxidation and induction in antioxidant enzymes activity, which protected the membrane from ROS, which generated during stress condition.

Table 1: Effect of exogenous cytokinin on leaf relative water content (RWC) of 10 days old wheat seedlings under normal and stress conditions

Genotypes	Treatment	Without BAP	With BAP	% Change (±)	Mean
C-306	Control	90.53	93.42	3.192194	91.98
	Drought	80.15	86.00	7.303277	83.07
	High Temperature	81.37	86.30	6.063088	83.83
	Drought+ High Temperature	67.83	76.73	13.12039	72.28
KO-307	Control	80.00	84.00	5	82.00
	Drought	66.62	76.00	14.08556	71.31
	High Temperature	70.23	78.88	12.31134	74.56
	Drought+ High Temperature	58.67	75.00	27.84091	66.83
	Mean	74.42	82.04	11.1146	78.23
Factors	L.S.D. (P<0.05)	L.S.D. (P<0.01)	S.E.M.	Sign. F	
Treatment	1.713	2.303	0.595	**	
Genotype	1.211	1.629	0.421	*	
Cytokinie	1.211	1.629	0.421	**	

Table 2: Effect of exogenous cytokinin on membrane stability index of 10 days old wheat seedlings under normal and stress conditions

MSI (%)					
Genotypes	Treatment	Without BAP	With BAP	% Change (±)	Mean
C-306	Control	85.50	88.43	3.43	86.97
	Drought	77.60	81.03	4.42	79.32
	High Temperature	78.50	81.57	3.91	80.03
	Drought+High temperature	74.03	80.73	9.05	77.38
KO-307	Control	78.93	82.80	4.90	62.62
	Drought	67.30	74.43	10.60	50.87
	High Temperature	71.10	76.73	7.92	53.92
	Drought+High Temperature	58.57	65.00	10.98	46.52
	Mean	73.94	78.84	9.48	67.20
Factors	L.S.D. (P<0.05)	L.S.D. (P<0.01)	S.E.M.	Sign. F	
Treatment	2.321	3.120	0.806	**	
Genotype	1.641	2.206	0.570	**	
Cytokinie	1.641	2.206	0.570	**	

Table 3: Effect of exogenous cytokinin on total chlorophyll of 10 days old wheat seedlings under normal and stress conditions

Total Chlorophyll (mg g-1FW)					
Genotypes	Treatment	Without BAP	With BAP	% Change (±)	Mean
C-306	Control	2.04	2.13	4.68	2.08
	Drought	1.38	1.60	16.11	1.49
	High Temperature	1.65	1.85	12.12	1.75
	Drought+ High Temperature	1.24	1.49	20.55	1.36
KO-307	Control	1.87	1.99	6.54	1.93
	Drought	0.90	1.09	21.11	1.00
	High Temperature	1.26	1.44	14.21	1.35
	Drought+ High Temperature	0.87	1.10	26.44	0.99
	Mean	1.40	1.59	15.22	1.49
Factors	L.S.D. (P<0.05)	L.S.D. (P<0.01)	S.E.M.	Sign. F	
Treatment	0.252	0.339	0.088	**	
Genotype	0.178	0.240	0.062	**	
Cytokinie	0.178	0.240	0.062	*	

Table 4: Effect of exogenous cytokinin on chlorophyll stability index of 10 days old wheat seedlings under normal and stress conditions

CSI (%)					
Genotypes	**Treatment**	**Without BAP**	**With BAP**	**% Change (±)**	**Mean**
C-306	Control	90.27	97.67	8.20	96.97
	Drought	80.67	89.33	10.74	86.50
	High Temperature	90.00	98.00	8.29	93.75
	Drought+ High Temperature	80.07	88.53	10.57	85.50
KO-307	Control	80.33	91.00	9.54	86.17
	Drought	70.00	81.33	16.19	75.67
	High Temperature	73.00	83.67	14.61	78.33
	Drought+ High Temperature	68.70	81.00	17.90	74.85
	Mean	572.92	639.66	80.39	612.24
Factors	L.S.D. (P<0.05)	L.S.D. (P<0.01)	S.E.M.	Sign. F	
Treatment	3.562	4.789	1.236	**	
Genotype	2.519	3.386	0.874	**	
Cytokinie	2.519	3.386	0.874	**	

Table 5: Effect of exogenous cytokinin on lipid peroxidation of 10 days old wheat seedlings under normal and stress conditions

Lipid Peroxidation (TBARS g-1 FW)					
Genotypes	**treatment**	**Without BAP**	**With BAP**	**% Change (±)**	**Mean**
C-306	Control	40.80	39.53	-3.11	40.16
	Drought	49.68	47.96	-3.46	48.82
	High Temperature	48.39	44.09	-8.89	46.24
	Drought+ High Temperature	55.70	52.04	-6.56	53.87
KO-307	Control	50.54	48.58	-3.87	46.56
	Drought	73.33	61.94	-15.54	67.63
	High Temperature	65.59	49.66	-24.30	54.62
	Drought+ High Temperature	77.42	64.97	-16.08	71.19
	Mean	57.68	51.09	-10.23	53.64
Factors	L.S.D. (P<0.05)	L.S.D. (P<0.01)	S.E.M.	Sign. F	
Treatment	11.059	14.867	3.839	**	
Genotype	7.820	10.513	2.715	**	
Cytokinie	7.820	10.513	2.715	*	

Conclusions

Combined effect of drought + high temperature stress was more detrimental than the individual stress however, the interaction effect was hypo-additive in nature, which may be due to cross adaptation effect and further exogenous application of cytokinin (BAP) ameliorates the negative effects of combined stress as well individual stress.

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