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Application of Biostimulants Ameliorates Terminal Heat Stress in Lentil (*Lens culinaris* Medik.)

Rahul Nandkishor Ingle [a] and Kavita [a*]

[a] *Department of Botany, Plant Physiology and Biochemistry, College of Basic Sciences and Humanities, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur – 848125, Bihar, India.*

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Lentil is a second major winter sown legume after chickpea. Due to climate change, lentil crop sustains heat stress at various phases of growth mainly at flowering and pod filling, which causes major yield loss. To reduce the adverse effects of terminal heat stress, a pot experiment was carried out in Pusa (Samastipur), Bihar during 2021-22 with the objective to study the response of biostimulants on antioxidant system and yield of lentil grown under late sowing date vis-à-vis normal sowing date. Two genotypes *viz.*, IPL 220 and KLS 218 were sown in pots with two sowing dates *i.e.,* normal (control) and late sown (to expose plants to terminal heat stress) in completely randomized design with three replications. Experiments comprised of eight treatments having various combination of seaweed extract (SWE) and humic acid (HA) applied as seed priming and/or foliar spray at 40 and 60 Days after sowing. Results showed that lipid peroxidation and activities of antioxidative enzymes decreased by the application of humic acid and seaweed extract as seed priming and foliar application. Seed priming + foliar application (40+60 DAS) with SWE followed by

**Corresponding author: E-mail: kavitaphysiology72@gmail.com;*

seed priming + foliar application (40+60 DAS) with HA was found significantly superior in reducing the adverse effects of terminal heat stress in lentil. Hence, it is concluded that application of humic acid and seaweed extract with the combination of seed priming or foliar spray helps in ameliorating terminal heat stress in late sown crop of lentil.

Keywords: Lentil; heat stress; humic acid; sea weed extract; antioxidant system.

1. INTRODUCTION

Lentil (*Lens culinaris* Medik.) is cultivated as a winter-season food legume. It is highly susceptible to heat stress [1]. It requires lower temperatures during vegetative growth and comparatively warmer temperatures at maturity. Temperature between 18 to 30°C is considered as the optimum temperature [2]. Lentil is cultivated in comparatively warmer parts of central and southern India, where supra-optimal temperatures, particularly at the time of reproductive stage, significantly inhibit its yield.

Heat stress is one of the most ominous abiotic factors which limit the productivity and quality thereby resulting in huge economic losses [3]. High temperature affects the morphological, anatomical and physiological and biochemical changes in plant system. Global climate change has shortened the cold period and lengthened the heat periods due to which lentil crop is subjected to heat stress at various phases of growth, especially flowering and pod filling which causes major economic loss. Temperature above 32°C can retard metabolic pathways, photosynthesis, respiration rate and electron flow [4] which causes flower abortion, infertile pollen and reduced number of pods [5,1]. Reactive oxygen species (ROS) accumulate in the plant cells under environmental stress condition and are inactivated by antioxidant enzymes and by biological antioxidants that are small organic compounds or peptides. Cytokinins, a class of phytohormones, also function as antioxidants and have been shown to improve drought resistance [6]. Natural products, which contain phytohormones or exhibit hormone-like activity, have received increasing attention for use as nutrient supplements in agriculture and horticulture [7,8]. Zhang and Ervin [9] have reported that both humic acids (HA) and seaweed extract (SWE) contain substantial cytokinin, mainly zeatin riboside. SWE and HA are in common use as major components in turfgrass and ornamental biostimulant formulations. According to reports [9-12], HA and SWE both contain osmo-protective compounds including polyamines and betaines as well as growth-promoting phytohormones like auxins and cytokinins. Therefore, present study was conducted with the hypothesis that seed priming and foliar spray or a combination of biostimulants like HA and SWE may help to increase terminal heat stress tolerance in lentil crop.

2. MATERIALS AND METHODS

2.1 Plant Material and Experimental Conditions

Two genotypes (IPL 220 and KLS 218) of lentil (*Lens culinaris* Medik.) obtained from the Department of Genetics and Plant Breeding, Tihrut College of Agriculture, Dholi, Muzaffarpur were taken for the study. The experiment was laid out in pots following a complete randomized block design with three replications at College of Basic Sciences and Humanities, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur during *rabi* 2021-22. Experiments comprised of eight treatments *viz.,* T_1-Control, T_2-High temperature stress (HT), T_3-HT+ Seed priming (SP) with humic acid (HA), T_4-HT+ Seed priming with Sea Weed Extract (SWE), T_5-HT + Foliar spray(FS) with SWE at 40 DAS and 60 DAS, T_6 - HT + Seed priming + Foliar spray with HA at 40 DAS and 60 DAS, T_7-HT + Foliar spray with HA at 40 DAS and 60 DAS, T_8 - HT + Seed priming + Foliar spray with SWE at 40 DAS and 60 DAS. We used Sagarika™ IFFCO make seaweed extract containing 28% seaweed extract (red and brown algae) and iAgriFarm™ Humic Acid for the experiment. For seed priming, seeds were soaked in 3% HA and SWE for 8 hrs and air-dried and sown in pots. Both HA and SWE were applied as foliar spray at the rate of 3 mL L^{-1}.

2.2 Relative Water Content and Membrane Stability Index

Leaf relative water content was estimated by the method given by Barrs et al. [13]. The cell membrane stability index was estimated by the method as described by Sairam et al. [14].

2.3 Sampling and Assay for Enzymes Activity, Lipid Peroxidation and Proline Content

Leaf samples were taken at 70 DAS during flowering stage. Assay was done for lipid peroxidation, peroxidase enzyme activity, catalase enzyme activity, superoxide dismutase activity, and content of proline. For the extraction of enzyme, 0.5 g of fresh leaf sample was triturated in 3 ml of pre-chilled extraction buffer (0.1 M phosphate buffer, pH 6.7) followed by centrifugation at 10000 × g for ten minutes. The supernatant was collected and used for the assays of enzymatic activities. All steps in the preparation of the enzyme extract were carried out at 4 °C.

The amount of lipid peroxidation was determined in terms of malondialdehyde (MDA) content, a product of lipid peroxidation measured by thiobarbituric acid reaction [15]. Peroxidase activity was determined spectrophotometrically using the method of Amako et al. [16] and Salama et al. [17]. Catalase activity was determined by consumption of H_2O_2 using the method of Dhindsa et al. [18]. Superoxide dismutase (SOD) activity was measured following photochemical method based on the reduction of NBT (nitroblue tetrazolium) according to Giannopolitis and Ries [19]. Proline content was determined in fresh leaf material, according to Bates et al. [20]. Extraction of proline was done by homogenizing 0.1 g of leaf sample in 10 ml of 3% sulpho salicylic acid. The reaction mixture was centrifuged for 10 minutes at 10000 × g. Supernatant was collected for the estimation of proline. The quantity of proline was calculated using standard curve which was prepared by taking 10-50 µg proline from the stock solution (10 mg/ 100 mL) of L-Proline dissolved in water.

2.4 Statistical Analysis

Data of three separate replications were reported as the mean ± SD. The data were subjected to analysis of variance (ANOVA) using statistical computing software. The F value, least significant differences (LSD) between means at 5% level of significance (P= 0.05) and the standard error (SE) of means were calculated. Microsoft Excel program was used to present the figures.

3. RESULTS AND DISCUSSION

3.1 Relative Water Content

Relative water content (RWC) showed a considerable decline under high temperature stress condition (65.44%) over control (84.98 %) (Table 1). Biostimulants application caused increase in RWC under high temperature stress condition. The maximum increase in RWC was observed in case of treatment SP + FS with SWE (40+60) DAS (82.63%) followed by FS with SWE (40+60) DAS (76.58%) which was at par with SP with SWE (75.41%), SP + FS with HA (40+60) DAS (75.01%) when compared to stress condition (65.44%). Biostimulant (HA and SWE) treated lentil plant prevented water loss under the heat stress condition and showed increased RWC because compatible solutes like proline is produced in cell [21], which is essential for osmoregulation and can decrease the loss of water. Decrease in RWC was also reported in mustard under terminal heat stress by Kavita and Pandey [22].

3.2 Membrane Stability Index

High temperature stress decreased the MSI under stress condition (63.06%) over control (94.19%). Treatment SP + FS with SWE (40 +60) DAS was the most efficient in improving MSI under high temperature stress condition *i.e.* 80.94%, followed by SP+FS with HA (40+60) DAS *i.e* 74.62 % (Table 1). Nardi et al. [23] reported that humic acid stimulates the production of hormone cytokinins in plants which increase the ability of plants to abiotic stress tolerance by inhibiting the oxidation of unsaturated fatty acids [24]. Botanical extracts are good source of glycine-betaine [25], which helps in ionic regulation in cell membrane, maintaining membrane integrity in heat stress condition [26].

3.3 Lipid Peroxidation

Among all the treatments, least MDA content was recorded in control (2.88 n mol MDA mg^{-1} fresh weight) followed by SP + FS with SWE (40 +60) DAS under stress condition (3.91 n mol MDA mg^{-1} fresh weight) (Table 2). Lipid peroxidation in plant is a reliable indicator of heat stress tolerance. Biostimulant application significantly decreased the lipid peroxidation [25] in rice plant under heat stress condition. SWE are rich in glycine betaine [26] which improve cell integrity, protein synthesis and ionic regulation of cell [27]. Humic acid stimulates the production of hormone cytokinins in plants [23] which enhances the ability of plants to tolerate abiotic stress condition by inhibiting the oxidation of unsaturated fatty acids.

Table 1. Effect of humic acid and seaweed extract application on relative water content and membrane stability index in leaves of lentil crop under terminal heat stress conditions

Treatments (T)	Relative water content (%)			Membrane stability index(%)		
	Genotypes (G)		Mean	Genotypes (G)		Mean
	IPL 220	KLS 218		IPL 220	KLS 218	
Control	84.95	85.02	84.98	94.90	93.47	94.19
HT	67.67	63.21	65.44	63.91	62.22	63.06
HT+SP with HA	73.53	73.34	73.43	73.30	71.25	72.27
HT+SP with SWE	75.49	75.34	75.41	67.15	74.99	71.07
HT+FS with HA (40+60) DAS	72.46	72.39	72.42	72.94	71.98	72.46
HT+FS with SWE (40+60) DAS	78.88	74.29	76.58	75.02	70.37	72.70
HT+SP +FS with HA (40+60) DAS	74.20	75.82	75.01	73.64	75.60	74.62
HT+SP +FS with SWE (40 +60) DAS	84.52	80.743	82.63	81.97	79.91	80.94
	LSD (p=0.05)	SEm±		LSD (p=0.05)	SEm±	
G	NS	0.52		NS	0.43	
T	3.03	1.04		2.50	0.86	
G×T	NS	1.47		3.54	1.22	

Control (Normal sown and without biostimulant treatment), HA: Humic Acid, SWE: Seaweed extract, HT: High temperature stress, SP: Seed priming, FS: Foliar spray, DAS: Days after sowing

Table 2. Effect of humic acid and seaweed extract application on lipid peroxidation and peroxidase in leaves of lentil crop under terminal heat stress conditions

Treatments (T)	Lipid peroxidation (n molMDA mg^{-1} fresh weight)			Peroxidase activity (units mg^{-1} fresh weight)		
	Genotypes (G)		Mean	Genotypes (G)		Mean
	IPL 220	KLS 218		IPL 220	KLS 218	
Control	3.00	2.75	2.88	2.78	2.55	2.67
HT	5.44	5.48	5.46	5.68	6.33	6.00
HT+SP with HA	5.26	5.04	5.15	5.81	6.31	6.06
HT+SP with SWE	4.98	4.66	4.82	5.09	4.46	4.78
HT+FS with HA (40+60) DAS	4.68	5.06	4.87	3.93	3.86	3.89
HT+FS with SWE (40+60) DAS	4.72	5.03	4.88	4.05	4.38	4.22
HT+SP +FS with HA (40+60) DAS	4.80	4.73	4.77	3.18	3.18	3.18
HT+SP +FS with SWE (40 +60) DAS	3.95	3.87	3.91	3.14	3.09	3.11
	LSD (p=0.05)	SEm±		LSD (p=0.05)	SEm±	
G	NS	0.04		NS	0.10	
T	0.24	0.08		0.56	0.19	
G×T	0.34	0.12		0.80	0.28	

Control (Normal sown and without biostimulant treatment), HA: Humic Acid, SWE: Seaweed extract, HT: High temperature stress, SP: Seed priming, FS: Foliar spray, DAS: Days after sowing

3.4 Peroxidase Activity

High temperature stress resulted in increase of peroxidase activity (6.00 units mg^{-1} fresh weight) over control (2.67 units mg^{-1} fresh weight) which was at par with SP with HA (6.06 units mg^{-1} fresh weight) and then decreased in treatment SP with SWE (4.78 units mg^{-1} fresh weight) (Table 2). POD activity is to prevent the cell from damage. HA treated plant increased the activity of peroxidase for scavenging ROS free radical like hydrogen peroxide in plant. Anjos Neto et al. [28] reported that the H_2O_2 content was significantly reduced in heat stress condition in SWE treated spinach plant due to higher synthesis of peroxidase enzyme or antioxidant enzymes over the control condition. Repke et al. [29] also found increase in POD activity in case of SWE treatment in soyabean plant exposed to heat stress.

3.5 Catalase Activity

Data revealed that there was higher activity in stress condition (64.89 µ mol. H_2O_2 $min^{-1}g^{-1}$ fresh weight) over control (42.11 µ mol. H_2O_2 $min^{-1}g^{-1}$ fresh weight) which then decreased notably in an order of different biostimulants application *viz.*, SP with HA (61,53 µ mol. H_2O_2 $min^{-1}g^{-1}$ fresh weight), SP with SWE (56.23 µ mol. H_2O_2 $min^{-1}g^{-1}$ fresh weight) (Table 3). The best treatment was SP + FS with SWE (40+60) DAS which had catalase activity 46.72 µ mol. H_2O_2 $min^{-1}g^{-1}$ fresh weight. Repke et al. [29] also reported increase in CAT activity in SWE treated soyabean plant in heat stress condition.

3.6 Superoxide Dismutase Activity

An enhanced specific activity of SOD was observed when high temperature stress was imposed (43.06 units mg^{-1} protein) over control (71.25 units mg^{-1} protein). It was evident that application of biostimulants further decreased the activity of SOD in the order SP with HA (70.89 units mg^{-1} protein) followed by FS with HA (61.56 units mg^{-1} protein) (Table 3). Treatment SP + FS with SWE (40 +60) DAS had least SOD activity (49.53 units mg^{-1} protein) after control followed by FS with SWE (40 +60) DAS (58.95 units mg^{-1} protein). HA and SWE treated lentil increased SOD activity significantly over the control condition. Repke et al. [29] found that with SWE treatment SOD activity increased in soyabean plants affected by heat stress. SOD dismutates the superoxide anion to create H_2O_2, reducing the damage caused by ROS by breaking down superoxide radicals generated by oxidative stress.

Table 3. Effect of humic acid and seaweed extract application on catalase and superoxide dismutase activity in leaves of lentil crop under terminal heat stress conditions

Treatments (T)	Catalase activity (µ mol.H_2O_2 $min^{-1}g^{-1}$ fresh weight)			Superoxide dismutaseactivity (units mg^{-1} protein)		
	Genotypes (G)		Mean	Genotypes (G)		Mean
	IPL 220	KLS 218		IPL 220	KLS 218	
Control	42.11	41.57	42.11	42.89	43.23	43.06
HT	64.89	64.10	64.89	70.78	71.72	71.25
HT+SP with HA	61.53	63.09	61.53	70.75	71.03	70.89
HT+SP with SWE	56.23	61.89	56.23	58.19	58.67	58.43
HT+FS with HA (40+60) DAS	53.09	51.12	53.09	62.63	60.50	61.56
HT+FS with SWE (40+60) DAS	50.21	51.13	50.21	57.94	59.96	58.95
HT+SP +FS with HA (40+60) DAS	48.75	54.04	48.75	58.75	63.88	61.32
HT+SP +FS with SWE (40 +60) DAS	46.72	50.78	46.72	47.89	51.17	49.53
	LSD (p=0.05)	SEm±		LSD (p=0.05)	SEm±	
G	1.46	0.50		NS	0.54	
T	2.91	1.01		3.11	1.08	
G×T	NS	1.42		NS	1.52	

Control (Normal sown and without biostimulant treatment), HA: Humic Acid, SWE: Seaweed extract, HT: High temperature stress, SP: Seed priming, FS: Foliar spray, DAS: Days after sowing

Table 4. Effect of humic acid and seaweed extract application on proline content in leaves, and seed yield per plant of lentil crop under terminal heat stress conditions

Treatments (T)	Proline content (μ mol g^{-1} fresh weight)			Seed yield per plant (g)		
	Genotypes (G)		Mean	Genotypes (G)		Mean
	IPL 220	KLS 218		IPL 220	KLS 218	
Control	189.42	171.47	180.44	3.385	3.383	3.384
HT	237.80	233.65	235.73	1.498	1.469	1.484
HT+SP with HA	212.38	210.53	211.46	1.607	1.953	1.780
HT+SP with SWE	196.90	188.87	192.89	1.59	1.578	1.584
HT+FS with HA (40+60) DAS	191.25	197.39	194.32	1.575	1.580	1.578
HT+FS with SWE (40+60) DAS	191.54	192.03	191.79	1.585	1.796	1.691
HT+SP +FS with HA (40+60) DAS	191.62	176.67	184.15	2.894	2.279	2.587
HT+SP +FS with SWE (40 +60) DAS	190.59	174.21	182.40	2.985	2.590	2.787
	LSD (p=0.05)	SEm±		LSD (p=0.05)	SEm±	
G	2.19	0.76		NS	0.078	
T	4.38	1.52		0.451	0.156	
G×T	6.19	2.14		NS	0.220	

Control (Normal sown and without biostimulant treatment), HA: Humic Acid, SWE: Seaweed extract, HT: High temperature stress, SP: Seed priming, FS: Foliar spray, DAS: Days after sowing

3.7 Proline Content

Proline content increased significantly from 180.44 μ mol g^{-1} fresh weight in control condition to 235.73 μ mol g^{-1} in response to terminal heat stress (Table 4). Similar result of increased proline content was reported by Kavita and Sowmya [30] in chickpea under saline condition and Kavita and Mohan [31] in mustard under drought stress condition. Application of biostimulants increased proline content, the maximum was in the treatment SP with HA (211.46 μ mol g^{-1} fresh weight). Nair et al. [32] found that application of *A. nodosum* extract increased the proline synthesis in *Arabidopsis* under freezing stress. They reported that lipophilic components of SWE changes proline concentration by regulating the expression of genes related to its biosynthesis and degradation (*viz.* P5CS1, P5CS2 and ProDH, respectively).

3.8 Seed Yield per Plant

Seed yield per plant significantly decreased under high temperature stress condition (1.484 g) over control (3,384 g). Biostimulant application notably enhanced seed yield per plant under the treatment of SP + FS with SWE (40 +60) DAS (2.787 g) which was at par with the treatment SP + FS with HA (40+60) DAS (2.587 g) (Table 4). SWE improved yield per plant of lentil in heat stress condition which is in tune with the findings of Repke et al. [29] who have reported attenuating effect of the biostimulant based on seaweed extract yield attributes of soybean during the period of heat stress. This might be due to the maintenance of photosynthesis and antioxidative defence system that allowed continuity of soybean plant development under stressful conditions. Jan et al. [33] observed that foliar application of HA significantly enhanced yield per plant of chilli which might be attributed to increase in fruit set, number of fruit per branch and number of fruit per plant [12].

4. CONCLUSION

Based on present study, it was concluded that terminal heat stress adversely affected morpho-physiological, and biochemical attributes in lentil. These parameters were improved under high temperature stress condition with the application of biostimulants (Humic Acid and Seaweed Extract) as seed priming alone or seed priming in combination with foliar application at 40 and 60 DAS. The treatments which showed the best response was seed priming + foliar application (40+60 DAS) with SWE followed by seed priming + foliar application (40+60 DAS) with HA, seed priming with SWE.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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