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Posted Date: 26 December 2023

doi: 10.20944/preprints202312.1944.v1

Keywords: Triticum aestivum; yield; water stress; vitamin C; vitamin B2 doi:10.20944/preprints202312.1944.v1

Article

Riboflavin or Ascorbic Acid Enhances Wheat Drought Tolerance by Improving Both Yield and Water Productivity

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Abstract: Wheat is widely grown in the Mediterranean region, where plants frequently face water shortages. Recently, more attention to rationalizing irrigation water has been increased due to the adoption of sustainable agricultural systems. Therefore, two field trials were conducted at the Agriculture Research Station of Nubaria, Egypt, in two consecutive seasons (2017/2018 and 2018/2019) to examine three irrigation regimes' effects, i.e., 60, 80, and 100% of crop evapotranspiration (ETc) combined with foliar ascorbic acid (AsA) or vitamin B2 (VB2) on yield and water productivity of wheat in two separate experiments. A split-plot design was used with three replicates. Irrigation treatments occupied the main plots, while the subplot in the first experiment was placed by ascorbic acid (AsA) and vitamin B2 in the second experiment. Results indicated that 100% ETc produced higher wheat yield and its attributes and insignificantly surpassed 80% ETc. Compared to the unsprayed plots, the foliar application of 6 mM ascorbic acid or 2.0 mM vitamin B2 significantly increased grain yield, components, and water use efficiency. The interaction between irrigation regimes (100% ETc or 80% ETc) and foliar application of 6.0 mM ascorbic acid or 2.0 mM vitamin B2 resulted in the highest values with no significant difference. Foliar application of ascorbic acid or vitamin B2 saved 20% of irrigation water while yielding a reduction that did not exceed 10%. The biplot graph of treatments x traits (TT) was a useful statistical tool for examining the impact of treatments on yield and its characteristics and identifying the links between these factors. Results suggest that riboflavin or ascorbic acid may have a potential role as an effective antioxidant for enhancing the tolerance for drought stress in wheat.

Keywords: *Triticum aestivum*; yield; water stress; vitamin C; vitamin B2

1. Introduction

Cereal food crops produce the majority of human nutrition. The top crop is wheat, which feeds one-fifth of the world's population and is farmed on about 220 million hectares worldwide [1]. It contributes 20% of the world's total calories and a comparable amount of total protein, making it one of the most significant elements of global food security. Egypt has gradually boosted its wheat production over the past few years due to high-yielding cultivars, favorable weather, efficient resource use, enhanced storage facilities, and official support for price strategies. However, wheat imports increase yearly to fulfill the demands of our expanding population [2]. Bread wheat is the primary source of carbohydrates and a staple of both rich and poor Egyptian diets [3]. Egypt imports the remaining 10 million tonnes of the 20 million tonnes of wheat it consumes. Egypt is also the world's largest wheat buyer [4]. Production of strategic crops can be increased by expanding the area under cultivation and emphasizing the scale of agricultural investment. Sustainable farm productivity and food security can also be developed through technological cooperation, information transfer, agricultural research, effective human resources, and modern technologies [5].

Water scarcity, particularly in Egypt, is one of the most critical factors in global food security [5]. Egypt's water consumption is increasing as the population grows, the level of life rises, and the agriculture sector expands. There is a supply and demand imbalance, which is bridged through recycling and increasing water use efficiency. Around 80% of the Nile system in Egypt is effective overall. More than 85% of Egypt's Nile water is used for agriculture, which uses the most water [6].

Drought stress is one of the primary abiotic variables that reduces crop production [7]. Water deficiency negatively impacts wheat growth at all phases, but the most noticeable effect is shown during the reproductive stage, particularly during grain filling, which results in smaller grains [8]. Water deficit hindered the activity of essential enzymes involved in the preparation of sucrose and starch synthetic processes, resulting in reduced grain filling [9,10]. A lack of water disturbed plants' availability, absorption, transport, and accumulation of nutrients [11].

Researchers have recently investigated novel irrigation techniques, buildings, and approaches to boost water productivity (WP). Using managed deficit irrigation systems is one of the most promising strategies to raise irrigation water productivity. Deficit irrigation maximizes WP for greater yields per irrigation water [12]. In this regard, Egypt's implementation of deficit irrigation could be a beneficial technique for alleviating the country's water scarcity [13–15].

Crop breeding prioritizes crop yield improvement under drought stress because it's crucial for food security. According to Ashour et al. [16], Egypt's present major issue is the urgent need for better development and management of the nation's constrained water, land, and energy resources to meet the demands of a growing population. According to Moghazy and Kaluarachchi [17], the Siwa Oasis can be used for future expansion to make up for Egypt's strategic crop production shortfall. However, a water shortage combined with two irrigations after sowing could result in roughly 82% of the maximum grain yield. The highest grain production is obtained when 75% of the recommended irrigation rate is used at the tillering, stem elongation, booting, and grain filling stages while saving between 13 and 17% of the recommended irrigation water [18]. Despite the low yield, Sarkar et al. [19] found that the treatment with the highest water production for wheat crops was the one that received irrigation only once at the crown root initiation stage, or 2.02 kg m^{-3}. Additionally, crowned root initiation was found to be the most susceptible stage to water stress, and water stress significantly impacts yield during the vegetative stage.

Exogenous antioxidants have been demonstrated to considerably lessen the adverse effects of water stress on plant growth and metabolism [20]. Ascorbic acid (AsA), vitamin C, is one of the most well-known non-enzymatic antioxidants. In addition to playing a crucial part in several critical metabolic processes, AsA is a powerful antioxidant [21]. According to specific research, ascorbic acid is well known for controlling plants' ability to tolerate stress, e.g., pea [22], common bean [23], canola [24], maize [25], etc. In plants, ascorbic acid is an antioxidant that helps cell division, differentiation, and metabolism [26].

According to Xu [27], foliar ascorbic acid spray lessens the detrimental effects of water stress on stomata closure, nutrient uptake, total chlorophyll content, protein synthesis, transpiration, photosynthesis, and plant growth. Gámez et al. [28] found that drought harmed most yield and yield characteristics, while AsA treatments mitigated the drought's inhibitory effect in wheat. Because it acts as an electron acceptor, riboflavin (vitamin B2) is also helpful as an antioxidant [28]. Furthermore, vitamin B2 (VB2) appears to promote the development of antioxidative components in plants in general [29]. In plants, riboflavin may play a role in stress priming, creating baseline defense responses that help defense mechanisms activate more quickly and/or intensely during subsequent stressors [30]. Furthermore, according to Deng et al. [31], tobacco plants have shown that riboflavin can boost their tolerance to drought, even at low concentrations.

The GGE biplot method (genotype plus genotype by environment) uses graphs to evaluate yield across multiple environments. Yan [32] recently created a technique that uses various biplot graph types to condense the effects of the applied treatment on numerous target attributes simultaneously. Assessments commonly employ information from a two-dimensional array created by the interaction of the treatment and the qualities [33]. Few references were observed in Egypt using the treatment x trait (TT) biplot graph approach. As a result, one of the aims of this study was to use the (TT) biplot technique to summarize and graphically depict the impacts of factorial treatments on the studied variables.

Although several studies have examined how wheat reacts to water stress, further research is needed to understand how exogenous ascorbic acid or vitamin B2 applications affect growth, productivity, and water productivity. Two well-known compounds that are potential non-enzymatic antioxidants and plant growth regulators are ascorbic acid and riboflavin. Therefore, the current study aimed to determine how much exogenous ascorbic acid or vitamin B2 could control wheat plants' growth and productivity during a water shortage. Under water scarcity conditions, the anticipated results would improve the efficiency of wheat growth, grain yield, and irrigation water use.

2. Materials and Methods

2.1. Experimental site

Two field experiments were conducted in the Nubaria Agricultural Research Station, North Tahrir, Egypt (30°54′21″ N 29°57′24″ E), throughout the two sequential growing seasons in 2017/18 and 2018/19. Due to its arid climate, the experimental area is characteized by hot and dry summer and cool winter. Figure 1 shows the climatic data at the experimental site.

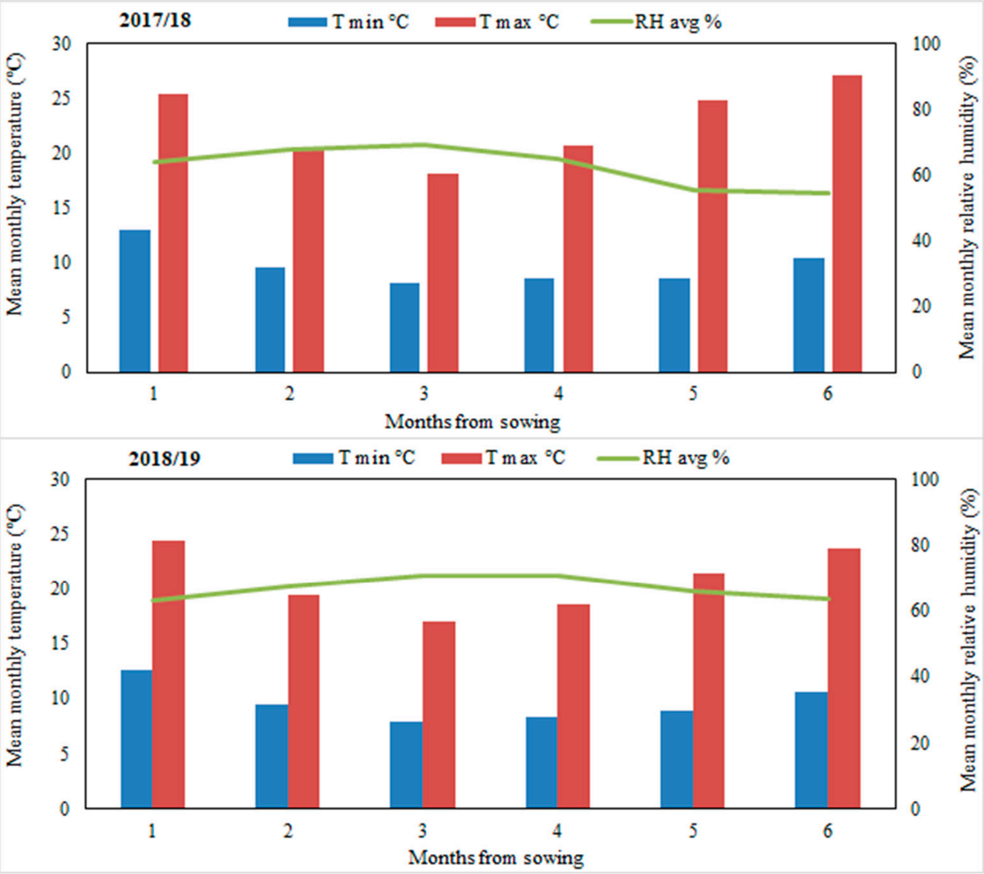

Figure 1. The experimental site's average monthly climatic information for the two growing seasons.

2.2. Soil type

The soil at the experimental site is classified as sandy-loam soil. Tables 1 and 2 show the physical and chemical characteristics of the experimental soil. The trial field was plowed before planting, and a combination driller that allowed for the simultaneous application of fertilizer and seeds was used. The River Nile branch close to the experimental area supplied the irrigation water. Some soil properties at the experimental site are presented in Table 1.

Table 1. Principal physical characteristics of the soil before sowing at the experimental site.

Texture	Soil depth (cm)	Bulk density ($Mg\ m^{-3}$)	Hydraulic conductivity ($cm\ h^{-1}$)	Particle size distribution		
				Sand %	Silt %	Clay %
Sandy loam	0 – 15	1.28	3.74	58.9	24.2	16.9
	15 - 30	1.26	3.12	60.3	24.5	15.2
	30 - 45	1.27	3.61	56.7	26.1	17.2
	45 – 60	1.27	3.05	57.0	25.5	17.5

Table 2. Principal chemical characteristics of the soil before sowing at the experimental site.

Soil depth (cm)	pH	E.C. (dS/m)	$CaCO_3$ (%)	Soluble cations (meq/l)				Soluble anions (meq/l)		
				K^+	Na^+	Mg^{2+}	Ca^{2+}	Cl^-	SO_4^{2-}	HCO_3^-
0 – 30	8.30	1.52	25.9	1.20	6.50	2.50	5.00	8.90	2.11	4.19
30 – 60	8.50	2.10	24.9	1.56	8.04	3.24	8.20	9.40	2.84	8.80

2.3. Fertilizers application

For the two following growing seasons, the wheat (*Triticum aestivum* L.) cultivar "Misr 1" was planted on 29/11/2017 and 30/11/2018, respectively. Wheat seeds weighing 70 kg per hectare were sown, and fertilizer applications were made following suggestions made by soil analysis. All plots got the same quantity of fertilizers during each growing season: 115 kg of potassium sulfate (48% K_2O), 70 kg of superphosphate (15.5% P_2O_5), and 285 kg of NH_4NO_3 (33.5% N). All other agricultural procedures followed the Egyptian Ministry of Agriculture and Land Reclamation's recommendations to achieve good wheat production.

2.4. The Experiments

The field experiments included a series of two main field experiments.

2.4.1. The first experiment

The first experiment included irrigation regimes with foliar spray with ascorbic acid. The ascorbic acid (AsA) experiments were conducted using a split-plot design with three replicates. The first factor given to the main plots was irrigation. The subplots were then dosed with ascorbic acid (vitamin C) (Sigma-Aldrich, St. Louis, MO, U.S.A.), and the number and timing of sprays were based on the results of the initial pot trial (data not shown). The main plots included three irrigation regimes, i.e., 1) 60% evapotranspiration (ETc) of the crop, 2) 80% ETc, and 3) 100% ETc during the season. Irrigation treatments of 60 and 80% ETc were applied to start after 19 days from sowing until the end of the growing season. Subplots were assigned four ascorbic acids (AsA) treatments, namely 1) no spray AsA, 2) 2 mM AsA, 3) 4 mM AsA, and 4) 6 mM AsA. Wheat plants received foliar spray treatments of ascorbic acid twice, 35 and 50 days following sowing.

2.4.2. The second experiment

The second experiment involved foliar spray with riboflavin (vitamin B2) regimens and water deficit treatments. The Vitamin B2 (VB2) experiment was also performed using a split-plot design with three replications. Irrigation was the first factor assigned to the main plots. A second factor occupied the subplots, the doses of vitamin B2 (Sigma-Aldrich, St. Louis, MO, U.S.A.), where the spray time depended on the results of the initial pot trial (data not shown).

Three irrigation treatments were devoted to the main plots included, namely: 1) 60% evapotranspiration (ETc) of the crop, 2) 80% ETc, and 3) 100% ETc during the season.

Subplots contained four treatments with vitamin B2 (VB2), *viz*. 1) null treatment of VB2, 2) 0.5 mM VB2, 3) 1.0 mM VB2, and 4) 2.0 mM VB2. Vitamin B2 was applied twice to wheat plants as foliar spraying after 25 and 40 days from sowing.

The total irrigation water was quantified in mm ha-1, as shown in Table 3. It is noted that spray treatments are done in the early morning and before 9:00 a.m. when there is less wind, using a hand-held sprayer with sufficient pressure to maintain small droplet sizes. The amount of sprayed water is about 500 m^3 ha^{-1}. Plants were sprayed from both sides of the rows to increase control and spray efficiency. Preliminary trials were conducted to check what ascorbic acid or vitamin B2 levels could be used for foliar spraying. It is revealed that the highest doses of 6 mM ascorbic acid or 2.0 mM vitamin B2 are not harmful and may enhance plant growth. The length of each plot is 4 m, the width is 1.25 m, the distance between each plot is 0.5 m, and the safe border boundary between irrigation treatments is 2 m. According to the suggestions of Egypt's Ministry of Agriculture and Land Reclamation, all other agricultural techniques are carried out when cultivating wheat.

Table 3. Applied irrigation water mm ha-1 in the 2017/18 and 2018/19 seasons.

Irrigation	ETo (mm/ha)		C.U. (mm/ha)		A.I.W. (mm ha^{-1})		Ecu (%)	
	2017/18	2018/19	2017/18	2018/19	2017/18	2018/19	2017/18	2018/19
IR60	316.8	297.9	191.1	181.5	420.2	393.9	45.5	46.1
IR80	422.4	397.2	209.0	210.1	576.7	541.0	36.2	38.8
IR100	528.0	496.5	230.2	230.0	733.1	688.1	31.4	33.4

ETo, reference evapotranspiration; C.U., water consumptive use; A.I.W., applied irrigation water.

2.5. Irrigation water requirements

Estimating crop evapotranspiration (ETc) or irrigation amounts under standard conditions was calculated according to Allen et al. [34]. The equation uses daily averages of the standard meteorological data of solar radiation (sunshine), air temperature (maximum and minimum), relative humidity, and wind speed calculated from daily measurements. The percentage of soil moisture content was measured gravimetrically. The wheat crop coefficient value [33] was obtained according to Allen et al. [34]. Water consumptive use was calculated according to Israelsen and Hansen [35]. Ali et al. [36] determined water productivity (WP) as crop yield per millimeter of water usage. The productivity of irrigation water (IWP) was calculated according to Ali et al. [36]. As explained by Doorenbos and Pruitt [37], the consumptive use efficiency (Ecu%) was determined.

2.6. The studied characters

The grown commercial bread wheat cultivar (Misr 1) was harvested on April 25th and 26th of April in the first and second seasons, respectively. Plants from the two center rows were randomly chosen for the grain yield and component data to minimize border impacts. Plant height (cm), number of spikes per m^2, spike length in cm, 1000 grain weight in g, grain yield in kg ha^{-1}, straw yield in kg ha^{-1}, and biological yield in kg ha^{-1} were all recorded.

2.7. Statical and data analysis

For each antioxidant vitamin treatment of ascorbic acid or vitamin B2, A combined analysis of variation across the two growth seasons was performed on the acquired data [38]. Levene test [39] was automated before combined analysis to test the homogeneity of individual error terms, and testing for normality distribution was performed [40]. The Least Significant Difference (LSD) test was used to calculate the significant differences among treatment means at a 0.05 probability level. The Least Significant Difference (LSD) test compared the differences between means at $P \leq 0.05$. GenStat 19th Edition software (VSN International Ltd, Hemel Hempstead, UK) was used for the target statistical analyses. The treatment by trait two-way data was presented using the Biplot method suggested by Yan and Rajcan [41] in a biplot graph known as a TT biplot utilizing principal components analysis.

3. Results and Discussion

3.1. Irrigation treatment and ascorbic acid (AsA) experiment

3.1.1. Response of grain yield and its components to water stress, AsA, and their interaction

The results proved that a 40% reduction in irrigation water (IR60) considerably affected four yield components (plant height, number of spike m^{-2}, spike length, and 1000 grain weight) (Table 4). The tallest plants were obtained under full irrigation (IR100) compared to the reduced irrigation (Table 4). With an increment up to 25%, No. of spike/m^2 significantly increased from 498 to 598 using IR60 % and IR100 %, respectively. Also, spike length and 1000 grain weight attained the highest values under the recommended irrigation (IR100), recording 6.0cm and 51.2g, respectively (Table 4). In this regard, wheat plants that were irrigated by 80% outperformed those irrigated by 60 percent and 100 percent [42].

Regarding foliar application with the tested concentrations of ascorbic acid, results showed that all concentrations of ascorbic acid increased yield components of the wheat plant compared with control plots (Table 4). The improvement in wheat plants due to AsA spraying may be attributed to increased chlorophyll content that enhanced the photochemical efficiency of the plant [23]. Also, ascorbic acid boosted the activity of several enzymes involved in regulating photosynthetic pigments, which could explain wheat plants' positive response [43].

The interaction effect between AsA application and irrigation treatments resulted in the uppermost values attained from plots with 6.0 mM of ascorbic acid foliar sprayed and irrigated with 100% water requirement treatment. There is no significant difference between the two treatments, i.e., IR80 x 6 mM AsA and IR100 x 6 AsA mM for all yield components except plant height, suggesting the potential for 20% irrigation water savings (Table 4).

Significant variances among the irrigation treatments in all grain yield characteristics were reported (Table 5). The highest values of grain, straw, and biological yields were obtained using full irrigation (IR100) compared to reduced irrigation (IR80 or IR60). The grain yield decreased significantly ($P<0.05$) from 5650 kg ha-1 in IR100 to 5123 and 4399kg ha^{-1} in IR80 and IR60, coinciding with a 9.33 and 22.14 % reduction, respectively. The same trend was noticed, with the straw yield significantly reduced by 9.7 and 19.91% under IR80 % and IR60 %, respectively. In comparison, the biological yield (grain yield plus straw yield) decreased by 9.61 and 20.43% under the reduced irrigation treatments (IR80 and IR60, respectively. Accordingly, upon saving 20% of irrigation water, it will lose about 10% of wheat crop yield; while saving 40% of irrigation water, the yield loss increases to about 20%. The positive impact of ascorbic acid on wheat productivity and growth analyses was attributed to enhanced irrigation water use efficiency, prevention of growth inhibition, lipid peroxidation, chlorophyll and protein degradation, and protection of the primary tissues in the flag leaf and stem, all of which helped the plant cope better with drought stress [44].

In contrast, the results demonstrated that foliar application of ascorbic acid significantly increased grain, straw, and biological yields compared to the control plots (Table 5). Foliar application with 6.0 mM AsA was an effective practice for promoting grain, straw, and biological yields. This treatment improved grain, straw, and biological yields by 21.35, 15.61 and 16.98%, respectively, over the unsprayed wheat plants. Hussein and Khursheed [45] concluded that applying ascorbic acid to wheat plants mitigated the adverse effects of drought stress by correcting nutritional disorders, photosynthetic pigment biosynthesis, water conservation, and increasing carbohydrates and proline levels.

The most effective combinations between irrigation treatment and AsA foliar applications for enhancing grain, straw, and biological yields of wheat were recorded using 6.0 mM AsA along with irrigated wheat plants by 100 or 80% (IR100 or IR80) of water requirement with no significant between them. These results help us to save irrigation water by 20% with a slight reduction in wheat yield.

Table 4. Impact of ascorbic acid and irrigation treatments on various yield components over the two growing seasons.

Treatment		Plant height (cm)	No. of spikes /m²	Spike length (cm)	1000 grain weight (g)
Irrigation (I.R.)					
IR60		103.9	498	5.3	46.3
IR80		111.2	564	5.6	48.6
IR100		117.5	598	6.0	51.2
LSD 0.05		4.0	50	0.3	3.7
Ascorbic acid					
Control		108.3	521	5.4	47.0
2.0 mM		109.9	541	5.6	48.6
4.0 mM		110.7	567	5.7	49.4
6.0 mM		114.6	584	5.8	50.0
LSD 0.05		2.9	31	0.4	2.9
Irrigation x Ascorbic acid interaction					
IR60	Control	102.4	454	5.1	44.6
	2.0 mM	103.2	482	5.4	46.0
	4.0 mM	103.6	516	5.3	47.1
	6.0 mM	106.3	538	5.4	47.6
IR80	Control	107.4	531	5.4	46.7
	2.0 mM	109.8	554	5.5	48.8
	4.0 mM	112.4	578	5.7	49.3
	6.0 mM	115.3	594	5.8	49.8
IR100	Control	115.0	579	5.8	49.7
	2.0 mM	116.6	587	5.8	51.0
	4.0 mM	116.0	606	6.0	51.7
	6.0 mM	122.2	619	6.3	52.4
LSD 0.05		5.0	53	0.7	5.0

Table 5. Impact of irrigation modifications and ascorbic acid, as well as how these two factors interact on grain, straw, and biological yields over the course of the two growing seasons.

Treatment	Grain yield (Kg ha^{-1})	Straw yield (Kg ha^{-1})	Biological yield (Kg ha^{-1})
Irrigation (IR)			
IR60	4399	14870	19269

IR80		5123	16765	21889
IR100		5650	18567	24217
LSD $_{0.05}$		464	1405	1304
Ascorbic acid				
Control		4461	15274	19735
2.0 mM		4857	16525	21381
4.0 mM		5240	17039	22279
6.0 mM		5672	18099	23771
LSD $_{0.05}$		461	1277	1529
Irrigation x Ascorbic acid interaction				
IR60	Control	3720	13350	17070
	2.0 mM	4167	14781	18948
	4.0 mM	4612	15272	19883
	6.0 mM	5097	16079	21176
IR80	Control	4629	15408	20038
	2.0 mM	4988	16574	21561
	4.0 mM	5256	17116	22372
	6.0 mM	5620	17963	23583
IR100	Control	5035	17064	22098
	2.0 mM	5416	18219	23635
	4.0 mM	5852	18730	24581
	6.0 mM	6299	20256	26555
LSD $_{0.05}$		798	2211	2648

3.1.2. Response on water productivity (WP) and irrigation water productivity (IWP) to water stress, AsA, and their interaction across the two seasons

The link between the appropriate yield and the amount of water used is measured by the concept of water productivity (WP). Irrigation water productivity (IWP) measures the ability of each unit of irrigated water to produce wheat yields. Each parameter is treated as a ratio based on the yield obtained as a nominator and the amount of water consumed or applied as a dominator [55]. The mean values of Water Productivity (WP) and Irrigation Water Productivity (IWP) of wheat crops as affected by irrigation treatments and rates of concentrations of ascorbic acid are presented in Table 6. According to the findings, there was no difference between irrigation treatments regarding water productivity (P>0.05). However, there was an inverse relationship between the amounts of supplied water and irrigation water productivity (IWP), recording 10.8, 9.2, and 8.0 kg mm^{-1} ha^{-1} for IR60, IR80, and IR100, respectively, with no significant difference between (IWP) mean values for IR80 and IR100.

Table 6. Water productivity (WP) and irrigation water productivity (IWP) across the two growing seasons due to irrigation treatments and ascorbic acid interaction.

Treatment	**WP (kg mm^{-1} ha^{-1})**	**IWP (kg mm^{-1} ha^{-1})**
Irrigation (IR)		
IR60	23.68	10.85
IR80	24.45	9.19
IR100	24.56	7.97

LSD $_{0.05}$		NS	1.00
Ascorbic acid			
Control		21.33	8.18
2.0 mM		23.25	8.95
4.0 mM		25.11	9.69
6.0 mM		27.22	10.52
LSD $_{0.05}$		2.20	0.80
Irrigation x Ascorbic acid interaction			
IR60	Control	20.01	9.16
	2.0 mM	22.42	10.27
	4.0 mM	24.83	11.37
	6.0 mM	27.46	12.58
IR80	Control	22.09	8.29
	2.0 mM	23.80	8.94
	4.0 mM	25.08	9.43
	6.0 mM	26.82	10.09
IR100	Control	21.88	7.09
	2.0 mM	23.54	7.64
	4.0 mM	25.43	8.26
	6.0 mM	27.38	8.89
LSD $_{0.05}$		3.80	3.80

Water was used more efficiently during water stress than during typical irrigation. The greater WP values found under water stress treatments than ordinary irrigation were mainly related to less water use and increased grain yield. These findings corroborate the findings of previous researchers [13,56,57]. This result indicates that, although the amount of irrigation water decreased by 20%, the wheat yield loss was insignificant, meaning that 80% of the irrigation water could be sufficient to maintain rewarding wheat yield. These results were previously confirmed by M'hamed et al. [58], who reported that with more irrigation water used, water productivity (WP) and irrigation water productivity (IWP) both dropped.

Foliar's application of AsA increased both WP and IWP, which gradually coincided with the rise in the AsA level. Thus, foliar spraying by 6 mM AsA compared to control significantly recorded the highest values of WP and IWP, 27.2 and 10.5 kg mm^{-1} ha^{-1}, respectively (Table 6). This result may be attributed to the positive physiological effect of AsA in reducing the impact of water shortage on the wheat crop.

The highest WP mean values were attained by the interaction effect between the high rate of AsA application with 6.0 mM under the three irrigation treatments (60, 80, and 100% of ETc) (Table 6). The result means that the WP of wheat plants sprayed with 6.0 mM AsA was not adversely affected by the decrease in irrigation water requirements, indicating that ascorbic acid has increased the ability of the wheat plants to withstand water deficit. The same conclusion can work well with WPI. Based on these results, 20% of the irrigation water can be saved if wheat plants are sprayed with 6.0 mM ascorbic acid, while in rain-fed areas, 40% of the irrigation water requirement can be saved. In this regard, Bakry et al. [42] reported that the interaction of 80% irrigation water requirements with 300 mg L^{-1} ascorbic acid foliar spray resulted in the highest grain, straw, and protein yields per acre and water use efficiency values.

3.2. Irrigation treatment and vitamin B2 (Riboflavin) experiment

3.2.1. Response on yield and yield components to water stress, VB2, and their interaction across the two seasons

Wheat yield components significantly responded to irrigation treatment and foliar applications of vitamin B2 and their interaction across the two seasons (Table 7). The recommended irrigation level (IR100) significantly increased the mean values of yield components, i.e., plant height, number of spikes/m^2, spike length, and 1000 grain weight by 10.44, 15.16, 14 and 13.39%, respectively, compared to the reduced irrigation (IR60).

Foliar spraying wheat plants with 2.0 mM vitamin B2 gave the most significant increases in yield components and surpassed the other foliar application treatments. Such a treatment showed increased mean values of yield components (plant height, number of spikes/m^2, spike length, and 1000 grain weight) by 5.15, 13.61, 9.8 and 6.3%, respectively, compared with the control one (without spraying) (Table 7).

The most effective interaction between irrigation treatment and foliar applications of vitamin B2 for enhancing wheat yield components was achieved by the full irrigation treatment (IR100) and spraying wheat plants with 2.0 mM vitamin B2 with significant differences from the control treatment (Table 7). The factorial treatments between the full irrigation (IR100) and vitamin B2 rate (1.0 mM) and between the reduced irrigation (IR80) with vitamin B2 (2.0 mM VB2) came in the second order with no significant difference between them. Subsequently, it is possible to obtain good wheat yield components by reducing irrigation water by 20% and using the highest rate of vitamin B2 (2.0 mM VB2). Foliar spraying of vitamin B2 seems to be essential for plant growth and health by activating metabolism as a necessary enzymatic cofactor and molecules for stress resistance, e.g., in tobacco [31], roselle [59], and Tecoma capensis [60].

Table 8 shows the significant impacts of irrigation treatment, foliar vitamin B2 treatments, and their interaction on grain, straw, and biological yields throughout the two growing seasons. Irrigation water considerably ($P<0.05$), reducing wheat crop grain, straw, and biological yield. The mean values of grain, straw, and biological yields were lower under the water deficit treatment (IR80) by 7.7, 9.8 and 9.3% compared to the recommended rate treatment (IR100), while under the lowest irrigation rate (IR60), they gave the reduction percent values of 19.9, 22.2 and 21.7 % in the same respect. This result reveals that the yield loss was less than 10% when saving 20% of irrigation water, which allows for reusing this surplus of water to irrigate new lands. Conversely, wheat yield losses were 11 and 25%, respectively, with irrigation treatments using 75% ETc during the growing season and 50% ETc throughout the growing season. Water saving values were 20 and 40% on average across the two growing seasons under these two irrigation treatments [15]. Similar results were obtained [61], who lowered the amount of water applied to wheat produced under surface irrigation by 22%, resulting in a 15% yield reduction. According to the study of Abdrabbo et al. [62], limiting the amount of water applied to wheat by 20% resulted in a yield loss of 12%. Moreover, El-Metwally et al. [49] stated that a 7% decrease in wheat grain yield might be expected if the irrigation water needed for the sprinkler irrigation system was reduced by 25%.

Table 7. Vitamin B2 (riboflavin) rates and irrigation treatments' effects on various yield components across the two growing seasons.

Treatment	Plant height (cm)	No. of spikes /m^2	Spike length (cm)	1000 grain weight (g)
Irrigation (IR)				
IR60	101.5	455	5.0	44.8
IR80	108.0	508	5.2	47.7
IR100	112.1	524	5.7	50.8
LSD 0.05	4.3	38	0.3	3.3
Vitamin B$_2$ (Riboflavin)				

Control		104.8	463	5.1	46.0
0.5 mM		105.9	486	5.2	48.0
1.0 mM		107.9	509	5.3	48.3
2.0 mM		110.2	526	5.6	48.9
LSD 0.05		3.3	21	0.3	2.1
Irrigation x vitamin B_2 (Riboflavin) interaction					
IR60	Control	97.7	411	4.7	43.4
	0.5 mM	100.6	443	5.1	44.6
	1.0 mM	102.8	471	5.0	45.2
	2.0 mM	104.9	496	5.0	46.0D
IR80	Control	106.3	473	5.1	45.7
	0.5 mM	107.2	501	5.0	48.1
	1.0 mM	108.1	522	5.4	48.4
	2.0 mM	110.5	538	5.5	48.6
IR100	Control	110.3	504	5.4	48.9
	0.5 mM	110.0	515	5.5	51.2
	1.0 mM	112.9	534	5.6	51.4
	2.0 mM	115.1	544	6.1	51.9
LSD 0.05		5.8	37	0.5	3.7

All foliar application treatments with vitamin B2 surpassed the control treatment in increasing the three yield characteristics (Table 8). Foliar application of the highest 2.0 mM vitamin B2 concentration provided the highest grain, straw, and biological yield values. This potent treatment increased the abovementioned yield traits by 12.3, 14.4, and 13.9%, respectively, compared with the unsprayed treatment. According to Deng et al. [31], riboflavin is necessary for appropriate plant development and growth.

Table 8. The impact of irrigation practices and vitamin B2 (riboflavin) interactions on grain, straw, and biological yields across the two growing seasons.

Treatment	Grain yield (kg ha^{-1})	Straw yield (kg ha^{-1})	Biological yield (kg ha^{-1})
Irrigation (IR)			
IR60	4217	13693	17910

IR80		4863	15879	20742
IR100		5267	17594	22861
LSD 0.05		314	1109	1242
Vitamin B$_2$ (Riboflavin)				
Control		4501	14638	19138
0.5 mM		4696	15387	20083
1.0 mM		4878	16121	21000
2.0 mM		5054	16743	21796
LSD 0.05		418	1126	1100
Irrigation x vitamin B2 (Riboflavin) interaction				
IR60	Control	3892	12087	15980
	0.5 mM	4129	13369	17498
	1.0 mM	4356	14310	18666
	2.0 mM	4491	15004	19496
IR80	Control	4574	14968	19542
	0.5 mM	4812	15535	20347
	1.0 mM	4956	16282	21238
	2.0 mM	5110	16732	21842
IR100	Control	5036	16858	21894
	0.5 mM	5148	17256	22404
	1.0 mM	5323	17772	23095
	2.0 mM	5560	18491	24052
LSD 0.05		725	1950	1905

Interaction effects presented in Table 8 showed that increasing the application of vitamin B2 concentration caused a significant gradual increase in the three yield traits under the three irrigation treatments compared to the untreated wheat plants (control). For grain, straw, and biological yields, the highest average values were attained by using the highest concentration of vitamin B2 (2.0 mM) under full irrigation, recording 5560, 18491, and 24052 kg ha^{-1}, respectively. When saving 20% of irrigation water (IR80) and using vitamin B2 rate (2.0 mM), the yield, as mentioned earlier, slightly decreased by only 8.1, 9.51, and 9.2%, respectively. As a result, it is determined that applying vitamin B2 to wheat through the foliar route will assist the plants withstand the harmful effects of water stress with just a tiny percentage of yield (less than 10%) lost. Many enzymes that decrease H_2O_2, such as glutathione reductase, require the flavin adenine dinucleotide (FAD) produced from riboflavin [63], which confirms the promoting role in plant growth. Our findings in Table 8 are in agreement with the results reported by Guhr et al. [29], who noted that the high degree of overlap between drought and riboflavin treatments shows a strong link between physiological responses and the possibility of riboflavin stress priming.

Additionally, they noted that riboflavin triggered defense mechanisms in hyphae under the stress of dryness. Moreover, [60] concluded that the effects of salinity stress on growth, physiological parameters, and leaflets' anatomy could be mitigated by spraying riboflavin at a concentration of 2000 ppm on salinized tecoma plants. The results imply vitamin B2 may be an efficient antioxidant by regulating osmotic and ionic equilibrium and improving salinity resistance in *Hibiscus sabdariffa* seedlings [59]. As a result, seedlings of *Hibiscus sabdariffa* may be developed under mild stress with a foliar treatment of 100 ppm riboflavin [59].

3.2.2. Response on water productivity (WP) and irrigation water productivity (IWP) to water stress, vitamin B2, and their interaction

The primary effect of irrigation treatments on water productivity (WP) did not appear to be significant according to the results (*P*>0.05). Still, irrigation water productivity (IWP) did, and it responded negatively to an increase in irrigation water (Table 9). This outcome could be explained by the fact that grain production increased less than the comparable increases in irrigation water. Water productivity (WP) and irrigation water productivity (IWP) did not significantly differ (*P*> 0.05) between the two irrigation treatments of IR100 and IR80, showing that only 80% of the irrigation water can be used without suffering a substantial reduction in grain output.

Mean values of foliar application treatment showed that spraying wheat plants with the highest concentration of vitamin B2 (2 mM) achieved the most significant increases in water productivity (WP) and irrigation water productivity (IWP) (Table 9). The mean water productivity (WP) values increased by 4.7, 8.8, and 12.7% under spraying wheat plants with 0.5 1.0 or 2.0 mM vitamin B2 compared to the control (Table 9). The same result was found for irrigation water productivity (IWP). Given that wheat plants were sprayed with vitamin B2 as opposed to the control, this outcome may be explained by the higher grain yield across irrigation treatments. Improved wheat crop yield and tolerance to deficit irrigation can be achieved by applying vitamin B2 at an appropriate rate.

It is obvious that the response of wheat plants sprayed with different levels of vitamin B2 was similar under the different irrigation regimes, reflecting that the interaction between irrigation treatment and foliar application of vitamin B2 was not significant (Table 9). Thus, foliar spray with 2.0 mM vitamin B2 resulted in the highest WP values among the three irrigation treatments, suggesting that less irrigation water may be used when spraying wheat plants with high concentrations of vitamin B2. The irrigation water productivity (IWP) was significantly influenced by the interaction between irrigation and foliar application of vitamin B2, where the irrigation water productivity decreased as irrigation water increased. However, the highest IWP values were recorded with the foliar application of 2.0 mM vitamin B2 under the IR60 irrigation treatment. This study aimed to investigate the role of riboflavin in drought stress tolerance in wheat plants. Slow chlorophyll degradation under drought conditions has been stated in plants pre-treated with riboflavin [31]. Riboflavin functions as a biphasic regulator in drought tolerance to boost water productivity (WP) and irrigation water productivity (IWP) during the two seasons.

Table 9. The impact of irrigation practices and vitamin B2 (riboflavin) levels, as well as how they interact, on water productivity (WP) and irrigation water productivity (IWP) over the two growing seasons.

Treatment		WP (kg mm^{-1} ha^{-1})	IWP (kg mm^{-1} ha^{-1})
Irrigation (IR)			
IR60		22.68	10.39
IR80		23.20	8.72
IR100		22.89	7.43
LSD 0.05		NS	0.66
Vitamin B$_2$ (Riboflavin)			
Control		21.55	8.30
0.5 mM		22.51	8.69
1.0 mM		23.40	9.04
2.0 mM		24.23	9.36
LSD $_{0.05}$		2.00	0.80
Irrigation x vitamin B$_2$ (Riboflavin) interaction			
IR60	Control	20.94	9.59
	0.5 mM	22.21	10.17

	1.0 mM	23.42	10.73
	2.0 mM	24.15	11.06
IR80	Control	21.82	8.21
	0.5 mM	22.96	8.63
	1.0 mM	23.65	8.89
	2.0 mM	24.38	9.17
IR100	Control	21.89	7.10
	0.5 mM	22.37	7.26
	1.0 mM	23.14	7.51
	2.0 mM	24.16	7.84
LSD $_{0.05}$		NS	1.40

3.3. Treatment x Traits (TT) biplot

This study used a biplot polygon graph, also known as a TT biplot graph, to examine the impact of treatments on the aimed traits in one chart [64]. Two polygon views were used to visually represent the effects of the twelve factorial irrigation treatments, foliar sprays of ascorbic acid, and vitamin B2 on the attributes under study (Figures 2 and 3 show two examples). The TT biplot's polygon view made it easier to identify which factorial treatments produced the most outstanding values for one or more attributes. Concerning the measured characteristics of wheat, the principal component analysis using the TT biplot method (Figure 2) explained goodness of fit, with the graph accounting for roughly 97.81% of the observed variation. The first and second PCs explained 87.03% and 10.78% of the data, respectively. Regarding vitamin B2 (Figure 3), the biplot model described 96.29% of the total variation of the standardized data for the attributes of wheat that were assessed after being sprayed with vitamin B2 foliar application treatments. The first principal components (PC1 and PC2) explained 84.23% and 12.05%, respectively.

A polygon graph of both vitamins (ascorbic acid and vitamin B2) illustrated which irrigation treatment combination won which for wheat traits. Over the two biplot graphs (Figures 2 and 3), it is observed that grain yield and its attributes were located in one sector close to each other, which means that there are strong positive interrelationships among them. In addition, it is clear that T12 (IR100 + 6 mM AsA or IR100 + 2.0 mM VB2) was the vertex treatment for grain yield and all studied traits followed by T11 (IR100 + 4 mM AsA or IR100 + 1.0 mM VB2), T8 (IR80 + 6 mM AsA or IR80 + 2 mM VB2), T10 (IR100 + 2 mM AsA or IR100 + 0.5 mM VB2) and T7 (IR80 + 4 mM AsA or IR80 +1 mM VB2). However, water productivity (WP) was laid outside the sector of the best treatments, indicating that WP was less responsive to the used treatments compared to grain yield and yield attributes. As opposed to that, all treatments on the left side of the graphs (T1, T2, T3, T4, and T5) had the lowest mean values for most measured traits. These treatments represent the low irrigation water with or without spraying vitamins (ascorbic acid and vitamin B2). According to Yan and Kang [65], the first two PCs must represent more than 60% of the total variation for the T.T. biplot model to have a decent fit. The current findings summarized and supported the conclusion reached using mean comparisons and analysis of variance (Tables 4–8). Similar results on wheat were also reported by other researchers [66,67].

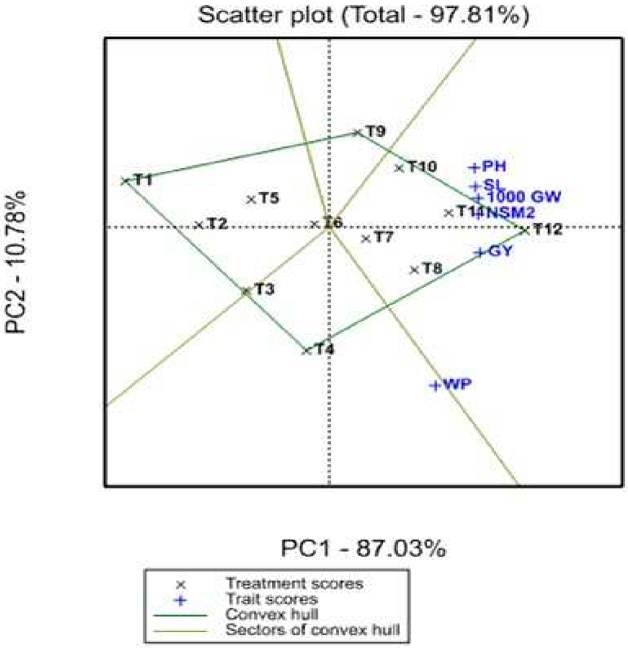

Figure 2. The irrigation and ascorbic acid factorial treatments with their corresponding greatest values for each trait are shown in a polygon view of the TT biplot.

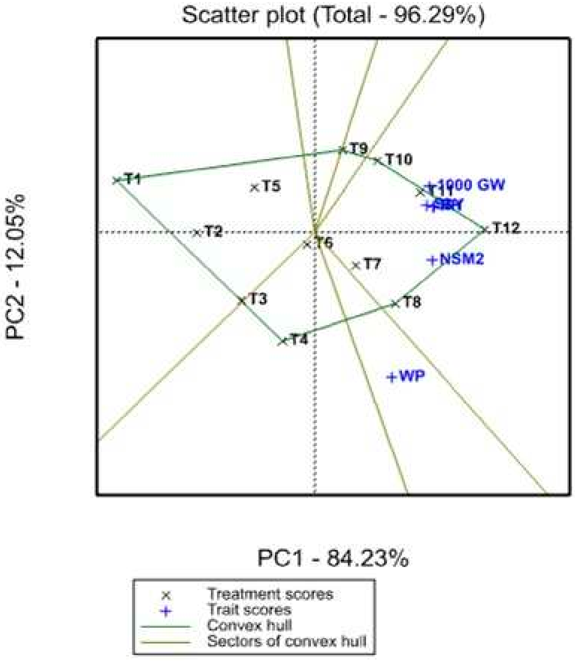

Figure 3. The TT biplot's polygon view reveals which factorial treatments—irrigation and vitamin B2—had the greatest values for which attributes.

4. Conclusions

Water scarcity, particularly in Egypt, is one of the most critical factors of global food security. As a result, every effort must be made to conserve and reuse every drop of water to develop freshly recovered fields. For example, vitamins like ascorbic acid (vitamin C) and riboflavin (vitamin B2) can help plants deal with and withstand water shortages. The current study found that full water requirement considerably boosted the yield of wheat grains and yield components compared to less amount. The results demonstrated that, in comparison to the control plots, the foliar spray of 6 mM ascorbic acid or 2.0 mM vitamin B2 considerably boosted grain yield and yield components as well as the water use efficiency indicated as water productivity (WP) and irrigation water productivity (IWP). The factorial treatments between the full irrigation IR100 or reduced irrigation IR80 with the highest concentration of ascorbic acid or vitamin B2 rate gave the most outstanding values of studied characters with no significant difference. Subsequently, when spraying wheat plants with a high concentration of ascorbic acid or vitamin B2, it is possible to save 20% of irrigation water requirement. At the same time, the loss of yield and its components is slight, not exceeding 10%.

Author Contributions: Conceptualization, A.KH.A., A.A.S., W.F., O.M., and M.T.A.; data curation, A.KH.A., A.A.S., W.F., O.M., and M.T.A.; formal analysis, A.KH.A., A.A.S., W.F.; investigation, A.KH.A., A.A.S., and M.T.A.; methodology, A.KH.A., A.A.S., and M.T.A.; resources, A.KH.A., A.A.S., O.M., and M.T.A.; software, W.F..; writing—original draft, A.KH.A., A.A.S., W.F., O.M., and M.T.A.; writing—review and editing, W.F., O.M., and M.T.A. All authors have read and agreed to the published version of the manuscript.

Funding: This work was part of Research Project No.11030129 and supported by the National Research Centre, Cairo, Egypt.

Data Availability Statement: The data presented in this study are available upon request from the corresponding authors.

Conflicts of Interest: The authors declare no conflicts of interest.

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