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Morpho-physiological attributes of Wheat (*Triticum aestivum* L.) genotypes as influenced by brassinosteroids under heat stress

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Abstract
High temperature is major environmental factor that limits productivity of cereal crops all over the world. Wheat (*Triticum aestivum* L.), one of the main staple cereal crops, is highly sensitive to heat stress. Heat stress adversely affects the morpho-physiological parameters, viz., germination, total leaf area, total dry weight and yield at different growth stage of plant. In this investigation heat was induced at early and late sowing of two wheat genotypes, viz., HUW-468, C-306.Prior to sowing, seeds of both the wheat genotypes were hardened with 2,4 epi brassinolide (BR) @1.0 µM for 6 hours and foliar application at pre-flowering stage with a view to alleviating the deleterious effect of heat stress on morpho-physiological parameters of plants.

Keywords: Germination, total leaf area, total dry weight, yield, wheat

Introduction
The average global temperature is reported to be increasing at a rate of 0.18°C every decade (Hansen *et al*. 2012; Annual Climate Summary, 2010) [7, 3]. Future temperature and increased frequency of hot days (Pittock, 2003).To adapt new crop varieties to the future climate, we need to understand how crops respond to elevated temperatures and how tolerance to heat canbe improved (Halford, 2009) [6]. Drought, being also a very important environmental stress, severely impairs plant growth and development, limits plant production and the performance of crop plants, more than any other environmental factor (Shao *et al*. 2009; Rad *et al*. 2012) [22, 20]. As a consequence of severe climatic changes across the globe, threat of the occurrence of more frequent drought spells is predicted. Available water resources for successful crop production have been decreasing in recent years. Furthermore, in view of various climatic change models scientists suggested that in many regions of world, crop losses due to increasing water shortage will further aggravate its impacts.
Wheat (*Triticum aestivum* L.) is very sensitive to high temperature and trends in increasing growing season temperatures have already been reported for the major wheat-producing regions (Alexander *et al*. 2006; Hennessy *et al*.,2008) [1,9]. Though, heat stress affects the metabolic pathways at every stage of life of wheat finally leading to yield reduction, the effect of high temperature is particularly severe during grain filling; these losses may be up to 40% under severe stress (Wollenweber *et al.* 2003, Hays *et al*. 2007) [26, 8]. Other effects of high temperatures are decreased grain weight, early senescence, shrivelled grains, reduced starch accumulation, altered starch-lipid composition in grains, lower seed germination and loss of vigor (Balla *et al.* 2012) [4]. End-of-season or 'terminal' heat stress is also likely to increase for wheat in the near future (Mitra and Bhatia, 2008; Semenov and Halford, 2009) [14, 23]. HT has strong deleterious effects on photosynthesis which are attributed to reduced photosynthetic capacity, photosynthetic efficiency of PSII and photochemical activity associated with PSI. Pretreatment with EBR (0.1 mg L-1) remarkably alleviated HT-induced inhibition of photosynthesis which was accompanied with increased activity of antioxidant enzymes and reduced lipid peroxidation in tomato. Exogenous application of EBR was found effective for both HT tolerant and HT-sensitive ecotypes of melon (Zhang *et al*., 2013) [29]. EBR pretreatment (1.0 mg L-1) significantly improved net photosynthetic rate, stomatal conductance, stomatal limitation and water-use efficiency of both ecotypes of melon under HT stress. EBR-induced improvement in photosynthesis is also associated with up-regulation of photosynthetic pigment contents and phototchemical activity of PSI. In eggplant, exogenous application of EBR (0.05-0.2 µM) significantly minimizes HT-induced harmful levels of ROS and increases reduced ascorbate (AsA), reduced glutathione (GSH), proline, soluble sugar and soluble protein content under HT stress (Wu *et al*.,2014). Therfore, keeping EBR's role into

active consideration in different crop plants, it appeared worthwhile to mitigate and to observe the degree of intensity of negative effects of heat stress for optimum morpho-physiological gain in two genotypes of wheat through seed hardening as well as pre-sowing foliar application for effective germination, seedling establishment and morpho-physiological performance.

Materials and Methods

The experiment was conducted in the Agronomy Research farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. Wheat had been raised in field and spacing between plants to plant is 25×10cm and all physical precautions will be kept in view in order to protect the crop from the external damage. The site of the experiment was unaltered during both the years. A small laboratory experiment was conducted in view of identifying the effective concentration of brassinolide among the optimum (i.e., 0.5, 1.0, 2.5, 5.0 µM) concentrations on growth response of wheat. Finally, 1µM was taken as effective concentration for further studies. The two different genotypes of wheat viz.,: HUW - 468 (susceptible variety) and C-306(tolerant variety) and the individual and combined treatments were taken as T_1 = Control (at normal temperature and without brassinolide), T_2 = Hardened seeds(S) T_3 = Foliar spray (F),T_4 = Hardened seeds+ Foliar spray (S+F)at early and late sowing to create heat stress. The treatments were replicated three times. Long term studies were made at 65 and 85 days after germination (DAG) in order to study physio-morphological parameters.

Measurements of various morpho- physiological parameters

Germination (%)

Seven days after sowing, germination was calculated in percentage by the following formula:

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

Total leaf area (cm^2)

The leaf area of total no. of leaves plant^{-1} was measured using leaf area meter (Systronics Leaf Area Meter 211).

Total dry weight plant^{-1}(g)

The plants were harvested carefully and oven dried for killing at 105^{0}C for 1 hr followed by keeping the plant material at 65-70^{0}C upto 72 hr., weighed with the help of electronic balance(Model., ADGR -200) at every 24 hr till constant weight was obtained.

Yield (Kg)

The plants were harvested carefully and yield (kg) were obtained for both the genotypes.

Statistical analysis

Experimental data were recorded with average mean values for three replicates of each treatment and data were subjected to ANOVA for split plot design. Critical difference was taken at $p \leq 0.05$. Standard error of mean was calculated (Gomez and Gomez, 1984).

Results and Discussion

Present research was carried out to deal with effect of heat stress on morpho-physiological parameters of wheat genotypes (HUW- 468, C-306), their tolerance level and favourable effect of brassinolide for alleviating the deleterious effect of heat stress on germination, total leaf area, total dry weight and yield.

Germination (%)

Seed germination is one of the most fundamental and vital phases in the growth cycle of plants that determine plant establishment and yield of the crops. Under heat stress condition decline in germination percentage in both tolerant (C-306) and susceptible (HUW-468) genotypes was more as compared to control plants, while per cent reduction in germination percentage was more prominent in susceptible genotype. However, in absolute quantatative terms, genotype C-306 revealed relatively higher germination percentage than the HUW-468 under heat stress which is duly supported by (Sharma *et al*.,2008; Ahammed *et al*., 2012), most thoroughly the influence of brassinosteroids was described in agricultural crops. 24 – epibrassinolide @ 1 µM seed treatment accelerates germination percentage in both the genotypes, though the amount was lesser in HUW-468 under heat stress. Accelerated germination and faster growth of the primary root were witnessed in barley (*Hordeum vulgare* L.) (Kartal *et al*., 2009) [12].

Total leaf area (cm^2)

Leaf area represents a measure of plant growth which can be affected by heat. Under heat stress condition decline in both tolerant (C-306) and susceptible (HUW-468) genotypes was more as compared to control plants, while per cent reduction in leaf area per plant was more prominent in susceptible genotype. 24 - epibrassinolide @ 1 µM seed treatment and foliar spray (T_3) increases leaf area per plant (cm^2) in both the genotypes, though the amount was lesser in HUW-468 under heat stress condition in both pre-anthesis and post-anthesis. Similarly, (Prasad *et al*., 2006) stated that exposure of sorghum plants to heat stress decreased the plant height due to reduction in shoot growth. There was an increase in plant height, leaf area and fresh and dry weight of *L. chinensis* plants by the treatment of BR, which indicated that BR exerted positive effects on physiological processes and responses including cell division and differentiation and further improved the photosynthetic activity (Vriet *et al*., 2012) [25].

Total dry weight per plant (g)

Under heat stress condition decline in total dry weight per plant (g) in both tolerant (C-306) and susceptible (HUW-468) genotypes was more as compared to control plants, while per cent reduction in total dry weight per plant (g) was more prominent in susceptible genotype. 24 - epibrassinolide @ 1 µM seed treatment and foliar spray (T_3) accelerates total dry weight per plant (g) in both the genotypes, though the amount was lesser in HUW-468 under heat stress condition in both pre-anthesis and post-anthesis. These results are in accordance with (Rivero *et al.* 2001) [19], who reported that heat stress decreased the shoot dry weight of tomato and watermelon plants. The results of our study are in agreement with (Nassar, 2004) [15], who perceived the increase in plant height and fresh weight of banana by the treatment with BR.

Yield (kg)

Under heat stress condition decline in yield (kg) in both tolerant (C-306) and susceptible (HUW-468) genotypes was more as compared to control plants, while per cent reduction in yield (kg) was more prominent in susceptible genotype. 24 - epibrassinolide @ 1 µM seed treatment and foliar spray (T_3)

accelerates yield (kg) in both the genotypes, though the amount was lesser in HUW-468 under heat stress condition in both the years.. For example, in case of drought, homobrassinolide stimulates wheat yield, which is manifested by an increase in the number of grains per spike, spikes per m2 and weight of 1,000 grains (Sairam, 1994). Root application of this hormone results in an enhanced total grain yield and 100-grain weight of both salt-stressed cultivars referred to above (Ali *et al.*, 2008) [2].

The values of all growth parameters reduced under heat stress and highest increase were obtained at seed and foliar application of 24-epi brassinolide (@ 1 µM as compared to control plants. Genotype HUW-468 exhibited utmost reduction in all studied parameters than genotype C-306. Comparable findings were recorded in the present investigation under heat stress where brassinosteroids (BRs) are steroidal hormones that are essential for growth and development of plants. A large number of physiological processes and responses has been known to be altered by BRs such as cell division and differentiation, functioning of stomata, photosynthesis, respiration as well as ion transport (Divi and Krishna,2009; Vriet *et al*., 2012) [25]

Table 1: Effect of brassinolide on germination (%) of two wheat genotypes (*Triticum aestivum* L.) under heat stress at 10 DAS during 2015-16 and 2016-17

Treatments	2015-2016				2016-2017			
	Early		Late		Early		Late	
	HUW-468	C-306	HUW-468	C-306	HUW-468	C-306	HUW-468	C-306
T_0	90.00	96.00	81.67	83.33	92.67	96.33	80.67	85.00
T_1	91.67	99.67	86.67	91.67	96.33	96.67	85.00	88.33
T_2	90.00	96.00	81.67	83.33	92.67	96.33	80.67	85.00
T_3	91.67	99.67	86.67	91.67	96.33	96.67	85.00	88.33
Mean	90.83	97.83	84.20	87.50	94.50	96.50	82.80	86.70
Interactions	SEm±		CD 5%		SEm±		CD 5%	
G	0.86		2.50		1.05		3.04	
S	0.86		2.50		1.05		3.04	
T	1.22		3.53		1.49		4.30	
G X S	1.22		3.53		1.49		4.30	
GXT	1.73		5.00		2.10		6.09	
SXT	1.73		5.00		2.10		6.09	
GXSXT	2.45		7.07		2.98		8.61	

G =Genotype, T = Treatment, S= Season, DAS= Days after sowing; T_0 = Control, T_1 = Seed hardened (1 µM BL), T_2 = Foliar application (1 µM BL), T_3 = Seed & foliar both application (1 µM BL)

Table 2(a): Effect of brassinolide on total leaf area plant^{-1}(cm^2) of two wheat genotypes (*Triticum aestivum* L.) at pre-anthesis (65 DAS) and post –anthesis (85 DAS) under heat stress during 2015-16

Treatments	2015-2016 Pre-anthesis				2015-2016 Post –anthesis			
	Early		Late		Early		Late	
	HUW-468	C-306	HUW-468	C-306	HUW-468	C-306	HUW-468	C-306
T_0	45.89	48.16	42.38	47.29	56.90	57.73	53.04	55.26
T_1	49.25	50.42	44.13	49.77	59.32	60.54	56.70	57.99
T_2	47.92	48.83	44.25	48.06	57.66	58.37	55.41	56.28
T_3	51.43	53.43	49.96	51.05	61.60	62.04	60.32	60.52
Mean	48.62	50.21	45.18	49.05	58.87	59.67	56.37	57.76
Interactions	SEm±				CD 5%			
G	0.212				0.601			
S	0.300				0.850			
T	0.300				0.850			
G X S	0.425				1.202			
GXT	0.425				1.202			
SXT	0.601				1.700			
GXSXT	0.850				2.404			

G =Genotype, T = Treatment, S= Season, DAS= Days after sowing; T_0 = Control, T_1 = Seed hardened (1 µM BL), T_2 = Foliar application (1 µM BL), T_3 = Seed & foliar both application (1 µM BL)

Table 2(b): Effect of brassinolide on total leaf area plant^{-1}(cm^2) of two wheat genotypes (*Triticum aestivum* L.) at pre-anthesis (65 DAS) and post –anthesis (85 DAS) under heat stress during 2016-17

Treatments	2016-2017 Pre-anthesis				2016-2017 Post –anthesis			
	Early		Late		Early		Late	
	HUW-468	C-306	HUW-468	C-306	HUW-468	C-306	HUW-468	C-306
T_0	46.62	48.81	42.61	47.38	47.13	50.04	44.43	48.36
T_1	49.53	54.69	45.16	49.18	50.06	54.81	47.03	49.20
T_2	48.40	51.51	43.41	48.37	49.80	51.17	45.88	48.33
T_3	50.42	56.40	47.93	51.48	51.78	56.78	48.75	51.91
Mean	48.75	52.86	44.78	49.11	49.70	53.21	46.53	49.45
Interactions	SEm±				CD 5%			
G	0.171				0.483			
S	0.242				0.684			

T	0.242	0.684
G X S	0.342	0.967
GXT	0.342	0.967
SXT	0.484	1.368
GXSXT	0.684	1.935

G =Genotype, T = Treatment, S= Season, DAS= Days after sowing; T_0 = Control, T_1 = Seed hardened (1 µM BL), T_2 = Foliar application (1 µM BL), T_3 = Seed & foliar both application (1 µM BL)

Table 3(a): Effect of brassinolide on total dry weight per plant (g) of two wheat genotypes (*Triticum aestivum* L.) at pre-anthesis (65 DAS) and post –anthesis (85 DAS) under heat stress during 2015-16

Treatments	2015-2016 Pre-anthesis				2015-2016 Post –anthesis			
	Early		Late		Early		Late	
	HUW-468	C-306	HUW-468	C-306	HUW-468	C-306	HUW-468	C-306
T_0	12.50	12.83	11.67	12.50	13.00	13.50	12.17	12.83
T_1	13.00	13.17	12.83	13.17	13.50	14.00	12.83	13.83
T_2	12.67	13.00	12.16	13.07	13.33	13.50	12.53	13.50
T_3	13.50	14.00	13.50	14.17	14.07	14.17	13.83	14.33
Mean	12.91	13.25	12.54	13.22	13.47	13.79	12.84	13.62
Interactions	SEm±				CD 5%			
G	0.064				0.183			
S	0.091				0.259			
T	0.091				0.259			
G X S	0.129				0.367			
GXT	0.129				0.367			
SXT	0.183				0.518			
GXSXT	0.259				0.733			

G =Genotype, T = Treatment, S= Season, DAS= Days after sowing; T_0 = Control, T_1 = Seed hardened (1 µM BL), T_2 = Foliar application (1 µM BL), T_3 = Seed & foliar both application (1 µM BL)

Table 3(b): Effect of brassinolide on total dry weight per plant (g) of two wheat genotypes (*Triticum aestivum* L.) at pre-anthesis (65 DAS) and post –anthesis (85 DAS) under heat stress during 2016-17

Treatments	2016-2017 Pre-anthesis				2016-2017 Post –anthesis			
	Early		Late		Early		Late	
	HUW-468	C-306	HUW-468	C-306	HUW-468	C-306	HUW-468	C-306
T_0	12.67	12.83	11.67	12.50	13.00	13.50	12.17	12.83
T_1	13.03	13.17	12.17	13.17	13.80	14.00	13.33	13.83
T_2	12.77	13.00	12.03	13.07	13.50	13.60	12.83	13.50
T_3	13.40	14.00	13.50	14.17	14.17	14.27	13.53	14.33
Mean	12.96	13.25	12.34	13.22	13.61	13.84	12.97	13.62
Interactions	SEm±				CD 5%			
G	0.064				0.183			
S	0.091				0.259			
T	0.091				0.259			
G X S	0.129				0.367			
GXT	0.129				0.367			
SXT	0.183				0.518			
GXSXT	0.259				0.733			

G =Genotype, T = Treatment, S= Season, DAS= Days after sowing; T_0 = Control, T_1 = Seed hardened (1 µM BL), T_2 = Foliar application (1 µM BL), T_3 = Seed & foliar both application (1 µM BL)

Table 4: Effect of brassinolide on yield (kg) of two wheat genotypes (*Triticum aestivum* L.) under heat stress during 2015-16 and 2016-17

Treatments	2015-2016				2016-2017			
	Early		Late		Early		Late	
	HUW-468	C-306	HUW-468	C-306	HUW-468	C-306	HUW-468	C-306
T_0	4.23	4.70	3.20	4.10	4.30	4.57	3.30	4.20
T_1	5.30	5.50	4.00	4.47	5.45	5.50	4.00	4.40
T_2	5.00	5.35	3.50	4.00	5.00	5.30	3.50	4.00
T_3	6.03	6.50	5.40	5.17	6.30	6.43	5.23	5.47
Mean	5.15	5.51	4.02	4.43	5.26	5.45	4.00	4.51
Interactions	SEm±		CD 5%		SEm±		CD 5%	
G	0.021		0.061		0.030		0.088	
S	0.021		0.061		0.030		0.088	
T	0.029		0.086		0.043		0.125	
G X S	0.029		0.086		0.043		0.125	
GXT	0.042		0.122		0.061		0.176	
SXT	0.042		0.122		0.061		0.176	
GXSXT	0.059		0.173		0.086		0.250	

G =Genotype, T = Treatment, S= Season, DAS= Days after sowing; T_0 = Control, T_1 = Seed hardened (1 µM BL), T_2 = Foliar application (1 µM BL), T_3 = Seed & foliar both application (1 µM BL)

Conclusion

From this present study, it is possible to conclude that application of 24- epibrassinolide mitigates the adverse effect of heat stress. It plays an important role in the protection of wheat crop against heat stress by increasing germination percentage, total leaf area, total dry weight and yield with 24-epibrassinolide@1µM in combination with heat stressed conditions in both HUW-468 and C-306 genotypes. Seed treatment as well foliar application together shows synergistic effect on growth parameters.

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