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Research Article

Improving the growth, Chlorophyll contents and productive tillers of wheat by exogenous application of fulvic acid under natural and heat stress conditions

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ABSTRACT

Terminal thermal stress, occurring under varying climatic conditions, significantly reduces wheat yield by affecting fertilization and grain filling processes. Wheat crop damage from terminal heat stress may be lessened by the use of biostimulants. The purpose of this study is to apply fulvic acid topically to wheat in order to reduce the effects of heat stress. Wheat crops were subjected to high temperature stress during booting and initial stages of grain in in-vivo, with heat stress applied in staggered intervals in the main plot. Different concentrations of fulvic acid (water spray, 1.25 mg L-1, 2.50 mg L-1, and 3.75 mg L-1) were applied under natural and heat stress conditions during the booting and initial stages of grain of wheat. Heat stress during booting and grain filling phases notably decreased chlorophyll content, growth related parameters and productive tillers. The impact was more significant during the booting stage compared to the filling stage of grain. Application of fulvic acid at concentrations of 3.75 mg L-1 and 2.50 mg L-1 during heat stress at booting and grain filling stages substantially increased chlorophyll content and growth related parameters compared to water spray and 1.25 mg L-1 fulvic acid. These findings imply that fulvic acid applied directly to wheat under heat stress causes thermotolerance, which has a beneficial impact on biochemical and physiological processes.

Keywords: Biostimulant, Growth, Thermotolerance, *Triticum aestivum*, Yield.

INTRODUCTION

Pakistan, being primarily agrarian, relies on its agriculture sector, which employs 45% of the country's population. Wheat holds significant importance in Pakistan as a staple food, contributing 8.97% to the value added in agriculture and 1.8% to the GDP. In the 2021-22 period, wheat cultivation covered an area of 8.97 million hectares, yielding 26.39 million tons of wheat (Govt. of Pakistan, 2022).

Various factors contribute to the decline in wheat yield, including the unavailability of certified seeds, late sowing, traditional sowing methods, inadequate land preparation, ineffective weed management, unpredictable rainfall, waterlogging, salinity, and poor marketing. Among these factors, terminal heat stress emerges as a significant future threat, exacerbated by the current scenario of climate change (Nagar et al., 2015).

Increasing ambient temperatures adversely affect crop growth, development, and yield. Higher temperatures accelerate the plant development process, resulting in shortened life cycles, reduced plant stature, and shorter reproductive cycles, ultimately leading to lower yields (Jerry and Prueger, 2015).

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Semenov (2009) predicts a future increase in terminal heat stress for wheat. While wheat is affected by terminal heat stress at various phenological stages, the reproductive stage is particularly vulnerable, leading to reduced grain number and weight (Wollenweber et al., 2003).

Elevated ambient temperatures impact global and Pakistani crop production. As temperatures rise beyond species' optimal levels, the rate of vegetative development increases (Jerry and Prueger, 2015). Terminal heat stress in wheat affects crucial processes such as flowering, pollen viability, photosynthate availability, and translocation during anthesis and grain filling stages, resulting in decreased grain weight, number, and quality (Reynolds et al., 2012; Gonzalez-Navarro et al., 2015).

Photosynthesis gets rapidly affected by high temperatures (Wahid et al., 2007) causing a reduced rate of grain production and yield (Mondal et al., 2013). Burn causes a shortage of photoassimilates at the stage of grain filling (Talukder et al., 2013) and reduces enzyme activity in starch accumulation (Zhao et al., 2008).

Overcoming heat stress in plants involves a variety of plant-breeding strategies, genetically modifying for heat tolerance, applying a number of molecular markers and management practices. For example, practices like conservation of moisture while employing no-tillage and stubble mulching (Farooq et al., 2011) can be used for managing soil moisture under high temperatures. Besides, amendment of nitrogen, phosphorus, and potassium in mild heat stress can enhance plant development in a significant way (Dupont et al., 2006). Besides sowing wheat undertaken early and the delay of pervious crop harvests, cotton picking and rice harvest, all these practices face up serious challenges due to cotton-wheat and rice-wheat cropping systems in Pakistan.

In the process of exogenous treatment using osmolytes, signaling molecules, and non-nutrient salts, and biostimulants is an additional approach that can be adopted to reduce heat stress in wheat. Biostimulants, such as humic acids, fulvic acids, microbial inoculants (MIC), protein hydrolysates, amino acids, and seaweed extracts have presented some potential benefits (Oosten et al., 2017). Fulvic acid use is closely connected with amplification of plant productivity and photosynthetic pigments as well as rising antioxidant enzymes (Ali et al, 2015). It should be mentioned that different groups of researchers did investigations on the influence of mixed applications of fulvic acid and humic acid on maize yield and show the fruitful results (Matysiak et al., 2011), as well as the regulation of plant growth under drought conditions (Nardi et al., 2002). The ability of Fulvic acid to give plants resistance against all kinds of abiotic stresses is one of the peculiar things that make it stand out (Yildrim, 2007). Nevertheless, the particular modes of action of the extract in case of mitigation heat stress in Pakistan’s areas are still ambiguous.

The hypothesis predicted that fulvic acid would mitigate terminal heat stress in wheat through the activation of enzymes, the acceleration of photosynthesis, the promotion of protein metabolism in cells, the elimination of reactive oxygen species, and the enhancement of tissue water status. The study aimed to assess the optimal potential of fulvic acid in enhancing wheat growth by monitoring antioxidant enzyme activities, photosynthetic pigments.

MATERIALS AND METHODS

Experimental site and layout plan

A research project was undertaken at the Research Area within the Department of Agronomy, University of Agriculture Faisalabad, during the winter (Rabi) season of 2016-17. Punjab-2011 wheat variety was manually sown using a hand drill, adhering to the recommended seed rate of 100 kg ha-1, with a spacing of 22.5 cm between rows. Phosphorus and Nitrogen were applied at rates of 60 kg ha-1 and 75 kg ha-1, respectively, placed as bands in the soil at sowing. An additional 75 kg nitrogen per hectare was applied with the first irrigation. The field received irrigation at critical wheat growth stages.

The experimental design followed a Randomized Complete Block Design (RCBD) with a split-plot arrangement, replicated three times. Each net plot measured 3.0 m × 0.90 m and contained four rows of wheat. Main plots were designated for heat treatments (H0= No heat stress, H1= Heat stress at booting stage, H2= Heat stress at grain initiation stage), while sub-plots received foliar applications of fulvic acid (F0= Water spray, serving as control, F1= 1.25 mg L-1, F2= 2.50 mg L-1, F3= 3.75 mg L-1). Heat stress was induced by covering plots with perforated polythene sheets, and foliar spray was administered one week after applying heat stress at the grain initiation stage. All other crop management practices remained consistent across all treatments.

Procedure for recorded observations

For flag leaf area, Flag leaves of 5 plants, 10 days after fulvic acid application were taken randomly from each treatment and area was measured by digital leaf area meter and average was taken. For flag leaf fresh and dry weight, Flag leaves of 10 plants, 10 days after fulvic acid application were taken at random from each plot and were weighed with electrical weighing balance. The average was taken of all ten leaves. After taking fresh weight the same leaves were kept in oven till constant dry weight. Then an average of all ten leaves was taken. For peduncle fresh and dry weight, Peduncles from 5 plants 10 days after fulvic acid application were taken out and weighed by electrical weighing balance. The average weight was taken per plant. After taking fresh weight, some peduncles were placed in oven for drying till constant dry weight. The average of peduncle dry weight per plant was taken. For productive tillers, 3 areas of 30 cm length were selected randomly from each treatment and produce tillers were counted and converted to productive tillers m-2 by unitary method.

Chlorophyll contents were determined by adding Chl a and Chl b. For Chlorophyll a, b (mg/g) contents, a sample of 0.5 grams from each experimental unit was soaked in 80% acetone overnight and using ELISA plate optical density was noted at 645 nm and 663 nm wavelength. Chlorophyll contents were determined by the following formulae (Arnon, 1949).

$$\text{Chl } a = [12.7\ \times \text{A663} - 2.9 \times \text{A645}] \times \left(\frac{\text{V}}{1000}\right) \times \text{W}$$

$$\text{Chl } b = [22.9\ \times \text{A645} - 4.68 \times \text{A663}] \times \left(\frac{\text{V}}{1000}\right) \times \text{W}$$

A663 and A645 are absorbances at 663 nm and 645 nm respectively Where V and W are volumes of extract and weight of sample, respectively.

The recorded data underwent statistical analysis utilizing Fisher's Analysis of Variances Technique, as described by Steel et al. (1997). Treatment means were compared using Tukey's Honestly Significant Difference (HSD) test at a 5% probability level.

RESULTS

The flag leaf area is an important indicator for performance of wheat because current photosynthesis occurs in flag leaf and role of flag leaf in grain filling is more than 90 %. Data in table 1 and 2 indicats significantly reduced leaf area due to heat stress. Maximum leaf area (24.22 cm2/plant) was recorded in treatment H0 (No heat stress) followed by treatment H2 (Heat stress at grain initiation stage) and H1 (Heat stress at booting stage) which were 19.96 cm2/plant and 17.82 cm2/plant respectively and were similar statistically. Reduction in leaf area as compared to no heat stress was 26.42 % under heat stress at booting stage and 17.58 % under heat stress at grain initiation stage. Among different concentrations of FA 2.50 mg L-1 produced highest leaf area (22.56 cm2/plant) followed by FA @ 3.75 mg L-1 which were statistically alike. Minimum was observed in control treatment where no FA was applied.

Table 1. Analysis of variance for effect of foliar applied Fulvic acid on growth parameters and Chlorophyll contents of heat stressed wheat (MEANS SUM O SQUARES).

SOV	DF	FLA	FLFW	FLDW	PFW	PDW	PT	Chl *a*+*b*
Blocks	2	1.83	0.0169	0.00047	0.042	0.00058	21.00	0.96
Heat Stress(H)	2	127.38**	0.1169**	0.02595**	0.528**	0.09994**	2234.33**	2.73*
Error I	4	6.21	0.0048	0.00069	0.010	0.00392	181.96	0.23
Fulvic Acid (F)	3	21.39**	0.0255**	0.00652**	0.173**	0.01309**	61.21^{NS}	0.60**
H × F	6	0.53^{NS}	0.0021^{NS}	0.00043^{NS}	0.015^{NS}	0.00050^{NS}	15.07^{NS}	0.10^{NS}
Error II	18	3.97	0.0048	0.00038	0.015	0.00099	36.45	0.09

* = Significant ($p \leq 0.05$); ** = Significant ($p \leq 0.01$); NS = Non-significant; FLA = Flag Leaf Area; FLFW= Flag Leaf Fresh Weight; FLDW= Flag Leaf Dry Weight; PFW= Peduncle Fresh Weight; PDW= Peduncle Dry Weight; PT= Productive Tillers; SL= Spike Length; Chl= Chlorophyll

Table 2. Effect of foliar applied Fulvic acid on growth parameters and Chlorophyll contents of heat stressed wheat.

Treatments	FLA (cm^2)	FLFW (g)	FLDW (g)	PFW (g)	PDW (g)	PT (m^{-2})	Chl *a*+*b* (mg g^{-1} FW)
Heat stress (H)							
H_0 (No heat stress)	24.22 a	0.78 a	0.22 a	1.66 a	0.51 a	335 a	2.79 a
H_1 (Heat stress at booting stage)	17.82 b	0.58 b	0.13 b	1.25 c	0.33 b	307 b	1.88 b
H_2 (Heat stress at grain initiation stage)	19.96 b	0.64 b	0.15 b	1.41 b	0.42 b	318 ab	2.10 b
Tukey's HSD ($p \leq 0.05$)	3.627	0.101	0.038	0.142	0.091	19.6	0.691
Fulvic acid foliar spray (FA)							
F_0 (Control)	19.09 b	0.60 b	0.14 b	1.28 c	0.38 c	317	1.94 b
F_1 (1.25 mg L^{-1})	19.78 b	0.64 ab	0.15 b	1.38 bc	0.41 bc	320	2.20 ab
F_2 (2.50 mg L^{-1})	22.56 a	0.72 a	0.19 a	1.58 a	0.46 a	321	2.56 a
F_3 (3.75 mg L^{-1})	21.21 ab	0.70 a	0.18 a	1.53 ab	0.44 ab	323	2.33 ab
Tukey's HSD ($p \leq 0.05$)	2.653	0.092	0.026	0.161	0.041	NS	0.402

Any two means not sharing a letter in common differ significantly at $p \leq 0.05$; FLA = Flag Leaf Area; FLFW= Flag Leaf Fresh Weight; FLDW= Flag Leaf Dry Weight; PFW= Peduncle Fresh Weight; PDW= Peduncle Dry Weight; PT= Productive Tillers; SL= Spike Length; Chl= Chlorophyll

Fresh weight of flag leaf is an indicator of flag leaf health. More the fresh weight could be health of flag leaf. Data in table (2) show significant decrease in fresh weight of flag leaf under heat stress. The maximum fresh weight of flag leaf (0.78 g/plant) was obtained under no heat stress condition, and minimum was under heat stress at booting stage which was statistically similar to that obtained under heat stress at grain initiation stage. Reduction in flag leaf fresh weight was 25.64 % and 17.95 % under heat stress at booting stage and heat stress at grain initiation stage, respectively. All concentrations of FA performed equally well in improving flag leaf fresh weight but were significantly higher than control. Overall improvement due to FA was recorded up to 20 %. The interactive effect of FA and heat stress was found to be non-significant.

Flag leaf dry weight is an indicator of total dry matter accumulation. Data in table (2) indicates the decrease in dry weight of flag leaf due to heat imposition. Maximum dry weight (0.22 g/plant) was calculated in treatment H0 (No heat stress) minimum (0.13 g/plant) by treatment H1 (Heat stress at booting stage) which was statistically similar to treatment H2 (Heat stress at grain initiation stage) that produced 0.15 g/plant dry weight of flag leaf. Decrease in flag leaf dry weight was recorded 40.90 % under heat stress at booting stage and 31.81 % under heat stress at grain initiation stage. FA @ 2.50 mg L-1 produced highest (0.19 g/plant) and statistically similar flag leaf dry weight (0.18 g/plant) produced with FA @ 3.75 mg L-1, while minimum (0.14 g/plant) was recorded where no FA was applied. Overall improvement due to FA was recorded at 35.71%.

The peduncle of wheat ear expands and exposes it to light and air for better photosynthesis. Data in table (2) showed that heat stress decreased the peduncle’s fresh weight significantly. Maximum peduncle fresh weight (1.66 g) was noted in H0 (No heat stress) followed by H2 (Heat stress at grain initiation stage) and minimum was recorded in H1 (Heat stress at booting stage). Heat stress decreased the peduncle’s fresh weight by 15.05 % under treatment H2 and 24.69 % under treatment H1. Fulvic acid significantly improved the fresh weight of peduncle under normal and heat stress conditions. Maximum fresh weight of peduncle was recorded in treatment F2 (FA @ 2.50 mg L-1) which was statistically similar to treatment F3 (FA@ 3.75 mg L-1), while smallest was observed in treatment F0 (Control) where the absence of FA was statistically comparable to a lower level of FA (1.25 mg L-1) Heat stress and FA did not significantly interact.

Peduncle dry weight was substantially minimum under heat stress described in data table (2). Maximum peduncle dry weight per plant (0.51 g) was recorded in treatment H0 followed by treatment H2 (Heat stress at grain initiation stage) which was 0.33 g/plant while minimum (0.33 g/plant) was recorded in H1 (Heat stress at booting stage). The decrease in peduncle dry weight was 17.64 % under heat stress at grain initiation stage and 32.29 % under heat stress at booting stage. FA significantly improved the dry weight under normal and heat stressed conditions. Maximum dry weight (0.46) was obtained in F2 (FA @ 2.50 mg L-1) which was statistically at par with treatment F3 (FA @ 3.75 mg L-1), while lowest (0.38) was obtained in treatment F0 (Control) that was without foliar application FA. Improvement in dry weight of peduncle was 21.05 % by application of FA @ 2.50 mg L-1.

Productive tillers are an indicator of yield performance of wheat as it is one of yield components. Data in table (2) shows that heat stress significantly reduced the number of productive tillers. Highest number of productive tillers (335) was observed in treatment (H0) where no heat stress was applied, followed by (H2) heat stress applied at grain initiation stage, which was statistically at par to treatment H0, while minimum (307) was observed in treatment (H1) where heat stress was applied at booting stage. As fulvic acid was applied at grain filling stage so it did not significantly affect the number of productive tillers. Interactive effect of fulvic acid and heat stress was also non-significant.

Chlorophyll a+b contents were decreased by heat stress application according to data in table (2) while FA improved these contents. More chlorophyll a+b contents (2.79 mg g-1) were recorded in treatment H0 (No heat stress), while statistically similar contents were recorded under heat stress at booting stage (H1) and heat stress at grain initiation stage (H2) which were 1.88 mg g-1 and 2.10 mg g-1 respectively. FA @ 2.50 mg L-1(F2) produced maximum chlorophyll a+b contents (2.56 mg g-1) followed by treatment F3 (FA @ 3.75 mg L-1) and F1 (FA @ 1.25 mg L-1) which were statistically at par with F2 and also with treatment F0 (Control) which produced minimum chlorophyll a+b contents (1.94 mg g-1). Heat stress decreased chlorophyll contents as compared to control treatment H0 (No heat stress) by 31.61 % and 27.73 % under heat stress at booting stage (H1) and heat stress at grain initiation stage (H2) respectively. FA @ 2.50 mg L-1 (F2) produced maximum chlorophyll contents (2.56 mg g-1) followed by treatments F3 (FA @ 3.75 mg L-1) and F1 (FA @ 1.25 mg L-1) which were statistically similar to F2, while minimum contents were recorded by treatment F0 (Control) which also statistically at par with F3 and F1. FA @ 2.50 mg L-1 improved chlorophyll a+b contents up to 31.95 % as compared to control treatment F0. The interactive effect of heat stress and FA was non-significant which showed that FA influenced equally to all heat stress treatments along with control (No heat stress).

DISCUSSION

Reduction in flag leaf area due to heat stress might be due to loss of chlorophyll contents, increased lipid peroxidation, membrane's depolarization and triggered programmed cell death in leaf due to accumulation of reactive oxygen species (Miller et al., 2009; Mittler et al., 2011). While improvement in flag leaf area could be due to increased chlorophyll contents in leaf, detoxification of reactive oxygen species by more production of antioxidants under FA application. Anjum et al., (2011) stated the improved leaf area due to foliar applied FA under drought stress.

Loss in chlorophyll contents, disruption of thylakoid membranes and reduced photosynthesis (Ristic et al., 2007) under heat stress might be responsible for reduction in flag leaf fresh weight. Improvement in fresh weight might be due to improved relative water contents of flag leaf and better photosynthesis under foliar applied FA. Anjum et al., (2011) recorded the increase in fresh weight of maize plants by foliar application of FA under normal and drought stress conditions.

Decrease in flag leaf dry weight might be due to reduced current photosynthesis and disturbed source sink relationship causing minimum dry weight accumulation in flag leaf. But improvement in dry weight might be due to improved photosynthesis and scavenging of reactive oxygen species which cause oxidative damage to membranes of cell.

Decrease in fresh weight of peduncle under heat stress might be due to transformation of assimilates as carbon reserves to grain filling under heat stress. As it was described by Mohammadi et al., (2009) that during heat stress after anthesis stage, stem reserves act as source of carbon for grains as heat stress limits photosynthesis. Improvement in fresh weight of peduncle might be due to improved photosynthesis and water relations of crop plants during heat stress by foliar application of FA which might retain the stem reserves.

Decrease in peduncle dry weight might be due to translocation of assimilates from stem to developing grains under heat stress as photosynthesis is limited. It might be also due to reduced water use efficiency under heat stress. While FA can increase the dry weight of peduncle by improving water use efficiency as stated by Zhang et al., (2016).

A more decrease (9%) in productive tillers was observed in H1 treatment followed by H2 (5.4%) which was statistically similar to H0 where no heat stress was applied. That decrease might be due to pollen sterility due to temperature stress. A 21.10 % decrease in productive tillers was observed by Ammarshettiwar and Berad, (2018) who stated the decrease due to limited supply of resources during heat stress. The results of Kalita et al. (2009) and Mukherjee (2012) further support the evidence.

Decrease in chlorophyll contents might be due to down regulation of pigment catabolic gene expression and degenerative effect of ROS for photosynthetic pigments which are produced under heat stress. Balouchi, (2010) also reported decrease in total chlorophyll contents in 8 wheat cultivars by application of heat stress upto 36°C, while decrease in chlorophyll contents in two wheat cultivars under heat stress of 37°C was observed by Efeoglu and Terzioglu, (2009). Improvement in chlorophyll contents might be attributed to alleviating effect of ROS in cell due to foliar application of FA. Haque et al., (2014) found a significant increase in chlorophyll contents in wheat cultivars under heat stress conditions.

CONCLUSION

Based on the aforementioned findings, it can be concluded that fulvic acid aids in alleviating heat stress by enhancing chlorophyll content and regulating growth. Therefore, it is concluded that fulvic acid can be applied as a foliar spray in wheat both under normal conditions and whenever the crop encounters heat stress during later stages of development

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COMPETING OF INTEREST

The authors declare no competing interests.

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