



RESEARCH ARTICLE

Effect of exogenous proline on drought stress tolerance in wheat (*Triticum aestivum* L.) cultivars

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Received: 03 May 2025; Accepted: 17 July 2025; Available online: Version 1.0: 07 September 2025; Version 2.0: 01 October 2025

Cite this article: Alkhafagi HHA, Al-Janabi YA, Al-Khafagi KF. Effect of exogenous proline on drought stress tolerance in wheat (*Triticum aestivum* L.) cultivars. Plant Science Today. 2025; 12(4): 1-8. <https://doi.org/10.14719/pst.9266>

Abstract

Due to the scarcity of irrigation water and to achieve maximum benefit from the available irrigation water, this study investigated the impact of exogenous proline application on drought tolerance in three wheat (*Triticum aestivum* L.) cultivars and evaluated their water use under stress conditions. A factorial field experiment was conducted using a split-split plot design with three factors at three levels each. The main plot factor was drought stress, with three levels: S1 (control) involved irrigation with 50 % of the available water between field capacity (FC) and wilting point (WP); S2 represented mild drought with 30 % available water (70 % reduction); S3 represented severe drought with 10 % available water (90 % reduction). The second factor (subplot) was proline concentration: P1 (0 mg L⁻¹), P2 (25 mg L⁻¹) and P3 (50 mg L⁻¹). The third factor (sub-subplot) was wheat cultivar: Ibaa 99, Adnaniyah and Sham 6. A 90 % reduction in available water significantly decreased vegetative and yield parameters, including plant height (58.53 cm), spikes per plant (3.98), seeds per spike (37.86), total yield (3.47 tons·ha⁻¹), flag leaf area (25.75 cm²), chlorophyll content and water use efficiency (1.6 kg grains m⁻³ water). The highest water use efficiency was recorded under 30 % available water, while the maximum grain yield (6.07 tons ha⁻¹) was achieved under 50 % available water. Foliar application of 50 mg L⁻¹ proline (P3) increased grain yield and improved water use efficiency to 1.61 kg grains m⁻³ water. Although interactions between drought stress and proline concentration were not statistically significant, Sham 6 consistently outperformed Ibaa 99 and Adnaniyah across most growth and yield parameters.

Keywords: amino acid; available water; drought stress; foliar application; water consumption

Introduction

Water scarcity presents a critical challenge to global agricultural productivity, particularly in arid and semi-arid regions. Addressing this challenge requires a clear understanding of crop water requirements and the development of optimized irrigation strategies that meet plant needs without excessive water usage. Implementing measures to mitigate irrigation water deficits is vital for conserving water resources. These measures also improve soil moisture distribution around the root zone and maintain soil health (1). Drought is one of the most severe abiotic stressors affecting crop growth and productivity, often resulting in substantial agricultural losses and, in extreme cases, complete crop failure (2). Drought stress in wheat significantly increases proline content in flag leaves while reducing chlorophyll levels and grain yield (3).

Proline, a key amino acid, plays a crucial role in plant responses to drought stress. It regulates osmotic pressure, enhances antioxidant enzyme activities, improves water retention and helps in maintaining the structural integrity of flag leaf tissue. These functions collectively contribute to increased drought resistance (4). Water stress decreases chlorophyll pigments and yield components, while proline application mitigates these effects through enhanced antioxidant activities.

Plants under drought stress also produce defensive enzymes like superoxide dismutase, peroxidase and catalase, which help in scavenging the reactive oxygen species and hydrogen peroxide (5).

The accumulation of compatible solutes, including proline, is a common adaptive response in plants facing drought (6). Interestingly, no significant differences were observed between 50 % and 70 % available water treatment, suggesting the possibility of conserving up to 40 % of irrigation water (7-9). Water deficits negatively impact wheat yield components such as the number of spikes, seeds per spike, 1000-seed weight and grain yield. Higher humidity levels positively influenced leaf area, spike number and yield (10, 11). Well-timed and quantitatively appropriate irrigation is essential to optimize production and water use efficiency, as it replenishes only the water deficit (12). Proper irrigation scheduling, defined as determining the timing and quantity of irrigation for maximum yield remains critical for sustainable agriculture (13, 14).

Furthermore, the application of proline increased chlorophyll a content in wheat, indicating its potential role in enhancing photosynthetic capacity (15). It was also demonstrated that proline accumulation increases with drought severity, thereby enhancing drought and heat tolerance

Additionally it was found that applying proline at a concentration of 20 mg L⁻¹ positively influenced most growth stages and yield components of wheat (16).

The present study aims to further elucidate the role of exogenous proline in alleviating the adverse effects of water stress on wheat crops, optimize irrigation water usage and estimate the actual water consumption of different wheat cultivars under varying drought and proline treatment conditions.

Materials and Methods

Experimental location and soil characteristics

A field experiment was conducted during the spring growing season of 2023 at a local farm in the northern Babylon Governorate, Iraq at latitude 32.79°, longitude 44.40°. The soil was classified as clay loam, with physical and chemical properties presented in Table 1.

Experimental design

The experiment was laid out in a randomized complete block design (RCBD) with a split-split plot arrangement. The main plots consisted of water stress levels, S1 (control): 50 % available water between FC and WP. The percentage of moisture present between field capacity and wilting point S2 (mild stress): 30 % available water (70 % reduction) and S3 (severe stress): 10 % available water (90 % reduction). Subplots: proline concentrations, P1: 0 mg L⁻¹, P2: 25 mg L⁻¹ and P3: 50 mg L⁻¹. Sub-subplots: wheat cultivars V1: Ibaa 99, V2: Adnaniyah and V3: Sham 6. Each treatment was replicated three times.

Irrigation and water management

The experiment was designed to assess plant response based on the principle of moisture consumption within the range of available water for plant uptake, defined by FC and WP. The soil's water retention capacity was evaluated by determining the percentage moisture content and constructing a soil moisture

characteristic curve, which represents the relationship between soil water content and matric potential (Fig. 1). Different water tension forces (33, 100, 300, 500, 1000 and 1500 kPa) were applied to soil samples to establish this curve. The experimental site was planted on November 30, 2023. Irrigation water was delivered to the plots using a 2-inch pump equipped with a water meter to quantify the applied volumes. Soil moisture content was monitored using a portable soil moisture meter calibrated



Fig. 2. Moisture measuring device in the field.

Table 1. Physical and chemical properties of the field soil

Property	Value	Unit
Silt	372	gkg ⁻¹ soil
Clay	78	gkg ⁻¹ soil
Loam	550	-
Soil texture	Salty loam	-
Bulk density	1.55	gcm ⁻³
Field capacity	34.68	% volumetric moisture
Wilting point	12.22	% volumetric moisture
EC (electrical conductivity)	4.98	dSm ⁻¹
K ⁺	214.45	mg.kg ⁻¹
N	94.8	mgkg ⁻¹

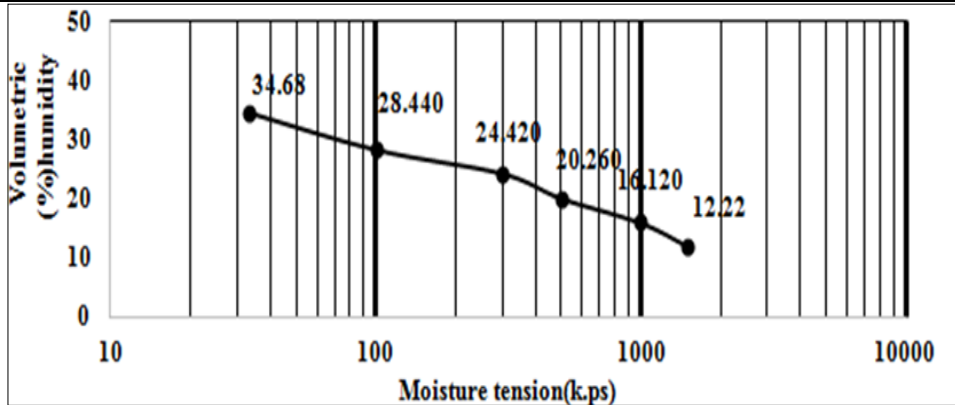


Fig. 1. Description curve of the experimental field.

against laboratory gravimetric measurements (17). Calibration ensured accurate field moisture readings (Fig. 2).

$$qv = qw \times (ab / aw) \quad (\text{Eqn.1}) \dots\dots\dots(18)$$

Soil moisture content was expressed on both a volumetric (qv) and gravimetric (qw) basis, where qv is in $\text{cm}^3 \text{cm}^{-3}$ and qw is in g g^{-1} .

The bulk density (pb) of the soil was calculated as:

$$pb = \frac{M}{V} \dots\dots (\text{Eqn. 2}) \dots\dots\dots$$

pb = Mass of dry soil (g) / volume of soil (cm^3). The density of water (ρ_w) is approximately 1 g cm^{-3} .

During planting, all experimental units received equal amounts of water to reach the desired water depletion level, ensuring uniform germination. Subsequently, the quantity of water to be added to each treatment to restore soil moisture to the required level was calculated based on the following equation (modified from the original presentation for clarity) (19).

$$w = a \times \ell b \left[\frac{\%Pw^{f.c} - \%Pw^w}{100} \right] \times \frac{D}{100} \quad (\text{Eqn. 3})$$

Where, W = the amount of water added (m^3)

a = experimental unit area (m^2)

ℓb = bulk density (microgram m^3)

$Pw^{f.c}$ = % moisture field capacity [after irrigation]

Pw^w = % moisture level before irrigation

D= irrigation water depth (m), calculated as:

$D = 100 (\% \text{ moisture at field capacity} - \% \text{ moisture before irrigation}) \times \text{rooting depth (m)} \times pb$

The water balance equation was used to calculate the water requirement of wheat.

$$ET = I + P \pm \Delta S - R - D \quad (\text{Eqn. 4})$$

Where, ET: actual evapotranspiration, I: depth water used in irrigation P: depth rain water (mm), D: ground water depth (equals zero because irrigation is done to the extent of field capacity, R: surface runoff (equals zero because presence of surface runoff), ΔS : humidity changes during the cultivation period until the end of the experiment.

Characteristics under study

Different characteristics such as plant height, volume of flag leaf, spike, seeds per plant, seed spike⁻¹, total yield tons ha^{-1} , efficiency $\text{kg m}^3 \text{water ha}^{-1}$ were studied.

Statistical analysis

All collected data were statistically analyzed using GenStat version 12. Means were compared using the least significant difference (LSD) test at a significance level of $p \leq 0.05$ (20).

Results and Discussion

Plant height

Based on the soil characteristics (Table 1) and water requirements (Table 2), the results indicated that plant height was greatest under the control treatment (Table 3) (S1: 50 % available water), as the average plant height reached 91.45 cm. Drought stress exerted a significant negative effect on plant height, with the most pronounced reduction observed under severe drought conditions (S3: 10 % available water), yielding an average height of 58.52 cm. This reduction in height under water deficit is primarily attributed to diminished cellular water content and restricted cell expansion caused by increased cell wall rigidity, which ultimately hampers plant development. Wheat seedlings

Table 2. Moisture depletion and corresponding water consumption $\text{m}^3 \text{ha}^{-1}$

Number of irrigations	Water added $\text{m}^3 \text{ha}^{-1}$	Depth of rain water (mm)	Depth of added water (mm)	Water consumption (m)	Depletion available water
8	3420	31	342	373	50 %
5	2510	31	251	282	70 %
3	2130	31	213	244	90 %

Table 3. Drought stress, proline, varieties and their interaction on plant height (cm)

Treatments			Cultivars			Mean	
Reduce available water	Proline concentrations mgL ⁻¹	V ₁	V ₂	V ₃	S*P	S	
1 Stress	S ₁ 50 %	P ₁ (0)	88.127	86.943	89.640	88.237	91.459
		P ₂ (25)	91.430	90.693	92.677	91.600	
		P ₃ (50)	93.740	93.293	96.583	94.539	
	S ₂ 70 %	P ₁ (0)	79.537	78.270	81.263	79.69	85.741
		P ₂ (25)	83.933	83.007	85.487	84.142	
		P ₃ (50)	92.700	91.763	95.710	93.391	
	S ₃ 90 %	P ₁ (0)	51.883	49.933	54.243	52.02	58.527
		P ₂ (25)	59.443	57.720	59.863	59.009	
		P ₃ (50)	63.817	62.920	66.917	64.551	
		LSD _{0.05}		S*P*V= NS	S*P= NS	S=0.4441	
	S*V	V ₁	91.099	90.310	92.967	Mean V	
		V ₂	85.390	84.347	87.487	78.290	
V ₃		58.381	84.347	87.487	77.171		
	LSD _{0.05}		S*V= NS		80.265		
P*V	V ₁	73.182	71.716	75.049	V=0.4698		
	P ₁	78.269	77.140	79.342	Mean P		
	P ₂	83.419	82.659	86.403	73.316		
	LSD _{0.05}		P*V= NS		78.250		
					84.160		
					P= 0.2948		

subjected to water stress rapidly lose cell wall plasticity in stems and leaves, leading to reduced cell division and consequently reduced plant size (21, 22). Among the evaluated cultivars, Sham 6 exhibited the greatest plant height (80.27 cm), whereas Adnaniyah recorded the lowest (77.16 cm).

Foliar application of proline at 50 mg L⁻¹ (P3) resulted in the tallest plants (84.16 cm). Exogenous proline enhances both shoot and root system development, thereby promoting increased plant height (23). In contrast, the control treatment without proline application (P1: 0 mg L⁻¹) resulted in the lowest average plant height (73.31 cm). Importantly, all interactions among the experimental factors were statistically non-significant.

Flag leaf area

Table 4 shows that there were no significant differences in flag leaf area between the S1 and S2 treatments, which recorded comparable values. However, both treatments were significantly superior to the severe drought condition (S3), which resulted in a markedly lower average flag leaf area of 25.75 cm². These results indicate that drought stress reduces leaf area, a phenomenon attributed to the role of the flag leaf in osmotic regulation under water deficit conditions (24).

Among the tested cultivars, Sham 6 exhibited the largest flag leaf area (38.21 cm²), followed closely by Adnaniyah (37.68 cm²). Application of the highest proline concentration (P3) yielded the greatest average flag leaf area (40.73 cm²), underscoring proline's role in osmotic adjustment, dehydration tolerance and cellular protection (25, 26). In contrast, the control treatment without proline application (P1: 0 mg L⁻¹) resulted in the smallest average flag leaf area (36.06 cm²). Notably, a significant interaction was detected between the control water treatment and the highest proline concentration (S1 × P3), producing the largest recorded flag leaf area (47.38 cm²). All other interactions among the experimental factors (S × V, P × V, S × P × V) were statistically non-significant.

Total spikes per plant

Table 5 reveals that the total number of spikes per plant did not significantly differ between the S1 and S2 available water treatments, both of which were superior to S3, which averaged 3.98 spikes per plant. The reduction in spikes per plant under severe drought stress may be attributed to the abortion of some tillers and a decrease in their overall number. Drought stress can also reduce nutrient availability during seedling emergence and development, leading to increased competition and a lower

Table 4. Drought stress, proline, varieties and their interaction on flag leaf area per cm²

Treatments		Cultivars			Mean	
Reduce available water	Proline concentrations mgL ⁻¹	V ₁	V ₂	V ₃	S*P	S
S ₁ 50 %	P ₁ (0)	41.997	41.833	42.340	42.057	44.044
	P ₂ (25)	42.827	42.467	42.783	42.692	
	P ₃ (50)	47.477	47.033	47.640	47.383	
S ₂ 70 %	P ₁ (0)	41.647	41.033	41.870	41.517	44.082
	P ₂ (25)	43.127	42.860	43.490	43.159	
	P ₃ (50)	47.620	47.340	47.753	47.571	
S ₃ 90 %	P ₁ (0)	24.500	24.307	25.013	24.607	25.751
	P ₂ (25)	25.390	25.153	25.653	25.399	
	P ₃ (50)	27.250	27.140	27.353	27.248	
LSD _{0.05}		S*P*V=NS			S*P=0.4307	S=0.3468
S*V	V ₁	44.100	43.778	44.254	Mean V	37.981
	V ₂	44.131	43.744	44.371		37.685
	V ₃	25.713	25.533	26.007		38.211
LSD _{0.05}		S*V=NS			V=0.1778	
P*V	P ₁	36.048	35.724	36.408	Mean P	36.060
	P ₂	37.114	36.827	37.309		37.083
	P ₃	40.782	40.504	40.916		40.734
LSD _{0.05}		P*V=NS			P=0.2438	

Table 5. Drought stress, proline, varieties and their interaction on the number of spike per plant

Treatments		Cultivars			Mean	
Reduce available water	Proline concentrations mgL ⁻¹	V ₁	V ₂	V ₃	S*P	S
S ₁ 50 %	P ₁ (0)	4.183	4.137	4.277	4.19	4.270
	P ₂ (25)	4.293	4.273	4.867	4.47	
	P ₃ (50)	5.233	5.167	5.243	5.21	
S ₂ 70 %	P ₁ (0)	4.170	4.170	4.253	4.19	4.245
	P ₂ (25)	4.283	4.277	4.293	4.28	
	P ₃ (50)	4.383	4.350	4.407	4.83	
S ₃ 90 %	P ₁ (0)	3.887	3.870	3.920	3.892	3.983
	P ₂ (25)	3.957	3.940	4.083	3.993	
	P ₃ (50)	4.037	4.023	4.133	4.064	
LSD _{0.05}		S*P*V=NS			S*P=0.091	S=0.0745
S*V	V ₁	4.570	4.526	4.796	Mean V	4.630
	V ₂	4.279	4.266	4.318		4.287
	V ₃	3.960	3.944	4.046		4.386
LSD _{0.05}		S*V=NS			V=0.0560	
P*V	P ₁	4.080	4.059	4.150	Mean P	4.096
	P ₂	4.178	4.163	4.414		4.252
	P ₃	4.551	4.513	4.594		4.553
LSD _{0.05}		P*V=NS			P=0.0511	

number of fertile tillers (27-29). The Ibaa 99 cultivar recorded the highest number of spikes per plant (4.63), while Adnaniyah had the lowest (4.28).

Proline application at 50 mg L⁻¹ (P3) resulted in 4.55 spikes per plant, compared to 4.09 spikes per plant in the control proline treatment (P1). A positive interaction between the control water treatment and the highest proline concentration (S1 × P3) yielded the highest number of spikes (5.21). Conversely, the interaction between severe drought and the control proline concentration (S3 × P1) resulted in the lowest number of spikes (3.89). The remaining interactions (S × V, P × V, S × P × V) were statistically non-significant.

Number of seeds per spike

Table 6 shows that the control treatment (S1) resulted in the highest number of seeds per spike (105.45), significantly higher than the severe drought treatment (S3), which produced the lowest average of 37.86 seeds per spike. Drought-induced seed reduction may be due to tiller abortion and decreased nutrient availability during seed development (30). Among the cultivars, Sham 6 exhibited the highest number of seeds per spike (79.51), while Adnaniyah had the lowest (79.02). Proline application at 50 mg L⁻¹ (P3) resulted in the highest average number of seeds per

spike (80.02), compared to 78.41 seeds per spike in the control proline treatment (P1). All interactions between the experimental factors were statistically non-significant.

Total grain yield

Wheat productivity was significantly affected by water availability, as shown in Table 7. The control treatment (S1) resulted in the highest grain yield (6.07 tons ha⁻¹) (Fig. 3), while the severe drought treatment (S3) yielded the lowest (3.47 tons ha⁻¹), likely due to the reduced yield components observed (29-31). The Sham 6 cultivar recorded the highest average grain yield (5.24 tons ha⁻¹) and Adnaniyah the lowest (4.78 tons ha⁻¹).

Proline application at 50 mg L⁻¹ (P3) resulted in the highest average grain yield (5.80 tons ha⁻¹), while the control proline treatment (P1) had the lowest (4.19 tons ha⁻¹), suggesting a protective role of proline under stress. A significant interaction was observed between water stress and proline application (P × S). Specifically, the combination of the control water treatment and the highest proline concentration (S1 × P3) resulted in the highest grain yield (6.87 tons ha⁻¹), demonstrating that proline accumulation enhances production under drought and heat stress by regulating osmotic pressure (32). The lowest grain yield (2.96 tons ha⁻¹) was recorded in the interaction between severe

Table 6. Drought stress, proline, varieties and their interaction on the number of seeds per spike

Treatments		Cultivars			Mean	
Reduce available water	Proline concentrations mgL ⁻¹	V ₁	V ₂	V ₃	S*P	S
S ₁ 50 %	P ₁ (0)	105.027	104.590	105.143	104.920	105.459
	P ₂ (25)	105.623	105.423	105.573	105.540	
	P ₃ (50)	105.713	105.920	106.117	105.917	
S ₂ 70 %	P ₁ (0)	93.660	93.327	94.123	93.703	94.441
	P ₂ (25)	94.097	94.080	94.513	94.230	
	P ₃ (50)	95.377	94.833	95.960	95.390	
S ₃ 90 %	P ₁ (0)	36.653	36.337	36.830	36.607	37.869
	P ₂ (25)	38.083	38.227	38.410	38.240	
	P ₃ (50)	38.837	38.450	38.990	38.759	
LSD _{0.05}		S*P*V = NS			S*P = NS	S = 0.3574
S*V	V ₁	S ₁	S ₂	S ₃	Mean V	
	V ₂	105.454	94.378	37.858	79.230	
	V ₃	105.311	94.08	37.671	79.021	
LSD _{0.05}		S*V = NS			V = 0.2404	
P*V	P ₁	V ₁	V ₂	V ₃	Mean P	
	P ₂	78.447	78.084	78.699	78.410	
	P ₃	79.268	79.243	79.499	79.337	
LSD _{0.05}		P*V = NS			P = 0.2286	

Table 7. Drought stress, proline, varieties and their interaction on total yield tons/ha⁻¹

Treatments		Cultivars			Mean	
Reduce available water	Proline concentrations mgL ⁻¹	V ₁	V ₂	V ₃	S*P	S
S ₁ 50 %	P ₁ (0)	5.44	4.90	5.59	5.31	6.07
	P ₂ (25)	5.96	5.95	6.20	6.04	
	P ₃ (50)	6.80	6.73	7.09	6.87	
S ₂ 70 %	P ₁ (0)	4.20	4.05	4.58	4.28	5.40
	P ₂ (25)	5.46	5.40	5.83	5.57	
	P ₃ (50)	6.26	6.19	6.64	6.37	
S ₃ 90 %	P ₁ (0)	2.98	2.77	3.14	2.96	3.47
	P ₂ (25)	3.19	3.09	3.59	3.29	
	P ₃ (50)	4.08	3.92	4.46	4.15	
LSD _{0.05}		S*P*V = NS			S*P = 0.1038	S = 0.0219
S*V	V ₁	S ₁	S ₂	S ₃	Mean V	
	V ₂	6.07	5.31	3.42	4.93	
	V ₃	5.86	5.21	3.26	4.78	
LSD _{0.05}		S*V = NS			V = 0.0603	
P*V	P ₁	V ₁	V ₂	V ₃	Mean P	
	P ₂	4.21	3.91	4.44	4.19	
	P ₃	4.87	4.81	5.21	4.97	
LSD _{0.05}		P*V = 0.1083			P = 0.0728	

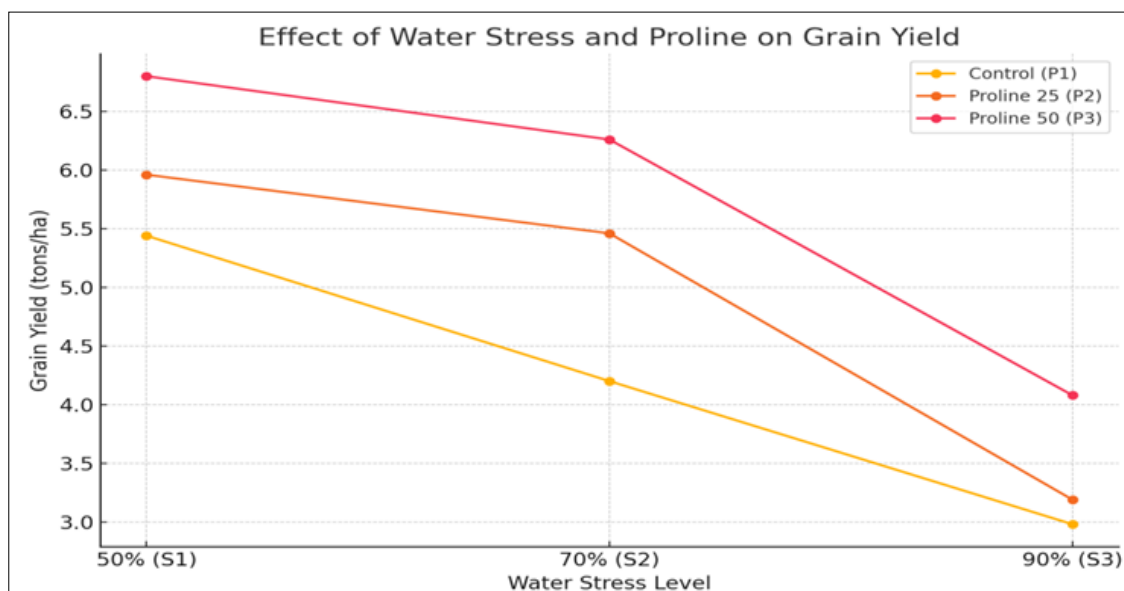


Fig. 3. Effect of water stress and proline on grain yield.

drought and the control proline concentration ($S3 \times P1$). Drought can deplete water and nutrients near wheat roots, leading to grain yield reduction (28, 33).

Water use efficiency

Table 8 demonstrates a significant increase in water use efficiency (WUE) under the mild drought treatment (S2), with an average of 2.15 kg grains m^{-3} water, compared to the control (S1). This suggests that moderate drought can enhance WUE without drastically compromising yield (34, 35). The lowest WUE was observed in the severe drought treatment (S3), averaging 1.6 kg grains m^{-3} water. Among the cultivars, Sham 6 exhibited the highest WUE (1.9 kg grains m^{-3} water), potentially due to its inherent productivity under the experimental conditions (36, 37). The Adnaniyah cultivar had a WUE of 1.7 kg grains m^{-3} water.

Proline application at 50 $mg L^{-1}$ (P3) resulted in the highest WUE (2.16 kg grains m^{-3} water). This result was observed under the mild stress treatment (70 %), while the control proline treatment (P1) had the lowest (1.5 kg grains m^{-3} water). The interaction between the mild drought treatment and the highest proline concentration ($S2 \times P3$) yielded the highest WUE (2.5 kg

grains m^{-3} water). The lowest WUE was recorded in the interaction between severe drought and the control proline concentration ($S3 \times P1$), reaching 1.3 kg grains m^{-3} water. Interactions between water stress and cultivars, proline concentration and cultivars and the triple interaction were statistically non-significant.

Conclusion

Reducing wheat water requirements by more than 80 % through depletion of available water is not advisable, as it significantly reduces yield. This finding is based on a study aimed at regulating the demand for irrigation water and mitigating its scarcity in Iraq, while also achieving the maximum benefit from the available water resources. However, irrigation water can be reduced to 70 % of field capacity without compromising economic viability, if proline is applied at the appropriate dosage and growth stage. Therefore, we suggest to use amino acid proline which functions as an anti-transpirants and nutrient, contributes to strengthening plant cell walls to reduce water loss from the plant.

Table 8. Drought stress, proline, varieties and their interaction on water use efficiency (kg grains m^{-3} water)

Treatments		Cultivars			Mean	
Reduce available water	Proline concentrations mgL^{-1}	V ₁	V ₂	V ₃	S*P	S
S ₁ 50 %	P ₁ (0)	1.5916	1.4327	1.6355	1.5533	1.7763
	P ₂ (25)	1.7437	1.7407	1.8138	1.7661	
	P ₃ (50)	1.9873	1.9669	2.0741	2.0094	
S ₂ 70 %	P ₁ (0)	1.6746	1.6135	1.8260	1.7047	2.1527
	P ₂ (25)	2.1766	2.1514	2.3240	2.2174	
	P ₃ (50)	2.4954	2.4661	2.6467	2.5361	
S ₃ 90 %	P ₁ (0)	1.4006	1.2989	1.4742	1.3912	1.6282
	P ₂ (25)	1.4961	1.4507	1.6854	1.5441	
	P ₃ (50)	1.9155	1.8388	2.0939	1.9494	
LSD _{0.05}		S*P*V = 0.0135			S*P = 0.0455	S = 0.0135
S*V	V ₁	1.7742	2.1155	1.6041	Mean V	1.8313
	V ₂	1.7135	2.0770	1.5295	1.7733	
	V ₃	1.8411	2.2656	1.7512	1.9526	
LSD _{0.05}		S*V = NS			V = 0.0257	Mean P
P*V	P ₁	1.5556	1.4484	1.6452	1.5498	
	P ₂	1.8055	1.7809	1.9411	1.8425	
	P ₃	2.1327	2.0906	2.2716	2.1650	
LSD _{0.05}		P*V = NS			P = 0.0316	

Authors' contributions

HHAA conceptualized, supervised and did the project administration. KFHA did the formal analysis, wrote the original draft. YAAJ done the fieldwork and prepared figures. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process: During the preparation of this work, the author used ChatGPT 4.0, for figure of the total seed yield and paraphrasing certain sections of the text to enhance clarity and coherence. After using this tool, the authors carefully reviewed and edited the content as needed and took full responsibility for the final version of the publication.

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