Improving Growth, Yield, Biochemical and Quality Attributes of Terminal Heat stressed Wheat by Exogenously Applied Fulvic Acid

Rubina Nawaz[1], Muhammad Shoaib[2], Umer Khurshid[3], Okasha Asif[4], Zulkaif Maqsood[5], Umar Sheher Yar[1], Muhammad Zafar[6], Nisa Mukhtar[7], Muhammad Arslan[8], Mustazhar Billah Zafar[9], Samman Gul Vaseer[10], Siraj Ahmad[11], Hafiz Muhammad Abdullah Tayyab[12], Basharat Ali[4]

[1]*Department of Plant Breeding and Genetics University of Agriculture, Faisalabad Pakistan*
[2]*Department of Agriculture Gomal University, D.I Khan Pakistan*
[3]*Department of Botany University of Agriculture, Faisalabad Pakistan*
[4]*University of Agriculture, Faisalabad Pakistan*
[5]*Pulses Research Institute, Ayub Agricultural Research Institute Faisalabad Pakistan*
[6]*Sugarcane Research Institute, Ayub Agricultural Research Institute Faisalabad Pakistan*
[7]*Soil Chemistry Section, Ayub Ayub Agricultural Research Institute Faisalabad Pakistan*[7]*Barani*
[8]*Agricultural Research Institute, Chakwal Pakistan*
[9]*Applied Linguistics GC University, Faisalabad Pakistan*
[10]*Wheat Program, Crop Sciences Institute, National Agricultural Research Center Islamabad, Pakistan*
[11]*Department of Agronomy University of Agriculture, Faisalabad Pakistan*
[12]*Department of Plant Pathology University of Agriculture, Faisalabad Sub-Campus Burewala Pakistan*

Abstract

Terminal heat stress causes a significant decline in yield of wheat by affecting its different reproductive phases. As an active biostimulant, fulvic acid can be used to ameliorate heat stress impacts in plants. To explore the effect of foliar applied fulvic acid a field experiment was carried out at Agronomic research area, University of Agriculture Faisalabad. Randomized complete block design with split plot arrangement having three replications was applied. In this experiment main plot treatments were comprised of heat stress (No heat, heat at booting stage and heat at grain initiation stage) and sub-plot treatments included foliar spray of fulvic acid at varying levels (0, 1.25, 2.50 and 3.75 mg L^{-1}). The data were recorded according to standard procedures regarding growth, yield, antioxidants and quality. These data were analyzed by using Fisher's analysis of variance technique to determine its variability and difference in treatments' means was compared by Tukey's HSD test at 5 % probability level. Heat stress application at booting stage caused more decline in flag leaf fresh and dry weight, flag leaf area, spikelet per spike, spike length, number of grains per spike, fertile tillers, 1000-grain weight, grain yield, total soluble protein, chlorophyll a+b, leaf relative water contents and crude protein than heat stress at grain initiation stage. During no heat stress FA @ 1.25 mg L^{-1} improved growth, antioxidants, chlorophyll and yield of wheat. During heat stress at booting stage FA @ 3.75 mg L^{-1} performed well to increase crop performance, while during heat stress at grain initiation stage FA @ 2.50 mg L^{-1} improved crop performance regarding growth, antioxidant activity, yield and quality. It is concluded that fulvic acid can be applied to improve crop growth and yield under normal and heat stress conditions.

Key words: Fulvic acid, Antioxidants, Heat stress, Growth, Quality, Wheat

Introduction:

Wheat is one of the most cultivated crops in the world because a third of the world's population depends on it in their food. Wheat shares 8.2% to value added in agriculture and 0.7%

to GDP. Area under wheat cultivation was 9043 thousand hactares and production of wheat was 27.634 million tonnes (Govt. of Pakistan, 2023).

Global climate change and shifts in weather patterns have significantly increased the frequency of heat events during reproductive growth phases of wheat crops in many parts of the world (Collins *et al*., 2022). Short episodes of heat during the early to mid-grain filling phase of wheat severely affect cell biochemistry, physiology and overall grain yields (Shenoda *et al*., 2021). Yearly variations in grain yield recorded in many wheat-growing countries are associated with the rapid changes in temperature during grain filling (Djanaguiraman *et al*., 2020). For wheat crop, 21-23°C is considered the optimum temperature during grain filling (Porter and Gawith, 1999), and grain yield is reduced by 6% for each 1°C rise above this optimum temperature (Asseng *et al*., 2015). During the last 100 years, the global mean air temperature has increased by 0.8°C, and a further 2-4°C increase is projected for 2050 (IPCC, 2014). Numerous workers have reported that high temperature during grain filling reduces grain number and weight in wheat (Ullah *et al*., 2019). Even a brief episode of heat (1 day) during early grain setting significantly diminishes wheat grain yield (Talukder *et al*., 2014)

Depending on severity and duration, post-anthesis heat stress limits carbohydrate formation and translocation toward developing grains (Sharkey, 2005). The reduction in growth and yield under a hot environment is associated with impaired physiological plant functioning. For example, high temperature in field crops disturbs the balance between reactive oxygen species (ROS) and the plant defensive system (Sarwar *et al*., 2021). Heat-induced cell membrane leakage during grain filling indicates oxidative damage to cellular organelles in wheat crops (Dias *et al*., 2010). Damage to leaf chlorophyll under hot environments results in poor photosynthesis, stomatal conductance, chlorophyll fluorescence and it inhibits the physiological functioning of wheat crops (Feng *et al*., 2014). Similarly, high temperature during grain-filling accelerates flag leaf senescence by 25% (Djanaguiraman *et al*., 2020), although wheat genotypes with superior leaf greenness could sustain grain yield hot heat stress (Mirosavljevic *et al*., 2021).

Several approaches have been used for improving heat stress tolerance in crops, i.e., by developing heat-tolerant genotypes (Mondal *et al*., 2016), adjusting sowing dates (Saeed *et al*., 2017) and applying compatible solutes (Siddique *et al*., 2018), signaling molecules (Sarwar *et al*., 2021) growth regulators (Hu *et al*., 2016) and nutrients (Sarwar *et al*., 2019).

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). Application of fulvic acid increased plant productivity, photosynthetic pigments and antioxidant enzyme activity (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). Fulvic acid resisted against the abiotic stresses in plants. But the effects of fulvic acid to mitigate heat stress under 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 water contents of flag leaf and yield related attributes.

Materials and Methods:

1.1. Plant material:

Punjab-11 wheat genotype was collected from Wheat Research Institute, AARI, Faisalabad for this study.

1.2. Experimental site:

A field experiment was carried out at Agronomic Research Area, Department of Agronomy, University of Agriculture Faisalabad during winter (Rabi) season.

1.3. Physiochemical traits of experimental site:

Soil samples were randomly collected before sowing the trial. Analyzed the samples to quantify different physiochemical attributes (ICARDA, 2013). Soil analysis was carried out in Environmental Sciences lab, Institute of soil and environmental science, UAF (Figure 1).

1.4. Weather elements:

Weather data were collected from metrological observatory cell, Department of Crop Physiology, University of Agriculture; Faisalabad situated at latitude 31° north, longitude 73° east and at altitude of 184.4 meter are presented graphically (Table 1). The experimental site was semiarid with annual mean rain fall of 375 mm.

1.5. Treatments:

The experiment was comprised of two variables a) heat stress in main plot (H_0 = No heat stress, H_1 = Heat stress at booting stage, H_2 = Heat stress at grain initiation stage) and b) foliar application of fulvic acid in sub plot (control (water spray), 1.25, 2.50, 3.75 mg L^{-1}).

1.6. Experimental design:

In this study randomized complete block design with split plot arrangement along with three replications was followed.

1.7. Imposition of treatments:

Heat stress was applied by covering the plots with perforated polythene sheet and foliar spray was applied one week after heat stress application at grain initiation stage. All other crop management practices were kept uniform for all the treatments.

1.8. Statistical analysis:

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).

1.9. Agronomic practices

Sowing was done 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. Irrigation was applied at critical growth stages of wheat. Net plot size was 3.0 m × 0.90 m and it comprised of four rows of wheat.

Observations recorded

Crop growth and yield components:

Flag leaf fresh weight was recorded by harvesting flag leaves of 30 cm row length and converted into m^{-2} by unitary method. Thereafter sub sample of 5 g fresh leaves was taken from each treatment and recorded oven dried weight. Similarly, leaf area of 5 g sub samples of flag leaves was measured by using leaf area meter (CI-202). Recorded leaf area and dry weight of sub samples of flag leaves were converted into m^{-2} by using their already calculated respective fresh weights. Ten plants were randomly selected from each experimental unit at maturity and spikelet per spike was counted and averaged; similarly ten spikes were randomly selected from each treatment and spike length was measured with scale and averaged. Number of grains of ten

spikes was manually counted and averaged. Three places of 30 cm row length were randomly selected in each treatment at maturity; number of fertile tillers in these selected lines was counted and converted into tillers for one square meter area by unitary method. The grain yield of each plot was weighed after threshing and was converted into tons per hectare.

Biochemical traits and leaf relative water contents:

To find out the total soluble protein a sample of 0.5 grams of frozen (at -80°C) leaves was taken from each experimental unit and enzyme extract was prepared by adding potassium phosphate buffer (pH 4), Vortexed and then centrifuged. After adding Bradford reagent absorbance was recorded at 595 nm using ELISA plate (Bradford, 1976). Chlorophyll *a*+*b* contents were calculated by adding chlorophyll *a* and chlorophyll *b*.

$$\text{Total Chlorophyll} = \text{Chl } a + \text{Chl } b$$

For LRWC leaves sample of 2 gram from each treatment was taken as fresh weight (F.W) and were floated on water for 24 hours to get saturated weight (S.W). After getting S.W theses leaves were placed in oven at 70°C till constant dry weight (D.W). Leaf relative water contents (LRWC) were measured by using following formula.

$$\text{LRWC} = \frac{\text{F.W} - \text{D.W}}{\text{S.W} - \text{D.W}} \times 100$$

Quality parameter:

Grain crude protein contents in percent were calculated as % Crude protein = % N × 6.25, where: 6.25 = Constant for wheat (Bremner and Mulvaney, 1982).

Results:

Crop growth and yield components:

Flag leaf fresh weight, flag leaf dry weight and flag leaf area were significantly affected by heat stress (H) and fulvic acid (F) (Table 3). Maximum values of all these parameters were observed with 2.50 mg L^{-1} fulvic acid application which was closely followed by 3.75 mg L^{-1} fulvic acid level. Among heat stress, highest flag leaf fresh weight (0.78 g $plant^{-1}$), flag leaf dry weight (0.22 g $plant^{-1}$) and flag leaf area (24.22 cm^2 $plant^{-1}$) were recorded with no heat stress and minimum in heat stress at booting stage. Fulvic acid did not affect number of fertile tillers and spikelet per spike. This may be due to delayed application at grain filling stage so it did not significantly affected the number of fertile tillers and spikelet per spike, while heat stress did

show significant effects (Table 3, 4) as heat stress application at booting and grain initiation stages maximum count of fertile tillers and spikelet per spike of wheat plant was recorded in no heat stress so, lowest number of fertile tillers (307 m^{-2}) and spikelet per spike (16.7) were recorded in plots where heat stress was applied at booting stage of wheat (Table 4). Heat stress significantly reduced the spike length, grains per spike and 1000-grain weight. More decrease in these traits were observed in H_1 treatment where heat stress was applied at booting stage than in H_2 treatment where heat stress was applied at grain initiation stage. Significant results of interaction show that FA performed differently in different heat stress levels. In H_0 treatment FA @ 1.25 mg L^{-1} produced more (12.47 cm) spike length, (49.0) grains per spike and (43.27 g) 1000-grain weight followed by FA @ 2.50 mg L^{-1} which was statistically at par with FA @ 3.75 for spike length and grains per spike while minimum values of these traits were observed in control. In H_1 treatment where heat stress was applied at booting stage, (FA @ 3.75 mg L^{-1}) produced more spike length (10.47 cm), grains per spike (36.0) and 1000-grain weight (35.70 g) followed by (FA @ 2.50 mg L^{-1}) for grains per spike and 1000-grain weight not for spike length and minimum in control. In H_3 treatment where heat stress was applied at grain initiation stage 2.50 mg L^{-1} produced more spike length (11.73 cm), grains per spike (42.3) and highest 1000-grain weight (37.54 g) which were statistically similar to 3.75 mg L^{-1}, while minimum were observed in control (Table 3,4). Heat stress also significantly decreased the grain yield. Grain yield was reduced from 5.64 to 3.18 t ha^{-1} which is in treatment H_1 followed by H_2 to 3.88 t ha^{-1} than control (No heat stress). Significant interactive effect of FA and heat stress shows that different behavior in FA and heat stress treatments. In treatment H_0 maximum grain yield (6.03 t ha^{-1}) was recorded where FA @ 1.25 mg L^{-1} was applied 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}) 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 as compared to control and statistically similar to treatment where FA @ 3.75 mg L^{-1} was applied.

Biochemical traits and leaf relative water contents:

Total soluble protein contents were influenced by heat stress and foliar applied FA. Heat stress increased TSP contents upto 11.32% under heat stress at booting stage and 12.57% during heat stress at grain initiation stage as compared to control. Significant interactive effect showed

different response of FA to different heat stress treatments. Under treatments H_0 and H_2, FA did not significantly increase TSP contents, while during treatment H_1 maximum TSP contents (0.583 mg g^{-1}) were recorded by FA treatment @ 3.75 mg L^{-1} which was statistically similar to all other treatments and minimum were recorded in control (Table 2). Chlorophyll a+b contents and leaf relative water contents (LRWC) were decreased by heat stress application while FA improved these contents (Table 3). More chlorophyll a+b contents and LRWC were recorded in treatment no heat stress, while statistically lower contents were recorded under heat stress at booting stage and heat stress at grain initiation stage. FA @ 2.50 mg L^{-1} produced maximum chlorophyll a+b contents (2.56 mg g^{-1}) followed by all other treatments and minimum in control (1.94 mg g^{-1}) while FA 3.75 mg L^{-1} produced more LRWC which was at par with other levels of FA and minimum in control . Interactive effect of heat stress and FA was non- significant for both traits (Table 3).

Quality parameter:

Heat stress decreased the grain crude protein contents significantly. FA significantly increased crude protein contents during normal and heat stressed conditions. Maximum (10.17 %) crude protein contents were recorded in treatment FA @ 2.50 mg L^{-1} which was statistically similar to treatment FA @ 3.75 mg L^{-1} while minimum was recorded in control. Interactive effect of FA and heat stress was non-significant (Table 4).

Discussion:

In wheat crop post-anthesis grains development and grain filling are highly sensitive to short and long-duration heat spells in most parts of the world. 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 condition. 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 damages to membranes of cell. 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 (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. Reduction in spikelets per spike may be due to production of reactive oxygen species which cause membrane damages and cause programmed cell death under heat stress condition. As FA was applied at later stage so it did not influence the number of spikelets per spike. Decrease in spike length might be due to reduced development of rachis during heat stress at booting stage which shortened the spike length and during heat at grain initiation stage it might be due to disrupted source-sink relationship due to production of ROS and lesser photosynthates accumulation in rachis and in grains which also produced shrunken grains causing spike to remain shorter. Kumar *et al*., (2016) reported the reduced spike length of wheat genotypes under heat stress which resulted in lesser grains per plant and low yield. As heat stress increases, it causes production of reactive oxygen species which causes membrane leakage, pollen sterility, destruction of photosynthesis apparatus and chlorophyll contents and lipid peroxidation (Farooq *et al*., 2011) and ultimately decreases grains per spike. These results are supported by Ghaffari *et al*., (2015) who conducted experiment for inducing thermos-tolerance in wheat cultivars. Improvement in number of grains per spike by application of FA was supported by Anjum *et al*., (2011) who stated the increase in kernals/cob in maize. More decrease (9%) in productive tillers was observed in H_1 treatment followed by H_2 (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. 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 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. As FA increased the all yield components. During heat stress total

soluble proteins were increased which might be due to increased activity of antioxidant enzymes. Moreover FA also increased TSP contents in wheat which might be due to improved chlorophyll contents, antioxidants activity and better nutrient uptake by foliar applied FA. Sharma *et al*., (2014) reported increased TSP in wheat genotypes which had higher activity of antioxidants. 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. Heat stress decreased relative water contents which might be coincided with more evapotranspiration due to increased temperature. Reduction in relative water contents reduces plant growth. Sairam *et al*., (2000) reported the decreased relative water contents due to heat stress. Farooq *et al*., (2011) reported that heat stress during reproductive stage of wheat decreased relative water contents and water potential. FA increased relative water contents that might be attributed to decreased water loss and improved water uptake by improved root hydraulic conductance (Li *et al*., 2005). Grain protein contents along with grain size are indicators of grain quality but heat stress decreased grain protein contents that might be attributed to decrease in sedimentation index with decreased amino acids levels in grains (Dias *et al*., 2008). Gooding *et al*., (2003) reported that heat stress affected the grain protein contents during grain filling stage. Castro *at el*., (2007) reported the decreased grain protein contents of crop as heat stress decreased the grain yield. FA improved grain crude protein contents that might be attributed to improved remobilization and accumulation of photosynthates in grain and thus increasing grain yield. FA might have improved the response and performance of enzymes responsible for protein accumulation in grain.

Conclusion:

Keeping above facts in view it was concluded that fulvic acid helps to ameliorate heat stress through improving total soluble protein, repairing and enhancing chlorophyll a+b 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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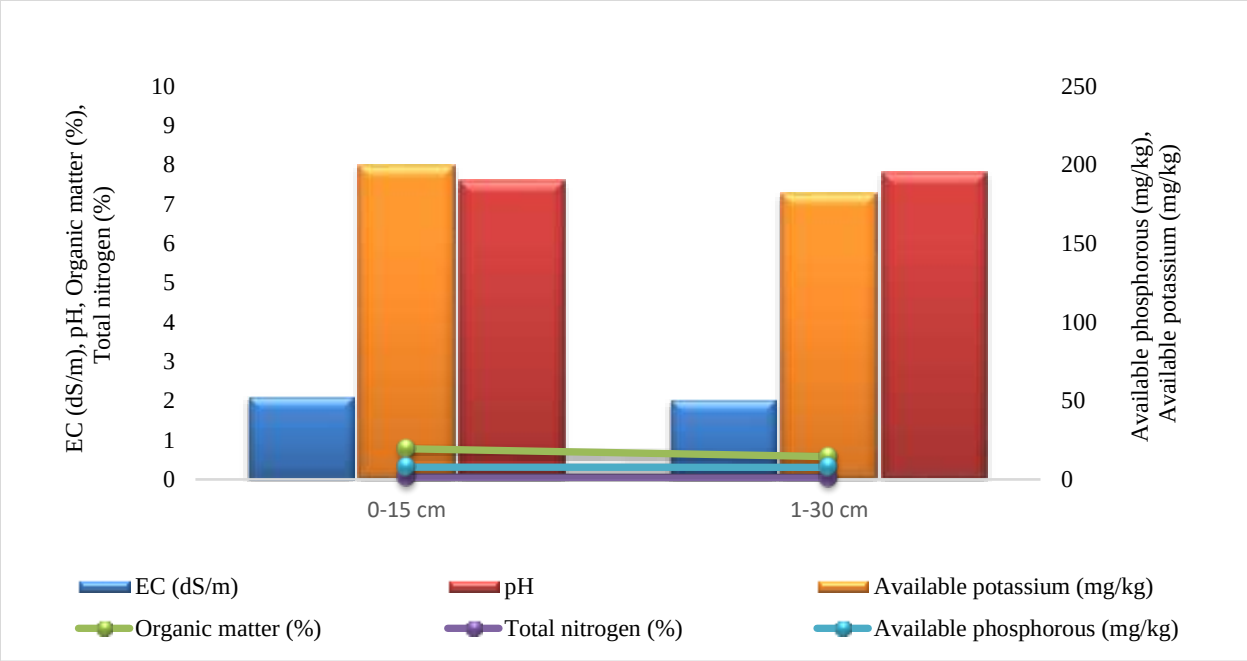

Figure 1: Monthly weather data during crop period

Table 1: Monthly averages of weather elements during growing season of wheat crop

Weather elements	November	December	January	February	March	April	May
Average temperature (°C)	18.9	12.2	11.7	16.5	19.1	27.0	31.8
Relative humidity (%)	61.7	75.0	75.3	66.0	64.0	43.9	27.5
Rainfall (mm)	10.0	0.0	12.2	20.5	67.9	32.8	17.0
Pan evaporation (mm)	1.8	1.5	1.0	2.1	13.0	5.3	7.6
Sunshine duration (hours)	7.6	4.7	5.0	5.6	4.9	9.1	10.4
Evapotranspiration (mm)	1.5	1.3	0.7	1.8	2.8	3.7	5.3
Wind speed (km h^{-1})	3.1	2.0	3.6	5.3	5.6	6.2	5.7

Table 2: Effect of foliar applied fulvic acid on total soluble protein, spike length and yield components of heat stressed wheat

Treatments	TSP (mg/g)	SL (cm)	Number of grains per spike	1000-grain weight (g)	GY (t ha^{-1})
H_0 (No heat stress)					
F_0 (Control)	0.467 a	11.33 a	43.0 b	39.50 b	5.30 c
F_1 (1.25 mg L^{-1})	0.490 a	12.47 a	49.0 a	43.27 a	6.03 a
F_2 (2.50 mg L^{-1})	0.476 a	11.87 a	48.3 a	42.74 a	5.75 ab
F_3 (3.75 mg L^{-1})	0.475 a	11.37 a	47.3 a	39.77 b	5.46 bc
H_1 (Heat stress at booting stage)					
F_0 (Control)	0.498 b	8.77 b	31.0 b	32.45 b	2.89 c
F_1 (1.25 mg L^{-1})	0.506 b	8.83 b	32.0 b	32.72 b	2.93 bc
F_2 (2.50 mg L^{-1})	0.538 ab	8.97 b	33.3 ab	33.79 ab	3.20 b
F_3 (3.75 mg L^{-1})	0.583 a	10.47 a	36.0 a	35.70 a	3.68 a
H_2 (Heat stress at grain initiation stage)					
F_0 (Control)	0.522 a	8.90 c	34.3 c	33.30 b	3.35 b
F_1 (1.25 mg L^{-1})	0.529 a	10.20 b	38.0 bc	34.13 b	3.62 b
F_2 (2.50 mg L^{-1})	0.553 a	11.73 a	42.3 a	37.54 a	4.37 a
F_3 (3.75 mg L^{-1})	0.543 a	11.40 ab	40.7 ab	37.33 a	4.17 a
Tukey's HSD ($p \leq 0.05$)	**0.0489**	**1.222**	**3.97**	**2.902**	**0.301**

Any two means not sharing a letter in common within a column differ significantly at $p \leq 0.05$; NS = Non-significant, TSP = Total Soluble Protein, SL = Spike length, GY = Grain Yield

Table 3: Effect of foliar applied Fulvic acid on leaf growth traits, chlorophyll a+b contents and leaf relative water contents of heat stressed wheat

Treatments	FLFW (g/plant)	FLDW (g/plant)	FLA (cm^2/plant)	Chl *a*+*b* (mg g^{-1} FW)	LRWC (%)
Heat stress (H)					
H_0 (No heat stress)	0.22 A	0.78 A	24.22 A	2.79 A	84.4 A
H_1 (Heat stress at booting stage)	0.13 B	0.58 B	17.82 B	1.88 B	74.2 B
H_2 (Heat stress at grain initiation stage)	0.15 B	0.64 B	19.96 B	2.10 B	76.9 AB
Tukey's HSD ($p \leq 0.05$)	**0.038**	**0.101**	**3.627**	**0.691**	**8.18**
Fulvic acid foliar spray (FA)					
F_0 (Control)	0.14 B	0.60 B	19.09 B	1.94 B	74.5 B
F_1 (1.25 mg L^{-1})	0.15 B	0.64 AB	19.78 B	2.20 AB	77.3 AB
F_2 (2.50 mg L^{-1})	0.19 A	0.72 A	22.56 A	2.56 A	81.1 A
F_3 (3.75 mg L^{-1})	0.18 A	0.70 A	21.21 AB	2.33 AB	81.2 A
Tukey's HSD ($p \leq 0.05$)	**0.026**	**0.092**	**2.653**	**0.402**	**5.52**

Any two means not sharing a letter in common differ significantly at $p \leq 0.05$; FLFW = Flag Leaf Fresh Weight, FLDW = Flag Leaf Dry Weight, FLA = Flag Leaf Area, Chl *a*+*b* = Chlorophyll *a*+b, LRWC = Leaf Relative Water Contents

Table 4: Effect of foliar applied Fulvic acid on number of fertile tillers, spikelet per spike and grain crude protein of heat stressed wheat

Treatments	Number of fertile tillers (m^{-2})	Spikelet per spike	Grain crude protein (%)
Heat stress (H)			
H_0 (No heat stress)	335 A	19.5 A	10.72 A
H_1 (Heat stress at booting stage)	307 B	16.7 C	7.86 B
H_2 (Heat stress at grain initiation stage)	318 AB	18.2 B	8.83 B
Tukey's HSD ($p \leq 0.05$)	**19.6**	**1.13**	**1.324**
Fulvic acid foliar spray (FA)			
F_0 (Control)	317	17.7	8.02 B
F_1 (1.25 mg L^{-1})	320	18.0	8.73 B
F_2 (2.50 mg L^{-1})	321	18.6	10.17 A
F_3 (3.75 mg L^{-1})	323	18.2	9.71 A
Tukey's HSD ($p \leq 0.05$)	**NS**	**NS**	**0.908**

Any two means not sharing a letter in common differ significantly at $p \leq 0.05$