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Research Article

Modulating the antioxidant defense systems and nutrients content by proline for higher yielding of wheat under water deficit

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Abstract

Numerous plant metabolites, especially amino acids, are accumulated as a result of stress. These amino acids are crucial for plant metabolism and development and have historically been viewed as the building blocks of proteins. Several studies suggested that there is a link between proline buildup and exposure plants to stress. Proline performs important functions under stress in addition to be a great osmolyte, antioxidant enzyme, acts as an antioxidant defense and signaling molecules. Two field trials were during two successive winter seasons (2020/2021 and 2021/2022). The effects of proline foliar application (0, 100, 200, 300 and 400 mg L^{-1}) and irrigation water levels (irrigation by 100, 80 and 60% of crop water requirements, CWR, CWR100, CWR80 and CWR60, respectively) on plant pigments, antioxidants activity, yield traits and nutrient contents of wheat were assessed. The experiment was designed in a split-plot involving 4 replicates. Drought stress (CWR80 and CWR60) led to reductions in photosynthetic pigments, and yield components. Under severe water stress (CWR60), proline 200 and 300 mg L^{-1} recorded the highest values of chlorophyll a, chlorophyll b, and total pigments. Application of proline 300 mg L^{-1} was the potent practice for enhancing the antioxidant activity%

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(DPPH radical scavenging) and phenols content under CWR100 and CWR80. Indole acetic acid (IAA) possessed the maximum values with proline 200 mg L^{-1} under all irrigation patterns. Under severe deficit water (CWR60), without proline spraying (for super oxide dismutase), 200, 300 or 400 mg L^{-1} proline (for peroxidase) and 200 or 400 mg L^{-1} proline (for polyphenol oxidase) recorded the highest values. Spraying proline 300 mg L^{-1} achieved the highest values of grain number $spike^{-1}$, weight of 1000 grains and grain yield ha^{-1} under different irrigation regimes. In conclusion, proline is considered a good mitigator for drought stress, due to it increased wheat plant tolerance to water deficiency throughout improving plant physiology and consequently yields quantity and quality. The most efficient concentrations of proline for coping the adverse impact of drought were 200 and 300 mg L^{-1}.

Keywords: cereal crops; deficit water; osmo-protectants; physiological defenders; wheat yield potential

Introduction

The limiting abiotic factor for agriculture globally is water scarcity. By 2025, crop production would gradually decline due to growing the deficiency of water (Kaya *et al.*, 2020; Darwesh *et al.*, 2021). Usually, drought stress stimulates the generation reactive oxygen species (ROS). Since ROS are venomous compounds, the degradation rate of vital cell components increases (Hussien *et al.*, 2015; El-Beltagi *et al.*, 2010, 2022). Accordingly, reducing in plant cell moisture content causes disturbance in physiological balance in crop plants (El-Bially *et al.*, 2022a; El-Metwally *et al.*, 2021; Makhlouf *et al.*, 2022; Saudy *et al.*, 2023). Therefore, plants that imposed to low water supply produce low yield (Saudy *et al.*, 2020a; Saudy and El-Metwally, 2023) and quality (Saudy and El-Metwally, 2019; Abd–Elrahman *et al.*, 2022; El-Metwally *et al.*, 2022a). Plants respond to the stress by producing more defensive enzymes such as superoxide dismutase which convert is the first which scavenge the H_2O_2 (Snezhkina *et al.*, 2019; El-Beltagi *et al.*, 2023a; Ramadan *et al.*, 2023). In response to different stresses plants accumulate large quantities of different types of compatible solutes (Serraj and Sinclair, 2002; Abd El-Mageed *et al.*, 2022; Shaaban *et al.*, 2023a; Shahin *et al.*, 2023).

Due to the natural genetic variety seen in grain crops, i.e., wheat, in addition to their food advantages, they rank among the most significant plants (Noureldin *et al.*, 2013; Miller *et al.*, 2019). Wheat meets about 21% of the global food needs while it is a unique fountainhead of protein (Hossain and da Silva, 2013; Saudy *et al.*, 2020b) as well numerous minerals and fibers (Saudy and Mubarak, 2015; Shewry and Hey, 2015).

Several attempts were performed to stimulate the growth and alleviate the negative effects of deficit water in several crop plants using various compounds (El-Bially *et al.*, 2018; El-Bially *et al.*, 2022b; El-Metwally *et al.*, 2022b; Saudy *et al.*, 2023). Plants protect themselves from ROS injury via enhancing the enzymatic antioxidant defense systems (Apel and Hirt, 2004; El-Beltagi *et al.*, 2023b; Shaaban *et al.*, 2023b). Proline as an amino acid known to occur widely and ordinarily cumulates in large quantities as a signal of ecological stresses in higher plants (Kavi-Kishore *et al.*, 2005). Proline is one of most solutes had the potential to adjust the osmotic pressure, detoxify ROS and stabilize cell membranes (Ashraf and Harris, 2004). Also, proline may act a potential role in maintaining and adjusting the balance between various forms of energy compounds ($NADP^+/NADPH_2$) in plant metabolism (Shamsult *et al.*, 2012).

The present work hypothesized that the external supply of proline could induce favorable changes in wheat metabolism helping the plants to be more drought tolerant. Therefore, the purpose of this work was to assess the prophylactic function of proline in relation to plant pigments and compatible solute as well as nutritional status and productivity of wheat exposed to drought.

Materials and Methods

The study area attributes

Along two growing seasons of 2020/2021 and 2021/2022, two field experiments were implemented at National Research Centre farm, El–Nubaria district, Egypt (30.8667 N and 31.1667 E). The study site had an arid climate with cool winters. Along the study period, there were no amounts of precipitations that could be taken into account. During the study seasons climate parameters were obtained monthly (Table 1). The initial physico-chemical traits and moisture status of the soil were estimated before sowing (Table 2). The acidity (pH) of irrigation water was 7.34 water with electrical conductance (EC) of 0.42 dS m^{-1}.

Table 1. Climatic parameters during 2020/21 and 2021/22 growing seasons of wheat at El–Nubaria district

Month	Air temperature (°C)			Wind speed (m sec^{-1})	Relative humidity (%)	Solar radiation (W m^{-2})
	Minimum	Maximum	Average			
2020/2021 season						
Decemberr	8.0	20.1	14.05	1.9	62.3	49.7
January	7.2	17.1	12.15	2.4	61.0	49.9
February	10.1	23.5	16.8	2.2	57.7	67.0
March	12.0	25.2	17.65	2.2	60.0	93.2
April	17.0	31.1	24.05	2.3	55.3	114.0
May	20.0	32.2	26.1	1.5	51.0	132.0
2021/2022 season						
Decemberr	9.1	22.6	15.85	2.1	61.7	49.5
January	7.3	18.1	12.7	2.6	66.0	50.0
February	11.2	23.4	17.3	2.4	57.0	68.0
March	12.3	28.3	20.3	2.6	56.0	95.0
April	18.2	30.6	24.4	2.4	52.0	113.0
May	21.1	33.8	27.45	1.7	49.0	135.0

Table 2. Initial physico-chemical traits and moisture status of the experimental site soil

Parameter	Unit	Value
Mechanical analysis		
Coarse sand	% by weight	47.8
Fine sand	% by weight	49.0
Clay+silt	% by weight	3.20
Chemical analysis		
pH (1:2.5)	--	8.40
Electrical conductivity (EC)	dS m^{-1}	0.30
Organic matter	%	0.75
Calcium carbonate ($CaCO_3$)	%	7.00
Water status		
Saturation percentage	% by volume	22.0
Field capacity	% by volume	10.8
Wilting point	% by volume	4.90

Experimental procedures and detail

The experiment was designed in a split-plot involving 4 replicates. The experimental unit size was 10.5 m2. Three irrigation patterns (100%, 80% and 60% of wheat crop water requirements, CWR) were arranged in the main plots. The sub-plots were assigned to five proline concentrations (0, 100, 200, 300 and 400 mg L^{-1}).

After preparing the land, wheat cultivar ('Giza 171') grains were sown at the end of November. About 168 kg seed per hectare was sown. The drilling machine was adjusted to place the seeds on 5 cm deep and distance of 13.5 cm between rows. All experimental units were fertilized with a similar amount of total fertilizers. Single superphosphate, 70 Kg P_2O_5 ha^{-1}, was added to the soil before planting. Nitrogen at a rate of 285 Kg N ha^{-1}, as NH_4NO_3, was added prior each irrigation in six portions, ceased before heading stage. Potassium sulphate, 60 Kg K_2O ha^{-1}, applied once after one month from sowing.

Irrigation application

According to the assessed climatic parameters of the study area, reference evapotranspiration (ET_0) was computed by the Penman-Monteith equation (Allen et al. 1998). Then crop evapotranspiration (ET_c) was obtained as follows:

$$ET_c = ET_o \times K_c \qquad \text{(Equation 1)}$$

where:
ET_c = Crop evapotranspiration (mm day^{-1})
ET_o = Reference crop evapotranspiration (mm day^{-1})
K_c = Crop coefficient

Afterwards, the amount of water for sprinkler irrigation system was computed according to the following equation:

$$AW = ET_c / E_a \text{ x } (1 - LR) \qquad \text{(Equation 2)}$$

where:
AW = depth of applied irrigation water (mm day^{-1})
E_a = application efficiency = 75%
LR = leaching requirements = 10%.

Biochemical compounds and nutrients analysis

At 90 days after sowing, samples of leaves were taken to measure the following:

Photosynthetic pigments

Some biochemical constituents, in fresh leaves, including photosynthetic pigments (chlorophyll *a* and *b*, carotenoids and total pigments) were estimated (Lichtenthaler and Buschmann, 2001).

Plant metabolites

The antioxidant activity, DPPH, (Liyana-Pathiranan and Shahidi, 2005); phenolic content (Maurya and Singh, 2010); total soluble sugars, TSS, (Gomez *et al.*, 2002 and Albalasmeh *et al.*, 2013), total soluble protein, TSP, (Bonjoch and Tamayo 2001) and indole acetic acid content (Gusmiaty *et al.*, 2019) were extracted and assessed.

Antioxidant enzymes

The activity of the enzymes was assayed differently based on their types. By exploiting the methodology of Chen and Wang (2006), the different enzyme extractions were prepared. The activities of super oxide dismutase (Chen and Wang, 2006); peroxidase (Kumar and Khan, 1982) and polyphenol oxidase (Cho and Ahn, 1999) were measured

Crude protein, P and K content

In wheat flag leaf, phosphorus and potassium Cottenie *et al.* (1982), and total crude protein (AOAC, 2012) were gauged.

Yield traits

At the beginning May harvesting was performed. Plants of 1.0 m^2 meter of each plot were gathered to evaluate the yield parameters. Hither, grain number $spike^{-1}$, 1000-grain weight, and grain yield were estimated

Statistical analysis

Being the homogeneity tested showed the non-significant the two seasons for most studied traits, the combined analysis was carried out. Further, the statistical analysis of the data was performed by MSTAT-C program. For distinguish the variations among treatments, the mean values were separated by the least significant range (LSR) with the probability level of 5% (Casella, 2008). Furthermore, the principal component analysis (PCA) and agglomerative hierarchical clustering (AHC) were estimated using XLSTAT 2022® (Addinsoft, Paris, France).

Results

Photosynthetic pigments

The data in Table 3 illustrated the influence of different tested concentrations of proline (0, 100, 200, 300 and 400 mg L^{-1}) on (chlorophyll a and b, carotenoids and total pigments) in wheat leaves under diversified regimes of CWR. Decreasing the CWR from 100 to 80 and 60% resulted in remarkable ($P<0.05$) decreases for all photosynthetic pigments, except carotenoids which showed the reverse picture (increased). As for proline spraying treatment effect, its worthy to mention that most tested concentrations significantly ($P<0.05$) incremented chlorophylls and total pigments, while it reduced carotenoids.

Generally, the interaction revealed that the maximal improvements in plant pigments were obtained via 300 and 200 mg L^{-1} proline under different CWR levels. Specifically, proline at concentration of 400 mg L^{-1} (for chlorophyll a), 300 mg L^{-1} (for chlorophyll b) as well as both of 300 and 400 mg L^{-1} (for total pigments) gave the highest values under normal irrigation. While, proline 200 mg L^{-1} exhibited higher values of chlorophyll a and b, and total pigments under 80% of CWR. Under 60% of CWR, proline 200 and 300 mg L^{-1} recorded the highest values of chlorophyll a and b, and total pigments. Under 60% of CWR, without proline spray as well as with spraying of 100 or 400 mg L^{-1} proline, the highest content of carotenoids was observed.

Table 3. Effect of proline concentration (mg L^{-1}) on photosynthetic pigments (mg/g fresh wt.) of wheat plants grown under different irrigation regimes

Variable		Chlorophyll a	Chlorophyll b	Carotenoids	Total pigments
Irrigation regime (Irr)					
CWR100		20.62^{a}	7.06^{a}	1.35^{c}	29.03^{a}
CWR80		17.58^{b}	7.02^{a}	1.62^{b}	26.23^{b}
CWR60		16.51^{c}	5.08^{b}	2.23a	23.82^{c}
Proline concentration (Pro)					
0		16.90^{b}	5.71^{c}	1.87^{a}	24.48^{c}
100		17.50^{b}	6.42^{b}	1.91^{a}	25.83^{b}
200		18.73^{a}	7.20^{a}	1.59^{b}	27.53^{a}
300		19.39^{a}	6.98^{a}	1.67^{b}	28.04^{a}
400		18.67^{a}	5.64^{c}	1.63^{b}	25.94^{b}
Irr x Pro					
CWR100	0	18.50^{cd}	6.65^{bc}	1.35^{hij}	26.50^{d}
	100	19.83^{bc}	7.28^{b}	1.62^{efg}	28.73^{bc}
	200	19.93^{bc}	7.40^{b}	1.44^{ghi}	28.77^{bc}
	300	21.25^{b}	8.87^{a}	1.20^{ij}	31.33^{a}
	400	23.59^{a}	5.13^{efg}	1.12^{j}	29.84^{ab}
CWR80	0	16.73^{ef}	5.95^{cde}	1.87^{cd}	24.55^{ef}
	100	16.49^{ef}	7.33^{b}	1.83^{de}	25.65^{de}
	200	19.42^{cd}	8.70^{a}	1.19j	29.32^{b}
	300	19.01^{cd}	6.42^{cd}	1.69^{def}	27.11^{cd}
	400	16.27^{f}	6.72^{bc}	1.54^{efg}	24.52^{ef}
CWR60	0	15.46^{f}	4.55^{g}	2.38^{a}	22.38^{g}
	100	16.17^{f}	4.64^{g}	2.30^{ab}	23.11^{fg}
	200	16.85^{ef}	5.51^{ef}	2.13^{b}	24.49^{ef}
	300	17.91^{de}	$5.65d^{ef}$	2.11^{bc}	25.67^{de}
	400	16.14^{f}	5.07^{efg}	2.24^{ab}	23.44^{g}

CWR100, CWR80 and CWR60: Irrigation by 100, 80 and 60% of crop water requirements, CWR, respectively. Each value indicates mean ± standard error (n=4). Means values in each column followed by the same lower-case letter are not significantly different according to the Duncan test ($p \leq 0.05$).

Plant metabolites

Findings in Table 4 depicted that deficit irrigation statistically ($P<0.05$) lowered the antioxidant activity (DPPH), total soluble protein (TSP) and indole acetic acid (IAA) in wheat leaves. Unlike, such stress increased total soluble sugars (TSS) and phenols contents. All foliar spraying of tested proline concentrations markedly ($P<0.05$) increased all compatible solutes in wheat plant leaves. Such effect was more evident for almost traits with 200 and 300 mg L^{-1} proline. However, TSS and TSP recorded the maximal values with application of proline 400 mg L^{-1}

Concerning the interaction effects on compatible solutes contents, it should be observed that there were diverse responses of proline concentrations under different water supplies. For instance, application of proline 300 mg L^{-1} was the potent treatment for enhancing DPPH% and phenols content under CWR100 and CWR80. While, these parameters achieved the maximal values with spraying of proline 200 mg L^{-1} under CWR60. Moreover, TSS and TSP values were higher under CWR100 x proline 400 mg L^{-1}, CWR80 x proline 400 mg L^{-1} and CWR60 x proline 300 mg L^{-1}. IAA possessed the maximum values with proline 200 mg L^{-1} under all irrigation patterns.

Table 4. Effect of proline concentration (mg L^{-1}) on compatible solutes contents of wheat plants grown under different irrigation regimes

Variable		Antioxidant compounds		Osmo-protectants (mg/100 g fresh wt.)		IAA (μg/g fresh wt.)
		DPPH %	Phenols (mg/100 g fresh wt.)	TSS	TSP	
Irrigation regime (Irr)						
CWR100		43.26^{a}	54.09^{c}	228.43^{c}	793.10^{a}	29.87^{a}
CWR80		35.44^{b}	61.85^{b}	275.73^{b}	775.53^{a}	27.51^{b}
CWR60		28.74^{c}	65.84^{a}	295.29^{a}	497.51^{b}	24.44^{c}
Proline concentration (Pro)						
0		32.28^{b}	28.63^{e}	201.88^{d}	424.96^{d}	23.16^{d}
100		34.96^{b}	33.69^{d}	245.51^{c}	679.72^{c}	26.69^{c}
200		43.46^{a}	69.29^{c}	250.86^{c}	650.30^{c}	32.20^{a}
300		40.63^{a}	96.82^{a}	288.41^{b}	781.78^{b}	28.53^{b}
400		27.74^{c}	74.51^{b}	345.78^{a}	906.80^{a}	25.79^{c}
Irr x Pro						
CWR100	0	42.24^{b}	13.05^{i}	173.91^{k}	548.06^{f}	24.90^{gh}
	100	45.83^{a}	25.26^{h}	186.52^{jk}	750.50^{c}	28.48^{bcd}
	200	45.61^{a}	59.11^{f}	225.22^{hi}	700.50^{cd}	36.97^{a}
	300	45.95^{a}	91.35^{b}	253.91^{fgh}	1041.83^{b}	30.35^{bc}
	400	36.68^{c}	81.67^{c}	302.61^{cd}	924.61^{b}	28.67^{cd}
CWR80	0	29.47^{e}	23.91^{h}	204.78^{ij}	405.39^{gh}	23.47^{hij}
	100	33.70^{d}	25.51^{h}	271.30^{efg}	747.28^{c}	26.94^{defg}
	200	38.20^{c}	66.20^{e}	247.37^{gh}	686.74^{cde}	31.60^{b}
	300	41.59^{b}	122.47^{a}	299.13^{cde}	748.72^{c}	29.45^{bcd}
	400	34.25^{d}	71.13^{de}	356.09^{b}	1289.50^{a}	26.11^{efg}
CWR60	0	25.12^{f}	48.92^{g}	226.96^{hi}	321.44^{h}	21.12^{j}
	100	25.35^{f}	50.31^{g}	278.70^{def}	541.39^{fg}	24.66^{ghi}
	200	46.58^{c}	82.56^{c}	280.00^{def}	563.65^{def}	28.03^{cdef}
	300	34.36^{g}	76.64^{cd}	312.17^{c}	554.78^{ef}	25.80^{fgh}
	400	12.29^{h}	70.74^{de}	378.65^{def}	506.28^{fg}	22.58^{ij}

CWR100, CWR80 and CWR60: Irrigation by 100, 80 and 60% of crop water requirements, CWR, respectively. Each value indicates mean ± standard error (n=4). Means values in each column followed by the same lower-case letter are not significantly different according to the Duncan test ($p \leq 0.05$).

The significant ($P<0.05$) effects of irrigation regimes (Figure 1) and proline concentrations (Figure 2) and their interaction (Figure 3) on the activity of super oxide dismutase (SOD), peroxidase (POX) and polyphenol oxidase (PPO) were observed. The activity of all tested antioxidant enzymes increased with increasing the degree of drought, where CWR60 caused increments in SOD by 1.21 and 1.31 times, POX by 1.34 and 1.86 times and PPO by 1.38 and 1.58 times greater than CWR80 and CWR100, respectively. Proline concentrations of 200 or 300 mg L^{-1} showed the highest values of SOD. Moreover, POX and PPO values were higher with spraying of 200 mg L^{-1} and 300 mg L^{-1}, respectively.

Under severe deficit water (CWR60), without proline spraying (for SOD), 200, 300 or 400 mg L^{-1} proline (for POX) and 200 or 400 mg L^{-1} proline (for PPO) recorded the highest values.

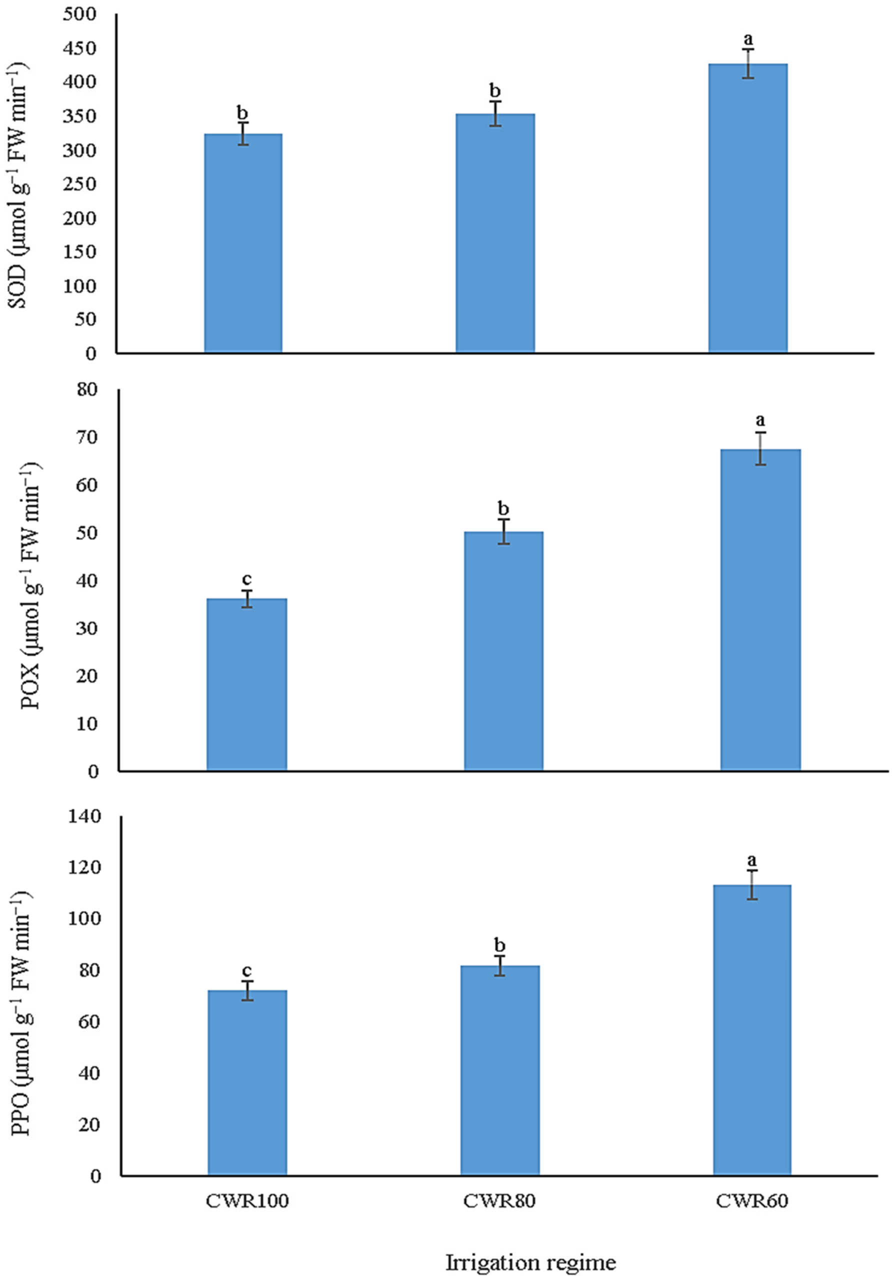

Figure 1. Effect of irrigation regime on super oxide dismutase (SOD), peroxidase (POX) and polyphenol oxidase (PPO) activity in wheat

CWR100, CWR80 and CWR60: Irrigation by 100, 80 and 60% of crop water requirements, CWR, respectively. Each value indicates mean ± standard error (n=4). Means values in each column followed by the same lower-case letter are not significantly different according to the Duncan test ($p \leq 0.05$).

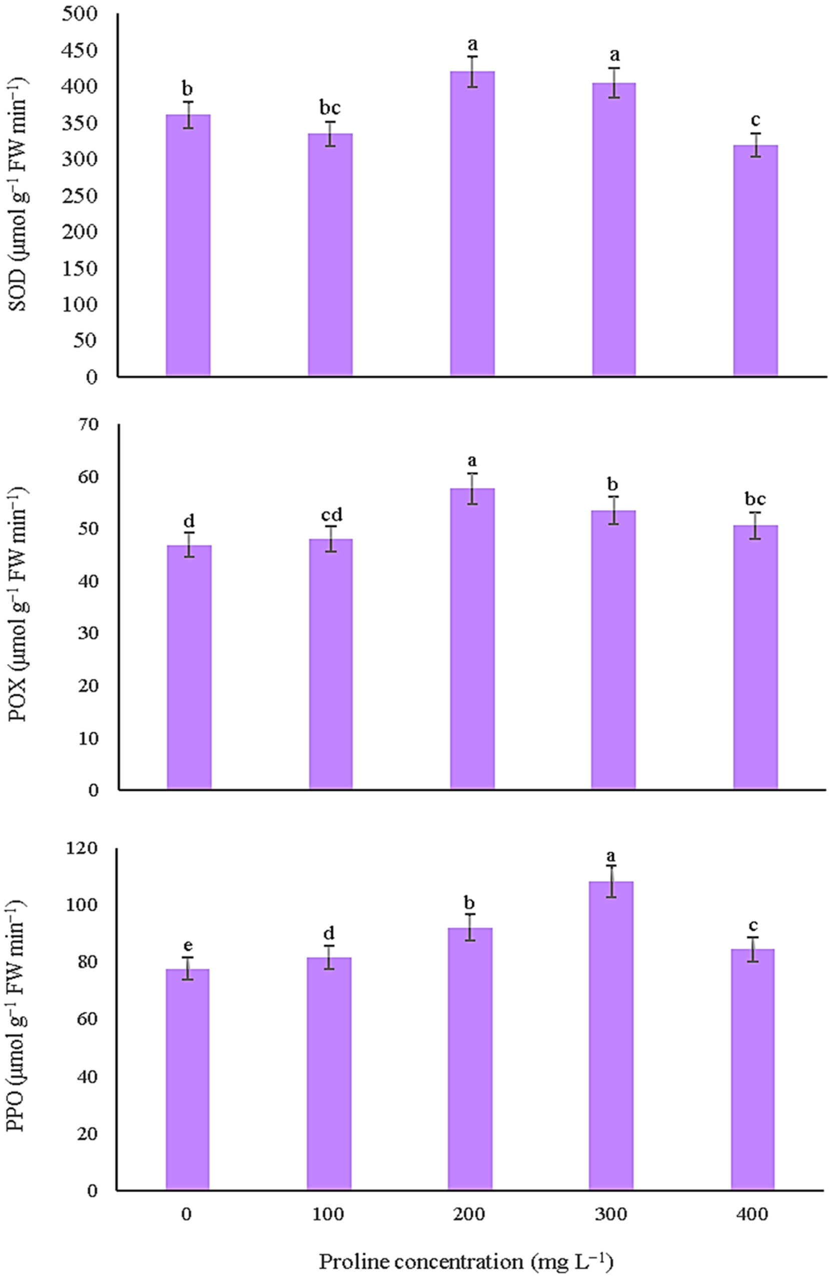

Figure 2. Effect of proline concentration on super oxide dismutase (SOD), peroxidase (POX) and polyphenol oxidase (PPO) activity in wheat

Each value indicates mean ± standard error (n=4). Means values in each column followed by the same lower-case letter are not significantly different according to the Duncan test ($p \leq 0.05$).

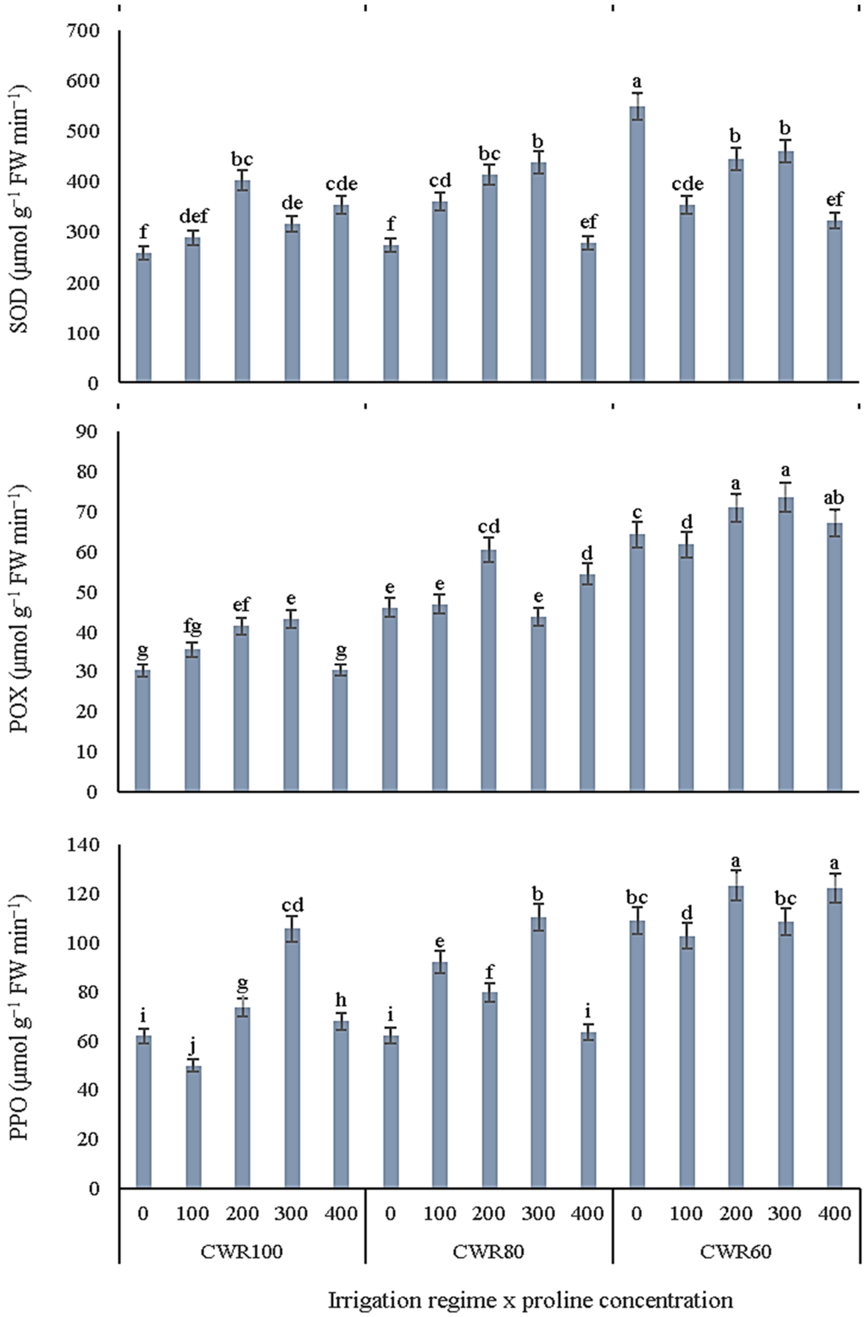

Figure 3. Interaction effect of proline concentration and irrigation regime on super oxide dismutase (SOD), peroxidase (POX) and polyphenol oxidase (PPO) activity in wheat

CWR100, CWR80 and CWR60: Irrigation by 100, 80 and 60% of crop water requirements, CWR, respectively. Each value indicates mean ± standard error (n=4).

Means values in each column followed by the same lower-case letter are not significantly different according to the Duncan test ($p \leq 0.05$).

Crude protein, P and K content

As shown in Table 5 low water supply caused distinctive ($P<0.05$) decreases in crude protein, P and K% in flag leaf of wheat. Treating wheat plants with 200 and 300 mg L^{-1} proline (for crude protein and P%) and 300 mg L^{-1} (for K%) caused the maximal increases. Under all irrigation regimes, proline 300 mg L^{-1} achieved the highest values of crude protein, P% and K%, significantly equalling proline 200 mg L^{-1} x any irrigation pattern (for crude protein), proline 200 mg L^{-1} x CWR60 (for P%) and proline 200 mg L^{-1} x CWR100 (for K%).

Table 5. Effect of proline concentration (mg L^{-1}) on crude protein, phosphorus and potassium content in flag leaf of wheat plants grown under different irrigation regimes

Variable		Crude protein %	Phosphorus %	Potassium %
Irrigation regime (Irr)				
CWR100		2.49^{a}	0.244^{a}	2.84^{a}
CWR80		2.37^{a}	0.232^{b}	2.67^{b}
CWR60		2.20^{b}	0.212^{c}	2.49^{b}
Proline concentration (Pro)				
0		2.16^{bc}	0.201^{c}	2.41^{c}
100		2.35^{b}	0.235^{b}	2.65^{b}
200		2.62^{a}	0.255^{a}	2.90^{b}
300		2.66^{a}	0.268^{a}	3.18^{a}
400		1.99^{c}	0.187^{c}	2.21^{c}
Irr x Pro				
CWR100	0	2.28^{ef}	0.212^{f}	2.62^{def}
	100	2.58^{bcd}	0.248^{d}	2.70^{de}
	200	2.80^{a}	0.270^{bc}	3.22^{ab}
	300	2.76^{ab}	0.290^{a}	3.33^{a}
	400	2.06^{gh}	0.199^{g}	2.35^{fg}
CWR80	0	2.14^{fg}	0.200^{g}	2.42^{fg}
	100	2.38^{de}	0.235^{e}	2.72^{de}
	200	2.60^{abc}	0.260^{c}	2.86^{cd}
	300	2.75^{ab}	0.275^{b}	3.18^{ab}
	400	2.00^{gh}	0.190^{g}	2.20^{gh}
CWR60	0	2.05^{gh}	0.190^{g}	2.20^{gh}
	100	2.10^{fgh}	0.223^{f}	2.55^{ef}
	200	2.46^{cde}	0.235^{e}	2.61^{def}
	300	2.47^{cde}	0.240^{de}	3.03^{bc}
	400	1.91^{h}	0.172^{h}	2.07^{h}

CWR100, CWR80 and CWR60: Irrigation by 100, 80 and 60% of crop water requirements, CWR, respectively. Each value indicates mean ± standard error (n=4). Means values in each column followed by the same lower-case letter are not significantly different according to the Duncan test ($p \leq 0.05$).

Yield and yield components

Deficit irrigation (drought stress) at 80% and 60% decreased wheat yield traits (grain number $spike^{-1}$, weight of 1000 grains and grain yield) Table 6, compared to the normal irrigation. Otherwise, spraying proline 300 mg L^{-1} caused increases in grain number $spike^{-1}$, weight of 1000 grains and grain yield amounted to 13.9, 16.0 and 31.2%, respectively, higher than the control. Application of proline 300 mg L^{-1} was the efficient treatment for enhancing all yield attributes under different irrigation regimes. Compared to the corresponding control treatment (without proline) under each irrigation pattern, the increases in grain yield owing to spraying proline 300 were 27.6, 31.2 and 35.3% under CWR100, CWR80 and CWR60, respectively.

Table 6. Effect of proline concentration (mg L^{-1}) on grain number spike^{-1}, weight of 1000 grains grain yield of wheat grown under different irrigation regimes

Variable		Grain number spike^{-1}	Weight of 1000 grains (g)	Grain yield (t ha^{-1})
Irrigation regime (Irr)				
CWR100		55.77^{a}	35.22^{a}	6.12^{a}
CWR80		53.78^{b}	33.77^{b}	5.83^{b}
CWR60		49.46^{c}	31.09^{c}	5.00^{c}
Proline concentration (Pro)				
0		50.28^{d}	31.60^{c}	5.10^{d}
100		52.65^{c}	33.28^{b}	5.78^{c}
200		55.02^{b}	35.40^{a}	6.30^{b}
300		57.28^{a}	36.67^{a}	6.69^{a}
400		49.78^{e}	29.85^{c}	4.38^{e}
Irr x Pro				
CWR100	0	52.6^{de}	33.40^{d}	5.66^{de}
	100	55.45^{c}	35.25^{c}	6.09^{c}
	200	58.25^{b}	37.00^{b}	6.89^{ab}
	300	61.00^{a}	38.70^{a}	7.22^{a}
	400	51.55^{ef}	31.75^{e}	4.77^{g}
CWR80	0	50.60^{fg}	31.85^{e}	5.23^{f}
	100	53.40^{d}	33.20^{d}	5.95^{cd}
	200	55.75^{c}	36.60^{b}	6.63^{b}
	300	58.25^{b}	37.70^{ab}	6.86^{ab}
	400	50.88^{f}	29.50^{f}	4.47^{g}
CWR60	0	47.63^{hi}	29.55^{f}	4.42^{g}
	100	49.10^{gh}	31.40^{e}	5.3^{ef}
	200	51.07^{ef}	32.60^{de}	5.39^{ef}
	300	52.60^{de}	33.60^{d}	5.98^{cd}
	400	46.90^{i}	28.30^{f}	3.90^{h}

CWR100, CWR80 and CWR60: Irrigation by 100, 80 and 60% of crop water requirements, CWR, respectively. Each value indicates mean ± standard error (n=4). Means values in each column followed by the same lower-case letter are not significantly different according to the Duncan test ($p \le 0.05$).

As presented in Figure 4, to correlate between the treatments and the biochemical and yield components, principle component analysis PCA- bi plot was performed, two groups of variables were observed in PCA- bi plot, whereas the first group contain all the high level of proline 300 and 400 mg.L^{-1} combined with CWR 80 and 100, correlated with TSS, TSP, T. phenols, Chlorophyll a, T. pigments and IAA. The second group correlated with lower levels of proline and the most important variables are the yield, grain number and weight of 1000 grain in addition to crude protein and carotenoids and DPPH antiradical scavenging. As shown in Figure 5, the agglomerative hierarchical clustering (AHC), cluster 1(C1) contain the lower levels of proline and C2 with higher level.

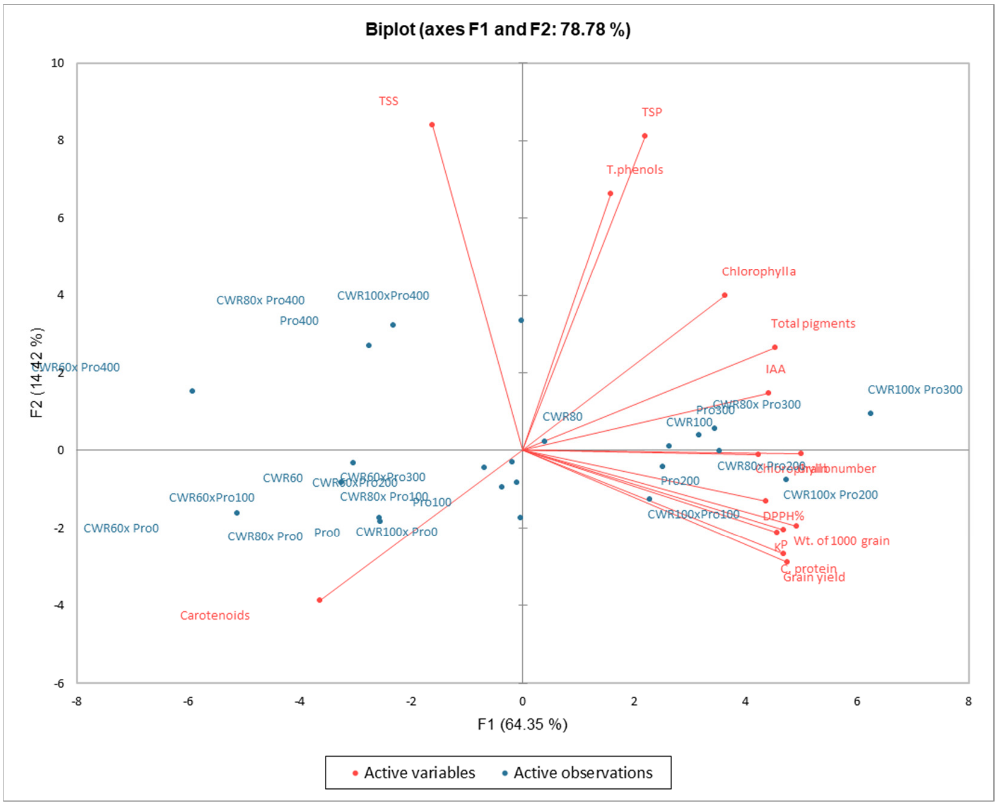

Figure 4. PCA-–bi Plot of wheat samples

Observations are: CWR100, CWR80, CWR60, Pro0, Pro100, Pro200, Pro300, Pro400, CWR100Pro0, CWR100Pro100, CWR100Pro200, CWR100Pro300, CWR100Pro400, CWR80Pro0, CWR80Pro100, CWR80Pro200, CWR80Pro300, CWR80Pro400, CWR60Pro0, CWR60Pro100, CWR60Pro200, CWR60Pro300, CWR60Pro400

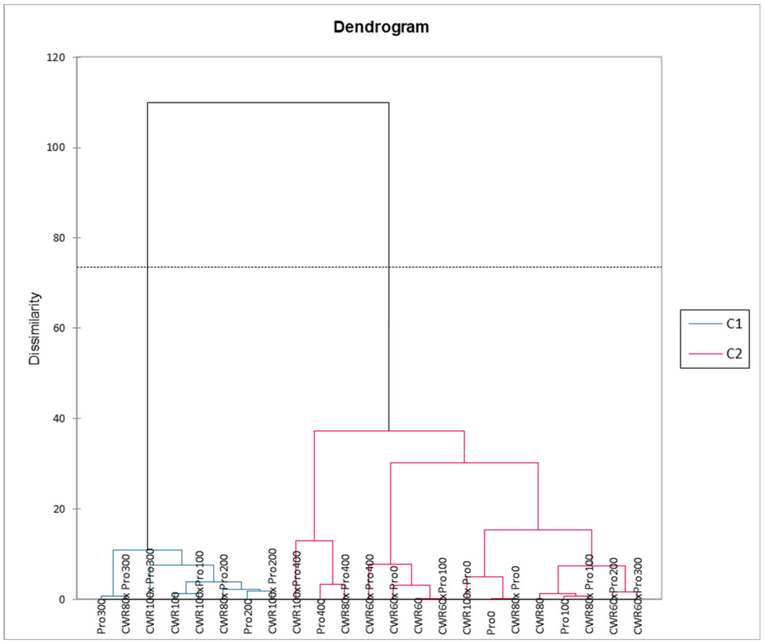

Figure 5. Agglomerative hierarchical clustering (AHC) / Number of clusters = 2.

Discussion

Photosynthetic pigments

The trend of photosynthetic pigments data obtained herein are in accordance with previous investigations. Herein, El-Metwally *et al.* (2016) stated that drought stress reduced the photosynthetic pigments of quinoa. Rong-Hua *et al.* (2006) attributed the reduced chlorophyll content in most stress-affected plants to the disruption of thylakoid membranes accompanied by more retrogradation than synthesis of chlorophyll through the fashioning chlorophyllase, an individual of proteolytic enzymes. Drought stress found to disrupt the plant pigments and cause incessant weakness in the plant photosystems and reduce gaseous mutuality resulting in decreasing growth parameters and productivity (Anjum *et al.*, 2011; Abd El Mageed *et al.*, 2023). In this domain, stem water potential of olive plants found to be affected by the obtainable moisture level, resulting in weak carbon assimilation rate and stomatal conductance when plants irrigated with only 60% of irrigation water requirements. It was suggested also that closing the stomata is the prevalent shackle to photosynthesis, since there is strong correlation between photosynthetic rate and the exchange of gases via stomata pores in the stressed plants. Moreover, plants under water deficiency conditions followed the physiological processes of chlorophyll dissolution and stimulating carotenoids synthesis as a maintenance strategy (Doupis *et al.*, 2013; Mohamed *et al.*, 2018).

Proline with various concentrations at 100, 200, 300 and 400 mg L^{-1} increased photosynthetic pigments comparing to the control, except carotenoids (Table 3). Carotenoids are one of the constituents in plants that plays more than one role, since these pigments have a major influence in photosynthesis and the defensive mechanisms against oxidative stresses (Gill and Tuteja, 2010). Noreen *et al.* (2018) emphasized the ROS was reduced and of chlorophylls content was increased under environmental stress of copper by proline spraying (80 mM) on wheat varieties. Reduction in photosynthetic pigments due to drought stress was ameliorated when quinoa plants sprayed with proline at the rates of 12.5 and 25 mM (Elew *et al.*, 2017). It has been proposed that proline enhances the biosynthesis and/or restrains the degradation of chlorophylls of salt-suffered bean plants (El-Metwally *et al.*, 2016). They attributed this effect to the improved efficiency in scavenging of ROS, which have damaged effect to chlorophyll, by antioxidant enzymes and cellular antioxidants constituents.

Compatible solutes content

Concerning the compatible solutes content in wheat leaf, it has been documented that TSS had a potential impact on adjusting the osmotic balance and scavenging the radicals in water stressed linseed (Bakry *et al.*, 2012). Moreover, increases in TSS and proline were obtained in plants that suffer low moisture conditions, preserving proteins, membranes and high cell moisture (Hellal *et al.*, 2019; Saudy *et al.*, 2021). As well, Anjum *et al.* (2011) demonstrated that proline acting as stabilizer for membranes and some macromolecules as well as a free radical scavenger via providing carbon and nitrogen. A decrease in proline oxidase activity was noticed with numerous abiotic stresses which motivate proline synthesis in plant tissues, and this might play an essential role to counter the damages caused by ROS (Bakry *et al.* (2012). Recently, Abdallah *et al.* (2019) and El-Sayed *et al.* (2020) A significant enhancement in the compatible solutes (free amino acids, soluble sugar and proline) production was recorded in wheat and peanut plants under water stress conditions, while depression was found in values of DPPH%, phenolic compounds and IAA. As a biochemical response to drought, soluble sugars increase (Murakeozy *et al.*, 2003) which may scavenge ROS, hence stabilizing the cell membranes (Hosseini *et al.* 2014). Moreover, phenolic compounds, as cell walls constituents, had distinctive effects on growth regulation and the different stresses tolerance (Cheynier *et al.*, 2013). Further, IAA performs a physiological action in inducing cell development, increasing the plant growth (Taize and Zeiger, 2006).

Antioxidant enzymes

As shown in Figure 1, drought caused remarkable ($P<0.05$) increases in SOD, POX and PPO of tested wheat plants. Also, foliar spraying with proline supported the stress tolerance by stimulating SOD, POX and PPO activities (Figure 2). Drought stress increases ROS (Ibrahim and Abdellatif, 2016), while the antioxidants endue vigorous precaution against oxidative harm via enhancing the lifetime of ROS (Sharma and Dubey, 2007; Afify *et al.*, 2012). The antioxidant enzymes are the first response strategy against stresses and express detoxification of superoxide and H_2O_2 mechanism (Esfandiari *et al.*, 2007; Jaleel *et al.*, 2007). Noreen *et al.* (2018) pointed out that proline stimulated the activities superoxide dismutase, catalase and peroxidase which are the first barrier of deleterious impacts of ROS.

Crude protein, P and K content

Since drought influenced the nutrient availability crop plants (Salem *et al.*, 2021; Mubarak *et al.*, 2021) nutrient accumulation reduced (Salem *et al.*, 2022; El-Metwally and Saudy, 2021). On the other hand, wheat plants retained maximal K^+ content owing to the foliage supply of proline (Noreen *et al.*, 2018). Also,

Chemical constituents expressed in P, K, N, protein and carbohydrates exhibited substantial decreases because of dropping irrigation (Shaaban *et al.*, 2023b). While, it is confirmed that proline acts as a molecular factor capable of protecting the integrity of the protein that enhances the activities of various enzymes (Murmu *et al.*, 2017). Similar actions are the obstruction of protein aggregation during stress, protection of nitrate reductase and steadiness of ribonucleases and proteases during stress (Sharma and Dubey, 2005). Khedr *et al.* (2003) suggested that stress tolerance could be improved by increased proline concentrations throughout enhancing the cumulation of stress-related protective proteins.

Yield and yield components

As expected, reductions in yield traits of wheat due to deficit water were obtained. This might attribute to the reduction in plant pigments (Table 3) with disturbance in plant physiological status (Table 4) as documented El-Sayed *et al.* (2020). It is well known that imposing crop plants to any stress type causes disturbance in plant physiology, hence the final economic product reduced (Saudy, 2014; Saudy, 2015; Saudy *et al.*, 2018; Saudy *et al.*, 2020c). Meanwhile, spraying wheat plants with proline could alleviate the adverse effects of drought-stressful, hence, increase yield traits. Herein, it should be noted that the potent treatment was 300 mg L^{-1} of proline under unstressed and drought stressed situations. The obtained results are in harmony with those recorded by Hoque *et al.* (2008) and Hasanuzzaman *et al.* (2012). At drought level, proline as osmo-protectant increased the water inflow (Joseph *et al.*, 2015), adjusting plant cell moisture status (Ahmed *et al.*, 2010), hence, improved wheat growth and yield.

Conclusions

Wheat is considered the first strategic crop worldwide. In the current study and during two successive seasons it has proved that the deleterious effect of drought in wheat could be mitigated by foliar treatments with proline. Not only the biochemical components (plant pigments, phenols, total soluble sugars, total soluble protein and indole acetic acid) were improved by foliar proline treatment but also the antioxidant activity (dismutase, peroxidase and polyphenol oxidase) and yield traits. Proline foliar treatment at different levels of crop water requirements reduced the oxidative damage of drought activating the antioxidant defensive components including enzymatic and non- enzymatic systems, hence grain number spike^{-1}, weight of 1000 grains and grain yield ha^{-1} improved. Further molecular researches are needed to assess the mode of action of proline to enhancing the tolerance to the physiological effects of drought stress.

Authors' Contributions

Conceptualization: MLH, KMAR, HSEB, AAR, IME-M, TAS, ESB and HSS; Data curation: MLH, KMAR, HSEB, AAR, IME-M, TAS, ESB and HSS; Formal analysis: AAR, IME-M, ESB and HSS; Funding acquisition: MLH, KMAR, HSE-B, and TAS; Investigation: HSEB, KMAR and HSS; Methodology: AAR, IME-M, ESB and HSS; Project administration: HSEB, KMAR and HSS; Resources: AAR, IME-M, ESB and HSS; Software AAR, IME-M, ESB and HSS; Supervision: HSEB, KMAR and HSS; Validation: MLH, KMAR, HSEB, AAR, IME-M, TAS, ESB and HSS; Visualization MLH, KMAR, HSEB, AAR, IME-M, TAS, ESB and HSS; Writing - original draft: AAR, IME-M, ESB and HSS; Writing - review and editing: MLH, KMAR, HSEB, AAR, IME-M, TAS, ESB and HSS; All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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