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Improving the Biochemical and Physiological Wheat Processes by Foliar Application of Fulvic Acid Under Natural and Heat Stress Conditions

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Abstract: Terminal heat stress under varying climatic conditions induces a significant reduction in yield by impacting the fertilization and grain filling. The use of biostimulants would be useful to boost terminal heat stress in the wheat crop. The research attempts to modulate the effect of heat stress on wheat by foliar application of fulvic acid. The wheat crop was exposed to high-temperature stress (3-5°C higher than ambient temperature) during booting and grain initiation under field conditions by staggering heat stress in the main plot. Various amounts of fulvic acid, i.e. water spray, 1,25, 2,50 and 3,75 mg L-1, were added under natural and heat stress conditions at booting and grain initiation stages of wheat crop. Heat stress during booting and grain filling phases significantly reduced chlorophyll *a* activity up to 32.58% and 25.44%, Chl *b* activity up to 31.48% and 20.37%, grain filling duration up to 42.43% and 20.81% and grain yield up to 45.47% and 36.79% but improved antioxidants viz Superoxide dismutase activity up to 31.74% and 22.29%, Total soluble protein activity up to 6.63% and 11.77%, Catalase activity up to 114.73% and 55.03%, Peroxidase activity up to 63.61% and 39.39%. The impact at the booting stage was more pronounced than at the grain-filling stage. Application of fulvic acid @ 3.75 mg L^{-1} and 2.50 mg L-1 during heat stress during booting and grain filling processes greatly increased chlorophyll, antioxidants, and grain yield relative to water spray and 1.25 mg L^{-1} fulvic acid. The results suggest that foliar application of fulvic acid during heat stress induces thermotolerance in wheat positively impacting the physiology and biochemical processes.

Keywords: Biostimulants, Growth, Thermotolerance, *Triticum aestivum*, Yield

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1. Introduction

Pakistan being an agricultural country employs 45% population of country in the agriculture sector. Wheat is the major staple food of the country. It shares 8.97% of value added in agriculture and 1.8% of GDP. The area under wheat cultivation was 8.97 million hectares and production of wheat was 26.39 million tons during 2021-22 (Govt. of Pakistan, 2022).

Along with other factors which are causing wheat yield decline *viz* unavailability of certified seeds, late sowing, the conventional method of sowing, poor land preparation, poor weed management, unpredictable rains, water logging, salinity and poor marketing, terminal heat stress is major future threat and it is increasing under present scenario of climate change (Nagar *et al*., 2015). As ambient temperature is increasing day by day it is affecting crop growth, development and yield. Higher temperature increases the development process in plants. This increase in development process shortens life cycle of plants which results in short statured plants, shorter reproductive cycles and lower yield of plants (Jerry and Prueger, 2015).

Higher ambient temperature is affecting the crop production in world as well in Pakistan. As temperature increases from species optimal temperature, it increases the rate of vegetative development like node and leaf appearance rate (Jerry and Prueger, 2015). Terminal heat stress in wheat affects flowering, pollen viability, photosynthates availability and translocation to developing kernel during anthesis and grain filling stage respectively which results in less grain weight, number and quality (Reynolds *et al*., 2012; Gonzalez-Navarro *et al*., 2015). Under high temperature stress, the most sensitive process is photosynthesis (Wahid *et al*., 2007). Increase in temperature increases development rate and reduces grain growth which causes reduction in wheat yield (Mondal *et al*., 2013). Due to heat stress supply of photoassimilates limits grain filling (Talukder *et al*., 2013) and reduces the enzymes' activity which are involved in starch accumulation in wheat grains (Zhao *et al*., 2008).

Heat stress can be mitigated in plants by selecting and breeding strategies (Vinh and Paterson, 2005), genetic engineering for heat tolerance, use of molecular markers and by some management strategies. To make water available to plants during heat stress, management strategies include moisture conservation by no tillage and stubble mulching (Farooq *et al*., 2011). Application of nitrogen, phosphorus and potassium can also improve the plant growth during mild heat stress (Dupont *et al*., 2006). Early sowing of wheat is another option to avoid

from heat stress. But in Pakistan as there is cotton-wheat and rice-wheat cropping system, it cannot be adopted due to late picking of cotton and late harvesting of rice.

Exogenous application of osmolytes, signaling compounds, inorganic salts and some biostimulants is another way to mitigate heat stress in wheat. Biostimulants including humic acids, fulvic acids, microbial inoculants, protein hydrolysates, amino acids, and seaweed extracts can be used (Oosten *et al*., 2017). Fulvic acid enhances the availability and absorption of essential nutrients by plants. It chelates or binds with minerals, making them more soluble and easily taken up by plant roots. This improves nutrient uptake efficiency and ensures optimal nutrient supply for plant growth (Kumar *et al*., 2019). Fulvic acid stimulates various metabolic processes in plants, including photosynthesis, respiration, and enzyme activities. It enhances chlorophyll synthesis, leading to improved energy production and plant growth. It also promotes root development and branching, leading to a stronger root system (Fang *et al*., 2020).

Fulvic acid helps plants cope with various abiotic stresses such as drought, salinity, and temperature extremes. It improves the plant's ability to withstand water stress by increasing water-holding capacity and reducing water loss through transpiration. It also aids in the detoxification of heavy metals, protecting plants from metal toxicity (Anjum *et al*., 2011). Fulvic acid enhances the plant's natural defense mechanisms against diseases and pathogens. It activates the plant's immune system, increasing the production of defense-related compounds and enzymes. This helps in suppressing the growth of pathogens and reducing the incidence of diseases (Voscancelos *et al*., 2021). Application of fulvic acid increased plant productivity, photosynthetic pigments and antioxidant enzyme activity in wheat (Ali *et al*., 2015). Matysiak *et al*., (2011) found that mixed application of fulvic acid and humic acid improved maize growth and yield. Humic and fulvic acids regulated plant growth under drought and well-irrigated conditions (Nardi *et al*., 2002). Yildrim, (2007) found that fulvic acid resisted against the abiotic stresses in plants. But the effects of fulvic acid to mitigate heat stress under the climate of Pakistan are still unknown.

It was hypothesized that fulvic acid will help to ameliorate the terminal heat stress in wheat by catalyzing enzyme reactions, increasing assimilation of photosynthates, stimulating metabolism of proteins in cell, scavenging the reactive oxygen species and by improving the plant’s tissue water status.

The objective of study was to indicate the optimum potential of fulvic acid to get better wheat growth and yield by monitoring antioxidant enzymes activities, photosynthetic pigments, and yield related attributes.

2. Materials and Methods:

2.1. Experimental site and layout plan

A study was conducted at Research Area of Department of Agronomy, University of Agriculture Faisalabad during winter (Rabi) season 2016-17. Wheat variety Punjab-2011 was sown through hand drill with recommended seed rate of 100 kg ha^{-1} by maintaining R × R distance of 22.5 cm. Phosphorus and Nitrogen at the rate of 60 kg ha^{-1} and 75 kg ha^{-1}, respectively were applied as band placement in soil at sowing, while 75 kg nitrogen per hectare was applied with first irrigation. Field was irrigated at critical growth phases of wheat.

In this study RCBD with split plot arrangement was followed and replicated thrice. Net plot size was 3.0 m × 0.90 m and it comprised of four rows of wheat. Heat treatments (3-5°C higher than ambient temperature) (H_0=No heat stress, H_1=Heat stress at booting stage, H_2=Heat stress at grain initiation stage) were kept in main plots and foliar applied fulvic acid (F_0= Water spray (control), F_1= 1.25 mg L^{-1}, F_2= 2.50 mg L^{-1}, F_3= 3.75 mg L^{-1}) was kept in sub plots. Heat stress was practiced by applying perforated polythene sheet on plots and foliar spray was applied one week after heat stress application at grain initiation stage. All other crop management practices were same for all treatments.

2.2. Procedure for recorded observations

SOD (unit/mg protein) activity was measured as amount of enzyme which blocked photochemical reduction of nitro blue tetrazolium (NBT). Reaction mixture was comprised of 100 µL enzyme extract prepared as for TSP + 100 µL NBT + 200 µL triton-X + 200 µL methionine + 800 µL water (distilled). After this reaction mixture was placed for 15 minutes under ultraviolet light. After adding 100 µL riboflavin, absorbance was recorded at 560 nm using ELISA plate (Giannopolitis and Ries, 1977).

POD (unit mg^{-1} protein) activity was measured as quantity of enzyme used in guaiacol oxidation. Enzyme extract same as for TSP was also used for POD measurement. Reaction mixture was comprised of 800 µL of potassium phosphate buffer (pH 5) + 100 µL guaiacol (20 mM) + 100 µL H_2O_2 (40 Mm). 100 µL reaction mixture and 100 µL of enzyme extract were taken to record absorbance at 470 nm using ELISA plate (Liu *et al*., 2009).

CAT (unit/mg protein) activity was measured as amount of H_2O_2 converted to H_2O and O_2 as consumed of enzyme. Catalase activity was determined by using same enzyme extract as for TSP. Absorbance was recorded at 240 nm wavelength after adding 100 µL enzyme extract and 100 µL H_2O_2 (5.9 mM) in ELISA plate (Liu *et al*., 2009).

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 following formulae (Arnon, 1949).

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

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

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

With an interval of five days, spikes from 5 selected plants were removed and placed in oven to take constant dry weight with the help of an electrical weighing balance. Grain filling rate (GFR) was determined with help of formula given by Hunt (1978).

$$\text{GFR} = \frac{W2 - W1}{T2 - T1}$$

W_1 and W_2 indicate the dry weights taken at time T_1 and T_2.

10 spikes from each treatment were selected and tagged to count number of days taken from heading to physiological maturity which describes the grain filling duration (Hunt, 1978).

After harvesting each plot separately, grains were threshed, weighed and grain yield was calculated in tons per hectare by unitary method. Statistical analysis of recorded data were done by Fisher Analysis of Variances Technique and treatments' means were compared by using Tukeys' HSD test at 5% probability level (Steel et al., 1997)

3. Results

3.1. Superoxide dismutase activity (unit mg^{-1} protein)

Data in table (1) showed that Heat stress and foliar application of FA significantly affected Superoxide dismutase (SOD) activity in wheat. Heat stress increased SOD activity and FA also shown similar behavior. Significant interactive effect shows different response of FA to different heat stress treatments. According to data in table (2) heat stress increased SOD activity upto 31.74 % and 22.29 % as compared to control under heat stress at booting stage (H_1) and heat stress at grain initiation stage (H_2) respectively. During no heat stress (H_0) FA

did not cause any significant improvement in SOD activity. During heat stress at booting stage (H_1) FA @ 3.75 mg L^{-1} produced highest SOD activity (166.78 unit mg^{-1} protein) and minimum (136.40 unit mg^{-1} protein) was recorded in treatment F_0 (Control). During heat stress at grain initiation stage (H_2) treatment F_2 (FA @ 2.50 mg L^{-1}) produced highest SOD activity (148.50 unit mg^{-1} protein) which was statistically at par with treatment F_3 (FA @ 3.75 mg L^{-1}) while minimum (126.61 unit mg^{-1} protein) was recorded in treatment F_0. Improvement due to FA application was 22.27 % and 17.28 % as compared to control during heat stress at booting stage (H_1) and heat stress at grain initiation stage (H_2) respectively.

3.2. Peroxidase activity (unit mg^{-1} protein)

Heat stress and FA significantly increased peroxidase (POD) activity (Table 1). According to data in table (2) heat stress increased POD activity upto 63.61 % and 39.39 % as compared to control H_0 (No heat stress) treatment, under heat stress imposition at booting stage (H1) and heat stress imposition at grain initiation stage (H_2). Significant interactive effect of FA and heat stress indicated that FA show different response under different heat stress treatments (Table 1). During H_0, FA @ 1.25 mg L^{-1} produced maximum POD activity (13.45 unit mg^{-1} protein) while minimum (8.30 unit mg^{-1} protein) was recorded in treatment F_0 Control). During H_1, FA @ 3.75 mg L^{-1} (F3) produced maximum POD activity (18.89 unit mg^{-1} protein) while minimum (13.58 unit mg^{-1} protein) was recorded in treatment F_0 which was statistically similar to treatment F_1 (FA @ 1.25 mg L^{-1}). During H2, FA @ 2.50 mg L^{-1} (F2) produced maximum POD activity (15.81 unit mg^{-1} protein) while minimum (11.57 unit mg^{-1} protein) was recorded in treatment F_0. Improvement in POD activity was 62.04 %, 39.10 and 36.64 % over control treatment (F_0) under H_0, H_1 and H_2 respectively.

3.3. Catalase activity (unit mg^{-1} protein)

Catalase (CAT) activity was also influenced by heat stress and FA significantly according to data in tables (1). According to data in table (2) heat stress and FA both improved CAT activity. Heat stress improved CAT activity upto 114.73 % and 55.03 % under heat stress at booting stage (H_1) and H_2 (Heat stress at grain initiation stage) respectively, over H_0 (No heat stress). FA had shown different response in different heat stress treatments as data (Table 1) indicated the significant interaction effect. During treatment H_0 (No heat stress) all FA treatments significantly improved CAT activity

Table 1: Analysis of variance for effect of foliar applied Fulvic acid on antioxidants, stay green, grain growth and grain yield of heat stressed wheat (MEANS SUM O SQUARES)

SOV	DF	SOD	POD	CAT	Chl *a*	Chl *b*	GFR	GFD	GY
Blocks	2	28.78	2.448	0.79	0.944	0.007	0.000034	4.08	0.020
Heat Stress(H)	2	5860.15**	91.34**	273.42**	1.815*	0.0947*	0.000428*	346.58**	19.271**
Error I	4	28.17	0.503	8.08	0.205	0.0083	0.000033	4.92	0.032
Fulvic Acid (F)	3	492.81**	22.81**	101.03**	0.395*	0.0221**	0.000108*	37.52**	0.699**
H × F	6	174.89**	7.68**	19.69**	0.102^{NS}	0.0002^{NS}	0.000660**	24.40**	0.339**
Error II	18	18.02	0.496	3.50	0.089	0.0031	0.000028	3.38	0.017

* = Significant ($p \leq 0.05$); ** = Significant ($p \leq 0.01$); NS = Non-significant; SOD = Superoxide dismutase; POD = Peroxidase; CAT = Catalase; Chl *a* = Chlorophyll *a*; Chl *b* = Chlorophyll *b*; GFR = Grain filling rate; GFD = Grain filling duration; GY = Grain yield

Table 2: Effect of foliar applied Fulvic Acid on antioxidant activity, grain growth and grain yield of heat stressed wheat

Treatments	SOD (U mg^{-1} protein)	POD (U mg^{-1} protein)	CAT (U mg^{-1} protein)	GFR (g day^{-1})	GFD (Days)	GY (t ha^{-1})
H_0 (No heat stress)						
F_0 (Control)	103.53 a	8.30 c	9.23 b	0.120 c	37 b	5.30 c
F_1 (1.25 mg L^{-1})	109.28 a	13.45 a	17.08 a	0.132 bc	42 a	6.03 a
F_2 (2.50 mg L^{-1})	105.30 a	9.94 b	15.47 a	0.143 ab	39 ab	5.75 ab
F_3 (3.75 mg L^{-1})	107.42 a	10.83 b	15.29 a	0.152 a	38 ab	5.46 bc
H_1 (Heat stress at booting stage)						
F_0 (Control)	136.40 c	13.58 c	19.82 c	0.160 a	25 c	2.89 c
F_1 (1.25 mg L^{-1})	140.00 c	15.40 bc	22.36 bc	0.154 a	25.7 bc	2.93 bc
F_2 (2.50 mg L^{-1})	150.43 b	16.67 b	24.35 b	0.140 b	29.7 ab	3.20 b
F_3 (3.75 mg L^{-1})	166.78 a	18.89 a	28.69 a	0.136 b	33.7 a	3.68 a
H_2 (Heat stress at grain initiation stage)						
F_0 (Control)	126.61 c	11.57 c	14.31 c	0.152 a	29.3 b	3.35 b
F_1 (1.25 mg L^{-1})	138.10 b	13.43 b	17.73 bc	0.141 ab	30.7 b	3.62 b
F_2 (2.50 mg L^{-1})	148.50 a	15.81 a	25.13 a	0.125 c	36 a	4.37 a
F_3 (3.75 mg L^{-1})	143.81 ab	14.21 b	20.41 b	0.130 bc	32.7 ab	4.17 a
Tukey's HSD ($p \leq 0.05$)	9.803	1.626	4.320	0.0122	4.245	0.301

Any two means not sharing a letter in common within a column differ significantly at $p \leq 0.05$; NS = Non-significant; SOD = Superoxide dismutase; POD = Peroxidase; CAT = Catalase; GFR = Grain filling rate; GFD = Grain filling duration; GY = Grain yield

Table 3: Effect of foliar applied Fulvic acid on chlorophyll *a* and chlorophyll *b* contents of heat stressed wheat

Treatments	Chl *a* (mg g^{-1} FW)	Chl *b* (mg g^{-1} FW)
Heat stress (H)		
H_0 (No heat stress)	2.24 A	0.54 A
H_1 (Heat stress at booting stage)	1.51 B	0.37 B
H_2 (Heat stress at grain initiation stage)	1.67 AB	0.43 AB
Tukey's HSD ($p \leq 0.05$)	0.659	0.074
Fulvic acid foliar spray (FA)		
F_0 (Control)	1.55 B	0.39 B
F_1 (1.25 mg L^{-1})	1.77 AB	0.43 AB
F_2 (2.50 mg L^{-1})	2.06 A	0.50 A
F_3 (3.75 mg L^{-1})	1.85 AB	0.47 A
Tukey's HSD ($p \leq 0.05$)	0.397	0.132

Any two means not sharing a letter in common differ significantly at $p \leq 0.05$; Chl *a* = Chlorophyll *a*; Chl *b* = Chlorophyll *b*

Table 4: Correlation Analysis of recorded parameters showing strength of association between each other:

	Chl *a*	Chl *b*	SOD	POD	CAT	GFR	GFD	GY
Chl *a*	**1.00**							
Chl *b*	0.92*	**1.00**						
SOD	-0.66 ^{NS}	-0.63 ^{NS}	**1.00**					
POD	-0.43 ^{NS}	-0.46 ^{NS}	0.91*	**1.00**				
CAT	-0.32 ^{NS}	-0.36 ^{NS}	0.90*	0.97*	**1.00**			
GFR	-0.14 ^{NS}	-0.43 ^{NS}	0.01 ^{NS}	0.01 ^{NS}	0.02 ^{NS}	**1.00**		
GFD	0.83*	0.91*	-0.55 ^{NS}	-0.36 ^{NS}	-0.31 ^{NS}	-0.59 ^{NS}	**1.00**	
GY	0.87*	0.93*	-0.75 ^{NS}	-0.56 ^{NS}	-0.51 ^{NS}	-0.46^{NS}	0.96*	**1.00**

Chl a= Chlorophyll a, Chl b= Chlorophyll b, SOD= Superoxide dismutase, POD= Peroxidase, CAT= Catalase, GFR= Grain filling rate, GFD= Grain filling duration, GY= Grain yield

but was statistically similar to each other, while minimum was in control F_0 (Control). During heat stress at booting stage (H_1) maximum CAT activity (28.69 unit mg^{-1} protein) was recorded by application of FA @ 3.75 mg L^{-1} (F_3), while minimum (19.82 unit mg^{-1} protein) was recorded in treatment F_0. During heat stress at grain initiation stage (H_2) FA @ 2.50 mg L^{-1} (F_2) produced maximum CAT activity (25.13 unit mg^{-1} protein), while minimum (14.31 unit mg^{-1} protein) was recorded in F_0 (Control) which was statistically similar to treatment F_1 (FA @ 1.25 mg L^{-1}).

3.4. Chlorophyll *a* contents (mg g^{-1})

According to data in tables (1) it is clear that chlorophyll *a* contents were significantly affected by heat stress and FA. Heat stress significantly reduced chlorophyll *a* contents. Data in table (3) indicated that during no heat stress (H_0) maximum chlorophyll *a* contents (2.24 mg g^{-1}) were recorded followed by heat stress at grain initiation stage (H_2) (1.67 mg g^{-1}) which was statistically at par with H_0, while minimum (1.51 mg g^{-1}) were recorded in treatment H_1 (Heat stress at booting stage). FA significantly improved chlorophyll *a* contents of wheat under no heat and heat stressed conditions. Maximum chlorophyll *a* contents (2.06 mg g^{-1}) were recorded by treatment F_2 (FA @ 2.50 mg L^{-1}) followed by F_3 (FA @3.75 mg L^{-1}) and F_1 (FA @ 1.25 mg L^{-1}) which were statistically at par with F_3, while minimum (1.55 mg g^{-1}) were recorded in control treatment (F_0) which was also statistically at par with F_1 and F_3. FA showed same response in all heat stress treatments as well in control as it was clear from non-significant interactive effect from data. Heat stress decreased chlorophyll *a* contents upto 32.58 % and 25.44 % treatment H_1 and H_2 over control treatment H_0, while FA improved chlorophyll a contents upto 32.90 % over control. Interactive effect of heat stress and FA was non-significant.

3.5. Chlorophyll *b* contents (mg g^{-1})

Chlorophyll b contents were affected by heat stress application and foliar applied FA as data in table (1) indicated. According to data in table (3), heat stress significantly decreased chlorophyll *b* contents. Maximum chlorophyll *b* contents (0.54 mg g^{-1}) were noted in treatment H_0 (No heat stress) trailed by treatment H_2 (Heat stress at grain initiation stage) which were statistically similar to each other, while minimum (0.37 mg g^{-1}) were recorded in treatment H_1 (Heat stress at booting stage). FA significantly increased Chlorophyll *b* contents. Maximum (0.50 mg g^{-1}) were recorded in treatment F_2

(FA @ 2.50 mg L^{-1}) followed by treatment F_3 (FA @ 3.75 mg L^{-1}) and F_1 (FA @ 1.25 mg L^{-1}) which were found statistically similar to F_2, while minimum were recorded in control treatment (F_0) where foliar spray of FA was not applied. Heat stress decreased chlorophyll *b* contents upto 31. 48 % due to heat stress at booting stage (H_1) and upto 20.37 % due to heat stress application at grain initiation stage (H_2) as compared to control treatment H_1, while FA increased chlorophyll *b* contents under normal (No heat stress) and heat stress conditions upto 22.20 % when FA @ 2.50 mg L^{-1} was applied as compared to control (F_0) where no FA was applied. Interactive effect of heat stress application and FA was noted as non-significant.

3.6. Grain filling rate (g day^{-1})

It is evident from facts in table (2) that during imposition of heat stress grain filling rate was increased significantly. Increase in rate was 33.33 % owing to heat stress at booting stage (H_1) and 26.66 % owing to heat stress at grain initiation stage (H_2) as compared to control (H_0). Significant interaction showed dissimilar response of FA to varying heat stress treatments (Table 1). During No heat stress (H_0) FA increased grain filling rate and more (0.152 mg day^{-1}) which was 26.66 % over control (F_0) was recorded in treatment F_3 (FA @ 3.75 mg L^{-1}) followed by F_2 (FA @ 2.50 mg day^{-1}) which was statistically at par with F_3 while minimum (0.120 mg day^{-1}) was recorded in control (F_0). During heat stress at booting stage FA @ 3.75 mg L^{-1} helped to decrease the elevated grain filling rate (0.160 mg day^{-1}) due to heat stress which was decreased upto 15 % and was similar (statistically) to treatment F_2 (FA @ 2.50 mg L^{-1}). During imposition of heat stress at grain initiation stage (H_2) FA @ 2.50 mg L^{-1} caused maximum decrease of 17.75 % from 0.152 mg day^{-1} to o.125 mg day^{-1}.

3.7. Grain filling duration (days)

According to data in tables (1) grain filling duration was significantly affected by application of heat stress and foliar applied fulvic acid (FA). Data in table (2) shows that heat stress significantly decreased grain filling duration from 37 to 25 days during heat stress imposition at booting stage (H_1) which was 42.43 % decrease and 37 to 29.3 days during heat stress at grain initiation stage (H_2) which was 20.81 % decrease. During no heat stress (H_0) FA @ 1.25 mg L^{-1} increased grain filling duration from 37 to 42 days which was 13.51 % improvement in duration and statistically similar to treatment F_2 (FA @ 2.50 mg L^{-1}) and F_3 (FA @ 3.75 mg L^{-1}). During heat stress at booting stage

FA @ 3.75 mg L^{-1} (F_3) increased grain filling duration from 25 to 33.7 days which was 34.8 % increase and statistically similar treatment F_2. During heat stress at grain initiation stage (H_2) FA @ 2.50 mg L^{-1} caused maximum increase in grain filling duration from 29.3 to 36 days which was 22.86 % increase and statistically at par with treatment F_3.

3.8. Grain Yield (t ha^{-1})

Heat stress also significantly decreased the grain yield. Data in table (2) shows that maximum grain yield was reduced from 5.30 to 2.89 t ha^{-1} which is 45.47% in treatment H_1 (heat stress at booting stage) followed by H_2 (heat stress at grain initiation stage) to 3.35 t ha^{-1} which is 36.79 % than control (No heat stress). Significant interactive effect (Table 1) of FA and heat stress shows that FA showed different behavior in heat stress treatments. In treatment H_0 (No heat stress) maximum grain yield (6.03 t ha^{-1}) was recorded where FA @ 1.25 mg L^{-1} was applied which is 13.77% improvement than control followed by treatment where FA @ 2.50 mg L^{-1} was applied which was statistically at par. Minimum was recorded in control where FA was not applied. In H_1 treatment FA @ 3.75 mg L^{-1} produced highest grain yield (3.68 t ha^{-1}) which was 24.56 % improvement and minimum was recorded in treatment F_0 where no FA was applied. FA @ 2.50 mg L^{-1} produced highest yield of 4.37 t ha^{-1} in H_2 treatment which was 30.44 % improvement in yield as compared to control and similar (statistically) to treatment where FA @ 3.75 mg L^{-1} was applied. Overall decrease was 43.61 % owing to heat stress at booting stage and 31.20 % owing to heat stress at grain initiation stage while overall improvement due to FA was recorded 15.32 %. A strong positive correlation was observed between chl *a*, chl *b* and grain filling duration with grain yield.

4. Discussion

Plants being immobile can't avoid biotic and abiotic stresses and the growth as well as development is affected by environmental stresses. These stresses result in enhanced production as well as accumulation of reactive oxygen species (ROS) due to osmotic stress caused by disturbed homeostasis and ion distribution in plants (Herbette et al., 2011). Generation of ROS in plants causes disruption of organelles, lipid peroxidation, membrane leakage and ultimately the cell death (Rajput et al., 2021). Antioxidants being critical for plant growth, are first line of defense in plants against these stresses (Rajput et al., 2016). Increase in SOD activity under heat stress

might be attributed to plants own expression under stress conditions. Alscher *et al*., (2002) reported the partial induction of SOD activity and down regulation of other antioxidants by plants under osmotic stress conditions. SOD activity might also be attributed to increased production of superoxides (O_2^-) as substrate for SOD gene expression. Bakalova *et al*., (2004) reported increased activity of SOD in wheat under heat stress while Sharma and Dubey, (2005) reported increased SOD activity in rice. FA application also increased SOD activity under heat stress conditions that might be attributed to improved performance of mechanisms which are responsible in antioxidants production. Anjum *et al*., (2011) reported increase in SOD activity in maize due to application of FA under drought stress.

During heat stress CAT activity increased that might be due to activation of plants tolerance mechanism which activates during less osmotic potential. As heat stress decreased osmotic potential in this study CAT activity increased. CAT activity increased in wheat under moderate high temperature (Luna *et al*., 2004; Kumar *et al*., 2012) in maize (Kolarovic *et al*., 2009). FA also increased CAT activity which might be attributed to increase in chlorophyll contents which cause to increase more energy interception and produce more antioxidants. Anjum *et al*., (2011) reported increased CAT activity due to foliar applied FA in maize under drought stress conditions.

Increased POD activity might be attributed to plants tolerance mechanism as Sairam and Saxena, (2000) reported that tolerant varieties have more POD activity, highest membrane stability and less lipid peroxidation. Tolerant barley variety with increased POD activity was reported by Acar *et al*., (2001). Antioxidants activity increases as osmotic potential decreases in plants so this increased activity might also be attributed to decreased osmotic potential under heat stress. Increase in antioxidants activity might be attributed to more energy intercepted by photosynthetic pigments (Anjum *et al*., 2011). Improvement in POD activity might be due to increased activity of chlorophyll contents during heat stress conditions. Aminifard *et al*., (2012) reported increased antioxidant activity in pepper by application of FA.

Heat stress damages chlorophyll contents by regulation of several chlorophyll catabolic genes expression under osmotic stress (Zimmermann *et al*., 2004) and by producing reactive oxygen species (ROS) which cause degradation of chlorophyll

contents (Ishikawa *et al*., 2001; Mach *et al*., 2001; Meskauskiene *et al*., 2001). Increase in chlorophyll contents might be due detoxification of ROS and change in catabolic genes expression by foliar application of FA and its alleviating nature of inhibitory effects of heat stress on chlorophyll contents. Anjum *et al*., (2011) found role of FA in improving chlorophyll contents in maize under drought stress.

Decrease in chlorophyll *b* contents might be due to production of ROS under heat stress which are cause of degradation of chlorophyll contents because leaves are burned or bleached under heat stress. Dall'Osto *et al*., 2006 found the reduction in photosynthetic pigments in higher plants under osmotic stress. Decline in photosynthetic pigments and photochemical reactions in thylakoids was observed by Su *et al*., (2014); Wang *et al*., (2014); Chen *et al*., (2016) under environmental stressed conditions. FA might have alleviating effect in degradation of photosynthetic pigments by changing gene expression and detoxifying ROS. Improvement in chlorophyll contents by FA application was stated by Khan *et al*., (2014).

Increase in grain filling rate was due to plant's internal mechanisms which activates during any osmotic stress conditions. Plant speeds up its metabolism to complete life cycle early. More grain filling rate is an indicator of high dry matter accumulation during high temperature after anthesis but reduced photosynthesis can't meet the rate so disruption of source-sink relationship occurs and photosynthates can't be accumulated. Shahid *et al*., (2017) reported the increased grain filling rate during heat stress. Decrease in grain filling rate by foliar application FA might be due to alleviation of ROS which cause oxidative damages and by changing gene expression which cause increment in grain filling rate to complete life cycle early. During normal conditions (No heat stress) FA improved grain filling duration which might be attributed to better photosynthesis by improved chlorophyll contents, relative leaf water contents, improved antioxidant activities in flag leaf.

Decreased grain filling duration during heat stress is attributed be "plant's escape mechanism" during which plant decreases its life cycle by accelerating its metabolism during any osmotic stress. Talukder *et al*., (2014); Streck *et al*., (2005) recorded decreased grain filling duration by 2.8 days by each 1°C rise in temperature. Rise in temperature by 5 °C above 20°C reduced grain filling duration by 12 days (Yin *et al*., 2009). Improvement in grain filling duration

due to foliar application FA might be attributed to alleviating effects to heat stress by detoxification of ROS, improved integrity of damaged membranes and lipid peroxidation.

Decrease in grain yield under heat stress could be due to reduced yield determining components such as productive tillers, grains per spike and 1000-grain weight. Sumesh *et al*., (2008) described the limited synthesis of starch under heat stress which is 60-75 % of total dry weight of grain (Sramkova *et al*., 2009). Mondal *et al*., (2013) described the altered growth and development due to very complex effects of heat stress which also caused physiological imbalance and reduced grains and yield in wheat. Improvement in grain yield might be due to ameliorative effects of FA on plants under heat stress.

Conclusion:

Keeping above facts in view it was concluded that fulvic acid helps to ameliorate heat stress through improving antioxidants activity, repairing and enhancing chlorophyll contents and regulating grain growth which ultimately results in improved yield, it was concluded that FA can be applied as foliar spray in wheat under normal and whenever it faces heat stress at lateral stages.

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