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Enhancing Barley Growth and Yield under Drought Stress through Exogenous Folic Acid Application

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CLIMATE change represents a pervasive global challenge and serves as a significant constraint on agricultural yields, jeopardizing food production worldwide. Drought stress stands out as one of the primary challenges confronting the agricultural sector. The scarcity of water resources adversely affects various physiological parameters, growth, and overall productivity of plants. The application of folic acid (FA) through foliar treatment may help mitigate the detrimental effects associated with water limitations. A study was conducted at the National Research Center's Experimental Station in Al-Nubaria District, El-Behaira Governorate, Egypt, to assess the effects of foliar-applied folic acid on barley plants under different irrigation levels. The study examined two concentrations of folic acid (5 and 10 mg/l) in combination with three irrigation levels (100%, 75%, and 50% of water irrigation requirements). The research aimed to assess the interaction of these factors on productivity and various physiological parameters. Moderate and severe water stress (at 75% and 50% of water irrigation requirements, respectively) significantly decreased yield and its components as well as adverse effects on physiological traits, including leaf photosynthetic pigments and endogenous Indole acetic acid (IAA). Conversely, there was an increase in certain osmolytes, such as total soluble sugars (TSS), proline, and phenolic compounds. Additionally, the application of folic acid at varying concentrations not only enhanced the yield of barley plants but also alleviated the negative impacts of water stress by improving the studied physiological parameters, including photosynthetic pigments and IAA, along with osmolytes and phenolic content. Moreover, folic acid treatment at different concentrations positively influenced the nutritional quality of the harvested grains, as indicated by higher levels of nitrogen, phosphorus, potassium, and protein percentages compared to untreated plants across the three irrigation levels. The higher concentration of folic acid (100 mg/l) proved to be more effective than the lower concentration (50 mg/l) in enhancing yield components through the improvement of various physiological parameters. In summary, foliar application of folic acid demonstrates potential for increasing productivity and enhancing stress tolerance in barley plants cultivated in sandy soil conditions.

Keywords: Folic acid, Water stress, Osmolytes, Yield, Barley.

Introduction

Agricultural productivity is profoundly affected by global climate change, soil fertility, air quality, and the availability of water resources. The direct and indirect impacts of abiotic stressors are leading to increasingly severe consequences for plant productivity, particularly in response to abrupt shifts in environmental conditions. (Noya *et al.*, 2018). Among the most frequent abiotic stressors on plant productivity is drought or water scarcity especially in arid and semiarid areas. (Sanchez *et al* 2002 and Qiao *et al.*, 2020). Decreased plant growth and stomatal closure are two physiological reactions influenced by plant water scarcity that reduce water consumption and total plant productivity. (Zhao *et al.* 2020). Understanding the impacts of water status, are essential for managing irrigation schedules and crop water stress in field crops. Plant begins to feel the effects of water Plants experience stress when their root water supply is reduced or their rate of growth slows of transpiration increases (Daryanto *et al.*, 2020). Even in semi-arid regions with 200–350 mm of annual rainfall, barley is one of the crops that may thrive in a range of environmental circumstances. Elham, *et al*. (2015a). After maize, rice, and wheat, barley is the fourth most produced cereal crop worldwide. (12% of total cereal grown). This cereal is regarded as a dietary fibre, niacin, magnesium, copper and iron rich source. The health advantages of barley have been mostly attributed to its presence, particularly to β-glucan in whole grain

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barley.(Makeri*et al.* 2013). Additionally, it also contains phytochemicals particularly phenolic acids, flavonoids, lignans, α-tocopherols, phytosterols, and foliate. These phytochemicals have substantial anti-inflammatory, antioxidant, anti-proliferative, and cholesterol lowering properties that may be helpful in reducing the risk of certain diseases (Zhu, 2017).

Recent attention has been focused on the need of both boost agricultural yield and reduces the pollution that results from using of synthetic chemical fertilizers and pesticides in crop cultivation. (Bakhoum *et al.*, 2018).Therefore, several studies have been undertaken to use environmentally friendly organic substances found in plant cells. Folic acid (is an essential member of vitamin B complex) among these natural compounds which present in plant cell. Folic acid is a water soluble vitamin, active in plant in its reduced form as tetra-hydro-folic acid and tetra- hydro-folic-coenzymes (Nelson and Cox, 2005). These derivatives serve a variety of functions, acting as central cofactor for one-carbon transfer reactions which involved in many cellular reactions such as synthesis of purines (Burguieres *et al.,* 2007, Bailey, Ayling 2009 and Heo *et al.,* 2019), glycine to serine conversion, methionine synthesis, amino acid metabolism, lignin, chlorophyll, and choline production, as well as the photorespiration cycle. (Hanson & Roje, 2001; Jabrin *et al.,* 2003). Thus, the current study aims to investigate the effect of foliar spraying of folic acid on barley plant grown under different irrigation water requirement as well as their interaction on productivity via some physiological aspects.

2. Material and Methods

2.1 Field Experiments

Two field studies were carried out at the Experimental Farm of the Agricultural Production and Research Station, National Research Centre, located in El Nubaria Province, El Behaira Governorate, and Egypt. These studies took place over two winter seasons, specifically 2019/2020 and 2020/2021. The mechanical and chemical properties of the soil are detailed in Table (1), measured at a depth of 30 cm, in accordance with the specified methodology by (Page, *et al.,* 1982).

The Cereals Crops Research Department of Egypt's Ministry of Agriculture provided the barley grains utilized in this experiment. Nitrogen fertilizer in the form of ammonium nitrate (33.5%) was applied at a rate of 75 kg N/fed, while P2O5 as calcium super-phosphate (15.5%) and K2O as potassium sulphate (48%), respectively, were added during seed bed preparation. Grains of barley were sown on the middle of November. The experiments were laid out in split plot design with three replicates. The experimental unit was 10.5 m^2 (1/400 fed.). The treatments consisted of three irrigation regimes (100%, 75% and 50% of water irrigation Requirement) located in the main plots, while, foliar treatments with folic acid (0, 5, 10, mg/L.) located in the subplots. The plants were sprayed twice at 45 and 60 days after sowing with freshly prepared solutions of folic acid at 5, 10 mg/L. Meanwhile, untreated plants were sprayed by distilled water. Plants were irrigated for two hours every five days utilizing a sprinkler irrigation type of irrigation. At harvest one square meter was taken randomly from each plot to determine: Plant height (cm), Spike length (cm), Number tillers/m^2, Number of spike/m^2, Weight of grains /spike (g), 1000-grains weight (g), Grain yield (ton/fed), Straw yield (ton/fed), Biological yield (ton/fed), Harvest index %, Grain protein percentage, Total nitrogen percentage, Total phosphorus percentage and Total potassium percentage.

2.2 Biochemical determinations

Photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids and total pigments) in fresh leaves were determined as the method described by (Lichtenthaler and Buschmann, 2001). Total soluble carbohydrates (TSS) were extracted as (Homme *et al*. 1992) and analyzed by (Chow and Landhausser, 2004). Free amino acid and proline contents were extracted according to the method described by (Vartainan *et al*. 1992). Free amino acid was determined with the method of (Tamayo and Pedrol 2001).Proline was assayed according to the method described Versluses, (2010). Calorimetric analysis of total carbohydrates was conducted using the technique of Albalasmeh *et al.*, (2013).

2.3 Statistical analysis

According to Snedecor and Cochran (1990), the average of two seasons was statistically examined using a split plot design. The MSTATC (1989) software was used to compute the least significant difference (LSD) for each season at the 0.05 level of probability.

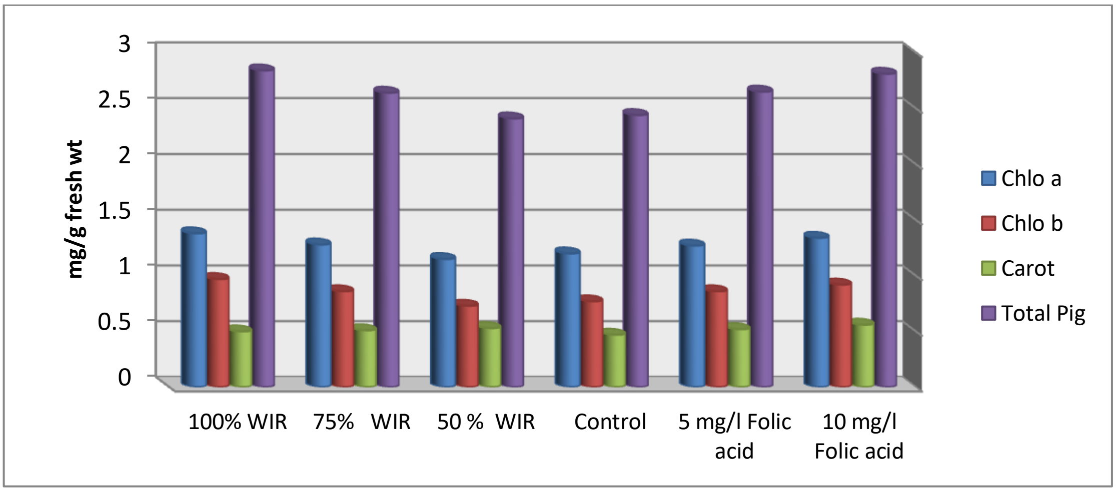
3. Results and Discussion

3.1 Changes of photosynthetic pigments content of barley plants

The data illustrated in Figure 1 demonstrate the impact of drought stress and foliar application of folic acid (FA). A reduction in water irrigation to 75% and 50% of the water irrigation rate (WIR) caused significant declines in the levels of photosynthetic pigments, including chlorophyll a, chlorophyll b, carotenoids, and total pigments, in barley plants as compared to those receiving 100% of WIR. Conversely, the application of folic acid at varying concentrations (5 and 10 mg/L) significantly enhanced the levels of the examined photosynthetic pigment components in comparison to barley plants that were not treated with folic acid. Moreover, the effect of interaction between levels of irrigation (100%, 75% and 50% WIR) and folic acid (FA) (0.0, 5 and 10 mg/L) are presented in Fig (2), the obtained results, revealed that, spraying barley plants with folic acid improved chlorophyll a, chlorophyll b, carotenoids and total pigments not only in plants grown under 100% of WIR but also under deficit of water (75% and 50% WIR) compared with control plants. Moreover, the interaction between folic acid with different levels of (WIR) on the photosynthetic pigments content was significant. However, the highest values were recorded by folic acid (10mg/L) under 100% of WIR (Fig.2). These reductions in photosynthetic pigment components are attributed to chlorophyll breakdown caused by ROS (Sadak *et al*., 2020b). Some investigations on different plant species have found that photosynthetic parameters decrease during water stress (Yang *et al*., 2016, Sadak *et al*., 2019, Tawfik *et al*., 2019, and Bakhoum *et al*., 2022).Under drought stress, the formation of ROS impeded the manufacture of several photosynthetic pigment components and reduced the photosynthetic electron transport chain in apples (Wang ***et al.,*** 2018). Furthermore, this decreased impact is produced by diffusion limitation due to stomatal closure and lower rubisco concentration, demonstrating co-dominance and biochemical restriction of the stomata under water stress circumstances, which might alter CO_2 assimilation rates. (Flexas *et al.*, 2016). Jabeen *et al*., (2020) stated that, drought triggered oxidative stress, nutritional disturbances, hormonal alterations, protein suppression/deterioration, enzyme deactivation and disruptions in secondary metabolism. On the other hand, the stimulative effect of folic acid on increasing *Chlo* a, *Chlo* b, carotenonoids and total pigments were in accordance with those of Al-Maliky *et al*., (2019) and Dawood *et al*., (2019). Folic acid improved chlorophyll content in barley leaves, this impact might be because of the fact that Glycine, which is necessary for the production of porphyrins and chlorophyll in chloroplast membranes, had its biosynthesis triggered by folic acid. (Stakhova *et al*., 2000). Moreover, these increments could be due to the impact of folic acid as an essential cofactor for one-carbon reactions that are utilized in several cellular activities like chlorophyll formation and photorespiration cycle.

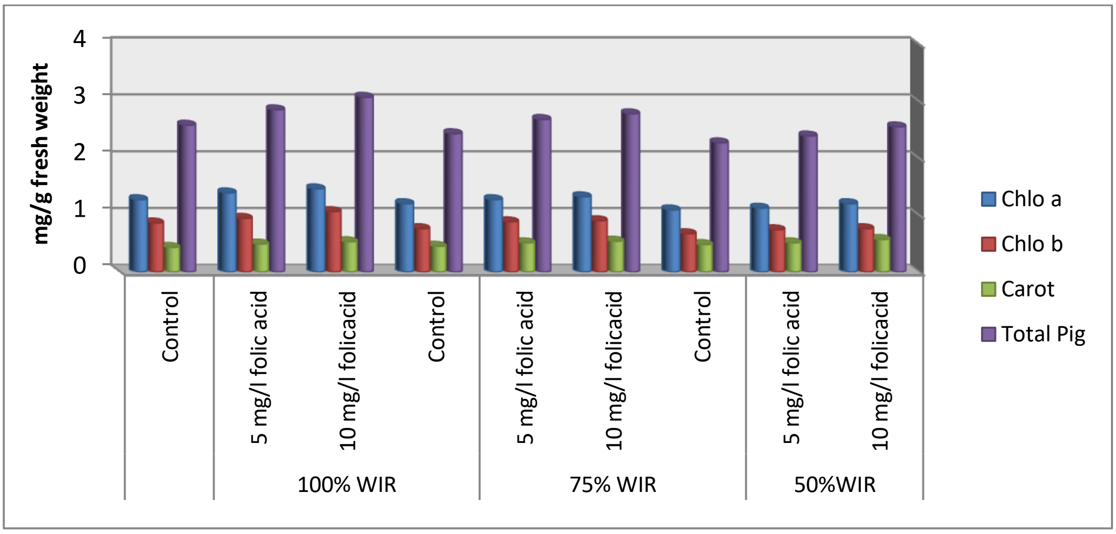
Table 1. Mechanical and chemical properties of experimental soil before treatments.

Sand %	Silt %	Clay %	pH	OM %	$CaCo_3$	EC ds/m	Soluble N (ppm)	Av. P (ppm)	Ex. K (ppm)
85.3	10.7	4.0	7.84	0.2	1.0	0.95	8.0	3.0	19.8



LSD at 5%: for WIR *Chlo* a: 0.11, *Chlo* b: 0.04, Carot :0.21and Total pig: 0.20
LSD at 5%: for Folic acid, *Chlo* a =0.1, *Chlo* b=0.02,Carot: 0.20 and Total pig: 0.19

Fig. 1. Effect of spraying folic acid (0, 5and 10 mg/l) and three levels of WIR on photosynthetic pigments (mg/g fresh wt.) on barley plant.



LSD at 5%: Chlo a =0.16, Chlo b =0.03, Carot=0.4and Total pig0.11

Fig 2. Effect of folic acid (0, 5 and 10 mg/l) on photosynthetic pigments (mg/g fresh wt.) under three levels of water irrigation WIR (100%, 75% and 50%) on barley plants.

3.2 Changes in endogenous IAA contents and phenol contents

The effects of exogenous application of folic acid at varying concentrations (0, 5, and 10 mg/L) on the endogenous levels of indole acetic acid (IAA) and phenolic compounds in barley plants subjected to different irrigation water requirements (100%, 75%, and 50% WIR) are illustrated in Figure(3). In comparison to the control group, which received 100% WIR, both moderate and severe water stress conditions (75% and 50% WIR) resulted in a significant and gradual reduction in endogenous IAA levels, while phenolic content exhibited a notable increase in response to escalating drought stress. Furthermore, the application of folic acid at concentrations of 5 and 10 mg/L significantly enhanced IAA levels relative to the untreated plants. The interaction between folic acid application and varying irrigation levels is depicted in Figure(4), which demonstrates that barley plants treated with folic acid showed a significant and progressive increase in both IAA and phenolic contents across all three irrigation levels (100%, 75%, and 50% WIR) when compared to the untreated control group. Folic acid is known to promote cell division and elongation, thereby enhancing root growth, which is particularly advantageous for root crops. The most well-known naturally occurring auxin, endogenous indole-3-acetic acid (IAA), is a hormone that plants make to promote growth. It is crucial for regulating development and growth. (Woodward and Bartel 2005; Teale *et al.*, 2006). The decrease caused by reduced water supply (75% and 50%), (Fig 3) were associated with declines in vegetative growth as stated in Abd Elhamid *et al.*, (2016) and Sadak (2022) on various plant species. Decreases in IAA level under water stress may be related to increased IAA breakdown or to the suppressing of its production. According to Kazan (2013), this decline is known as a stress responsive transcription factor that controls auxin and root developmental (Bano and Samina, 2010). On the other hand, the increased levels of endogenous IAA in response to folic acid treatments might be due to their enhancing role on its increased significantly with foliar application of folic acid treatments and those increases were resulted via enhancing alpha-ketoglutaric acid biosynthesis, which combines with NH_3forming amino acids and proteins, thus stimulating the creation of natural hormones such as IAA, cytokinins, and gibberellins (Samiullah *et al.*, 1988).

Drought stress raised the phenolic content of barley leaves by 75% and 50% of WIR, respectively. These increase in phenol concentrations haves the power to lessen harmful negative effects water stress. Plants produce more phenols because of many physiological processes being disturbed by water shortage (Hu *et al.*, 2022).Increased concentrations of free radicals are usually associated by variations in net carbon acquisition in plants under water stress, and According to Sachdev *et al.* (2021), these differences significantly affect the signaling pathways of secondary organic chemicals, particularly leaf polyphenols. Additionally, according to Huang *et al.* (2005), phenolics are vital antioxidants and scavengers of reactive oxygen species. Plants that experience water scarcity disruption through several physiological processes produce more phenolic chemicals.

3.3 Changes in osmoprotectants

Figure(3) illustrates the impact of folic acid (FA) application at three different irrigation levels (100%, 75%, and 50% of Water Irrigation Requirement, WIR) on the content of specific osmolytes in barley plants, namely proline and total soluble sugars (TSS). Water deficit conditions (75% and 50% of WIR) significantly ($p \leq 0.05$) enhanced the levels of osmoprotectants in comparison to the control group, which was cultivated under 100% of WIR. Additionally, the application of folic acid at varying concentrations (5 and 10 mg/L) resulted in notable increases in the measured compatible osmolytes when compared to plants that did not receive folic acid treatment. Furthermore, Figure (4) depicts the synergistic effect of folic acid foliar application across the three irrigation levels, highlighting its beneficial influence on the aforementioned parameters relative to the control plants. Without folic acid was observed under three different levels of water irrigation requirements (100%, 75%, and 50% of WIR). In contrast, the highest values were noted for each of these conditions when 10 mg/L of folic acid (FA) was applied under the 50% WIR. Osmolyte accumulation is an effective defensive strategy for plants in response to environmental challenges, notably drought conditions. (Nawaz et al., 2016). These compatible solutes, in conjunction with folic acid, contribute to the maintenance of cell turgor through osmotic adjustment (Turner, 2018) and function as scavengers for reactive oxygen species (ROS) (Zulfiqar et al., 2020). In the present study, barley subjected to water stress demonstrated elevated concentrations of osmolytes, including total soluble sugars (TSS) and proline, as illustrated in Fig 3. These results align with the findings of Elewa et al. (2017), Bakry et al. (2019), and Sadak and Ramadan (2021).They found that TSS and proline buildup occurred in quinoa, wheat, and white lupins under water stress, and that this accumulation is favorably related with drought stress resistance. Farooq *et al*. (2016) suggest that the observed increases may be related to a reduction in proline oxidase and proline catabolizing enzymes. Proline plays critical functions in osmotic adjustment, as well as in the stability and preservation of membranes, proteins, and enzymes from the harmful effects of drought-induced osmotic stressors (Ashraf and Foolad, 2007). (Ahmed *et al.*, 2020). Furthermore, folic acid treatment enhanced proline and TSS contents, our findings are in line with those of others as Ibrahim *et al.*, (2015), El-Metwally and Dawood (2017). Folic acid is regarded as the primary cofactor for one-carbon transfer reactions, which are involved in several physiological processes such purine synthesis, nucleic acid synthesis, and amino acid metabolism. (Jabrin *et al.*, 2003).

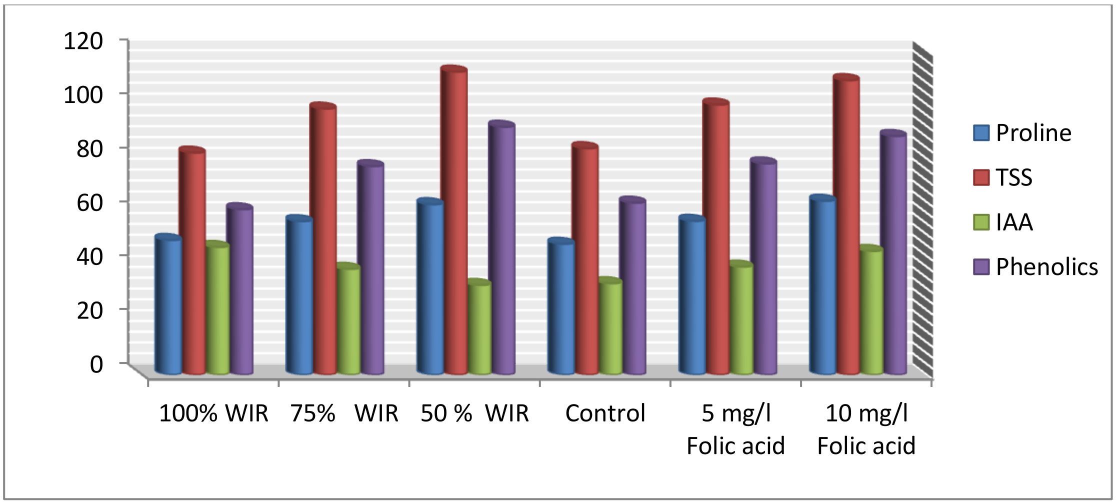
3.4 Changes in yield and its attributes of Barley Plants

The data presented in Table (2) indicate that a reduction in water availability from 100% to 75% and 50% of the water irrigation requirement (WIR) resulted in significant decreases ($p \leq 0.05$) in yield and its associated characteristics. These characteristics include plant height (cm), spike length (cm), weight of grains per spike (g), weight of 1000 grains (g), number of spikes per square meter, weight of spikes per square meter (g), as well as grain, straw, and biological yield (ton/fed), along with the harvest index (%), when compared to plants receiving 100% of WIR. Conversely, the application of exogenous folic acid at varying concentrations (5 and 10 mg/L) as foliar treatments led to significant enhancements in all aforementioned yield parameters relative to the untreated control plants. Furthermore, the interaction between foliar application of folic acid (FA) and the three levels of water irrigation (WIR) significantly affected yield and its attributes. The application of folic acid resulted in notable enhancements in yield and its associated characteristics, not only in plants cultivated under optimal irrigation conditions (100% of WIR) but also in those subjected to water deficit conditions (75% and 50% of WIR) when compared to control plants that did not receive folic acid treatment. Nevertheless, the most significant yield values were observed in plants treated with folic acid at a concentration of 10 mg/L under 100% of WIR (refer to Tables 2 &3).

Water stress negatively impacts plant-water interactions, lowers cell water levels, induces osmotic stress, and prevents cell division and expansion in addition to stunting plant development overall. **(**Anjum*et al.*, 2003**)**. The availability of water to plants at different phases of growth has an impact on both plant yield and the biochemical components of the seeds that are produced. This decrease in barley plant production is mostly caused by a decrease in growth factors and photosynthetic pigments. Folic acid may have a beneficial effect on controlling the synthesis of proteins and nucleic acids (Andrew *et al*., 2000), promoting cell division and expansion, production of natural hormones, and chlorophyll, which might explain why folic acid foliar spray improves plant development. (Jabrin *et al*., 2003) and increases nutrient uptake (Kilic& Ace, 2016). As well as increasing tolerance against abiotic stress (Poudineh *et al.*, 2015).The contribution of folic in these processes reflected by an increase in the size of the total vegetative growth, which was reflected by an increase of plant weight and accumulation of dry matter results were in agreement with (Elham *et a*l. (2015b), El-Metwally and Dawood (2017), Al-Elwany *et al*., (2022) and Sadak *et al*., (2022)

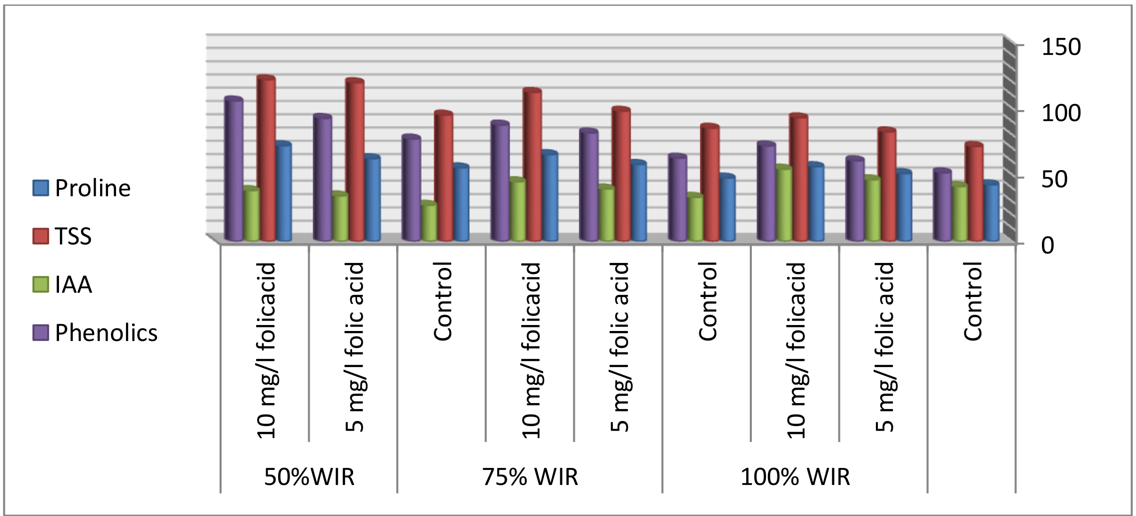
3.5 Changes in nutritional value of barley Plants

Table (3) demonstrates that a decrease in water availability, from 100% to 75% and then to 50% of Water Irrigation Rate (WIR), resulted in significant reductions ($p \leq 0.05$) in the nutritional values, specifically nitrogen (N), potassium (K), phosphorus (P), and protein percentage. Conversely, the application of folic acid (FA) at concentrations of 5 and 10 mg/L significantly enhanced the levels of nitrogen, potassium, phosphorus, and protein percentage in barley leaves when compared to the control treatment. The 10 mg/L concentration of folic acid yielded the highest values for N, P, K, and protein in comparison to the untreated control. Regarding the interaction between folic acid concentrations and the three levels of water irrigation (WIR) on barley plants grown in sandy soil, varying concentrations of folic acid considerably improved the nutritional value of the harvested grains, reducing the negative consequences of water stress (Table 3). Similar results were seen in other plant species. Ahmed and Sadak, (2016) and Sadak *et al.*,(2022) they stated that, nutrient contents were decreased in wheat and flax plants respectively under drought stress. These decreases are mainly due to the reduction in photosynthetic pigments (Fig 1 &2). Because they are directly related to physiological activities like respiration, photosynthesis, and translocation, nutrient differences in the grains that are generated are extremely important (Sadak and Ramadan, 2021).Growth in nitrogen, phosphorus, and potassium in folic acid-treated leaves as a result of folic acid's ability to improve root growth and nutrient and water absorption (Farouk &Abdul Qudos 2018).



LSD at 5% WIR IAA=1.2, phenolic=2.5, TSS=2.1, proline= 1.5, Folic Acid IAA=0.96 phenolic=2.11, TSS=2.1, proline= 0.91

Fig. 3. Effect of folic acid (0, 5 and 10 mg/l) and three levels of WIR on indole acetic acid,(µg/100 g fresh weight), phenolics and some osmolytes content (mg g-1 dry weight)on barley plants.



LSD at 5% IAA=1.04, phenolic=2.9, TSS=2.9, proline= 1.3

Fig. 4. The interaction Effect of folic acid (0, 5 and 10mg/l) on indole acetic acid (µg/100 g fresh weight), phenolics and some osmolytes content (mg g-1 dry weight) on barley plants grown under three levels of WIR.

Table 2. Effect of foliar spraying folic acid on yield and its components of barley plants grown under three levels of water irrigation requirements (WIR) (Data are means of two seasons).

Treatment		Plant height (cm)	Spike length (cm)	Weight of grains/spike (g)	Weight of 1000 grains (g)	Number. of spikes/m^2	Weight of spikes/m^2 (g)	Grain yield (ton/fed)	Straw yield (ton/fed)
100% WIR		94.51	10.57	2.98	58.90	255.62	776.13	2.12	2.84
75% WIR		87.65	9.77	2.52	53.07	245.75	700.29	1.91	2.66
50% WIR		75.43	8.83	1.92	45.82	195.81	671.52	1.80	2.50
L.S.D at 5%		2.25	0.23	0.10	1.40	7.32	21.41	0.04	0.18
FA0(control)		79.83	7.76	1.79	46.21	212.63	638.07	1.85	2.46
FA1(5 mg/l)		85.79	9.98	2.62	52.69	227.81	715.88	1.96	2.66
FA2(10mg/l)		91.96	11.45	3.01	58.87	256.73	793.98	2.03	2.87
L.S.D at 5%		2.11	0.21	0.09	1.37	4.71	20.19	0.02	0.16
100 % WIR	FA0(Control)	80.58	8.70	1.98	45.22	215.68	695.22	2.07	2.77
	FA1(5mg/l)	87.70	9.57	2.35	49.36	235.11	721.67	2.15	2.85
	FA2(10mg/l)	91.85	10.22	2.94	53.77	249.85	743.56	2.28	2.96
75 % WIR	FA0(Control)	78.90	7.95	1.88	40.73	190.54	640.88	1.76	1.99
	FA1(5mg/l)	83.28	8.90	2.11	45.86	201.32	668.99	1.85	2.14
	FA2(10mg/l)	86.00	9.32	2.42	49.98	217.45	682.22	1.99	2.31
50% WI R	FA0(Control)	66.73	7.65	1.70	37.68	184.38	520.11	1.61	1.89
	FA1(5mg/l)	71.15	8.74	1.89	40.93	196.75	541.98	1.73	2.00
	FA2(10mg/l)	74.93	9.05	2.01	44.96	200.11	562.77	1.85	2.10
L.S.D at 5%		2.10	0.30	0.12	1.36	2.91	13.56	0.31	0.06

Conclusion

The findings suggest that the productivity of barley plants diminished under water stress conditions (75% and 50% of WIR), attributed to alterations in various physiological processes. Nevertheless, the external application of folic acid at concentrations of 5 or 10 mg/L, especially at 10 mg/L, led to enhancements in the yield of both well-watered and drought-stressed barley. This improvement is primarily linked to an increase in photosynthetic pigment levels, endogenous IAA, and certain osmoprotectants, in addition to the nutritional benefits of the harvested grains.

Declarations

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Conflicts of interests/Competing interests

The authors declare that no conflict of interest / competing interest.

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