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RESEARCH ARTICLE

Phenotypic, physiological and molecular changes of some wheat varieties under drought stress

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Abstract

Water stress poses a significant challenge to wheat production, adversely affecting both field productivity and grain quality in the face of climate change and diminishing water resources. It reduces vegetative growth and disrupts physiological processes, which negatively impact yield components like grain size and protein content. Consequently, selecting drought-tolerant varieties is critical for enhancing resilience in arid regions. This study examined ten wheat varieties belonging to the genus *Triticum aestivum* L. (Abba 99, Adna 99, Baraka, Bohooth 10, Bohooth 22, Jihan 99, Bora, Dijla, Sham 6 and Wafia) under three water stress levels: 0 MPa (S0), -1.48 MPa (S1) and -2.95 MPa (S2) using polyethylene glycol (PEG6000). Phenotypic traits measured included plant height, leaf area, stem diameter, wet and dry weight, along with chlorophyll content. Real-time quantitative Polymerase Chain Reaction (RT-qPCR) was used to assess the expression of SOD and BADH-1 genes. Results indicated that Adana 99 exhibited significant drought resistance, recording the highest measurements in plant height (35 cm), leaf area (15.50 cm^2), stem diameter (1.80 mm), wet weight (0.80 g), dry weight (0.55 g) and total chlorophyll content (45.85 and 48.14) at 15 and 30 days, respectively, under S2. The SOD gene expression peaked at 8.57 in S2, an eightfold increase from S0. Similarly, BADH-1 gene expression was recorded at 8 in S2, also an eightfold increase. In contrast, Baraka and Wafia showed the lowest expressions for the SOD (0.001) and BADH-1 (0.002) genes under S2, negatively affecting their phenotypic and physiological traits. These findings underscore the importance of selecting drought-resistant varieties for sustainable productivity under harsh environmental conditions.

Keywords

BADH-1 gene; chlorophyll; drought-PEG6000; gene expression; SOD gene; wheat plant

Introduction

Iraqi territory faces great challenges due to extreme climate changes, such as global warming, increased desertification and water scarcity, highlighting the need to find agricultural crops that are resilient to volatile environmental conditions. About 37% of wheat globally is grown in arid and semi-arid regions, reinforcing the importance of studying the effects of drought on crops (1). Drought stress leads to an increase in the accumulation of reactive oxygen species (ROS) in plants due to an imbalance between ROS production and the antioxidant defence systems. This accumulation causes oxidative stress, which results in the oxidation of proteins, lipids and DNA, compromising cellular integrity and hindering normal growth processes. Additionally, the oxidative stress induced by ROS accumulation reduces photosynthesis efficiency and negatively impacts nutrient and water uptake. Excessive ROS accumulation is also known to contribute to programmed cell death (apoptosis), further exacerbating plant deterioration under drought conditions (2, 3).

Due to the importance of soft wheat and its key role in global food security as the basic food for most of the world's population, there was a need to identify a genetically, physiologically and phenotypically efficient variety with the ability to withstand water stress conditions. Recently, plants have been exposed to increased drought and water stress due to global climatic conditions, including high temperatures and prolonged droughts. This issue is especially severe in Iraq. It has harmed agricultural production and reduced arable land availability, exacerbated by outdated and primitive farming methods.

At present, water stress is one of the most prominent problems faced by agriculture and hinders agricultural expansion, as it is one of the biggest obstacles hampering the growth and productivity of the wheat plant in Iraq and many countries of the world (4). So, the need arose for researchers to study water stress and its negative effects on plants, as droughts occurred have 293 times around the world and have caused a total damage costing 107.2 billion US dollars between 2001 and 2018 (5).

The wheat crop (*Triticum aestivum* L.) is one of the most important strategic cereal crops in the world, as it ranks first in terms of cultivated areas globally and second in terms of production after yellow corn and provides about 70% of the food of the world's population and has strategic role in global food security (6). According to the Food and Agriculture Organization of the United Nations, the global wheat crop has reached about 781 million tons (7). The latest statistics showed that the total production of wheat in the Iraq reached 5 million tons, achieving self-sufficiency for the actual need (8).

Different plant species have developed complex networks of defence mechanisms to minimize damage caused by biotic and abiotic stressors. Global climate change and associated adverse abiotic stresses due to drought, salinity, extreme temperatures seriously affect plant growth and development. All of these biotic and abiotic factors lead to a significant reduction in the quantity and quality of agricultural products worldwide (9). Recent studies have indicated that water stress to which plants are exposed coincides with many significant changes at the morphological and physiological levels (10). Recent studies have focused on elucidating the role of superoxide dismutase (SOD) and betaine aldehyde dehydrogenase (BADH-1) genes in enhancing drought tolerance in wheat. These genes hold significant potential for incorporation into breeding programs aimed at developing drought-resistant crop varieties and improving agricultural resilience (11).

SOD is a crucial antioxidant enzyme that neutralizes ROS, which accumulate under drought stress leading to cellular damage. By mitigating ROS levels, SOD directly enhances plant performance and resilience under stressful conditions, contributing to improved drought tolerance. It emphasizes the significance of SOD in mitigating oxidative stress in wheat by scavenging ROS and enhancing the plant's drought resilience. Similarly, the BADH-1 gene is integral to the synthesis of glycine betaine, a compound that stabilizes cellular structures and maintains osmotic balance during drought conditions. It protects plant cells under water-deficit stress by enhancing osmoprotection and improving water retention capabilities (11, 12). The expression of these genes in wheat under polyethylene glycol (PEG)-induced drought stress revealed that elevated expression levels of SOD and BADH-1 correlate with enhanced drought tolerance. This body of research substantiates the role of these genes in improving the drought resistance of wheat presenting valuable genetic targets for the development of drought-resistant wheat varieties (13).

The current study aims to select the varieties most tolerant to water stress based on the gene expression of some genes responsible for plant tolerance to stress, which reflects positively on its physiological and phenotypic characteristics of the vegetative and root system.

Materials and Methods

The pots experiment was carried out in the greenhouse of the Department of Biology, College of Education for Pure Sciences - (Ibn Al-Haitham) - University of Baghdad, during the season 2023–2024. Ten varieties of wheat were cultivated as listed in Table 1.

Table 1. Wheat varieties under study

N	Variety	Sample ID	Parents
1	Abba 99	A1	Ures/ bow “S”/ 3/ Jup/ Biy “S” Yrse
2	Adna 99	A2	PFAU/ SERI-M-82/ BoBWHITE
3	Baraka	A3	IARI X STD
4	Bohooth 10	A4	Abba 99 X Abba 99
5	Bohooth 22	A5	CMSS96Y0 326M-050M-040M-020M-050SY-IM-0Y
6	Bora	A6	H31/ Traff 21/ Enesco
7	Jehan	A7	BJY ‘S’/ COC
8	Dijla	A8	8409644 HS2-6H
9	Sham 6	A9	W-3918-A/ JUPATECO-73
10	Wafia	A10	French / Certified by the Ministry of Agriculture

Completely Randomized Design (CRD) was adopted as an experiment consisting of two factors and three repeaters: first factor 10 varieties of wheat bread, second factor application of adding three levels of water stress, 0 MPa (S0), -1.48 MPa (S1) and -2.95 MPa (S2) of polyethylene glycol PEG6000 in the seedling and branching phases (20–35 days).

The stock solution was prepared at a temperature of 25 °C, with concentrations of100 g/L for a tension -1.48 MPa and 150 g/L for a tension -2.95 MPa (14), based on the following equation:

OP = (-1.18*10-2) * C – (1.18*10-4) * C2 + (2.267*10-4) CT + (8.39*10-7) C2T (Eqn. 1)

The experiment lasted for 35 days until the elongation stage, after which the samples were collected for the purpose of measuring the vegetative indicators.

Shoot parameter

Some of the shoot parameters were measured such as average plant length (cm) (15). Leaf area (cm^2) was measured by the following equation (16):

Leaf area (cm^2) = leaf length (cm) × sheet width (cm) × 0.95 (Eqn. 2)

Stem diameter (mm) and the dry and fresh weight of the shoot (g. $plant^{-1}$) were measured (17, 18).

Physiological parameter

Total chlorophyll

The total chlorophyll index was estimated directly on the plant leaves and the average of four leaves per pot was calculated using the Spadmeter 502 SPAD (Minolta, Tokyo, Japan) at days 15 and 30.

Detection of genes associated with microelement deficiency

The primer used in the study are shown in Table 2.

Gene expression

Gene expression analysis was conducted using the RT-qPCR for two genes, SOD and BADH-1, with the reference gene Ta Actin in wheat plants. The plants were grown in pots to simulate drought stress using PEG 6000 during the seedling and tillering stages (20–35 days). The leaves were isolated from each replicate, labelled and directly transferred to the scientific service company. RNA was extracted and converted to cDNA and the relative gene expression was quantified using the Sacycler-96 RT-qPCR system according to the following steps:

Total RNA extraction method

Total RNA was extracted using the FavorPrep™ Plant Total RNA Mini Kit (FAVORGEN, Korea). The fold change was calculated as follows (19):

$\Delta C_T = C_T$ of target gene - C_T of reference gene (Eqn. 3)

$\Delta\Delta C_T = \Delta C_T$ of each sample - average control ΔC_T (Eqn. 4)

Fold change = $2^{(-\Delta\Delta C_T)}$ (Eqn. 5)

Statistical Analysis

The statistical analysis system (SAS) was used in data analysis to study the effect of drought stress and lack of elements and their overlap in the studied traits of the selected varieties (20). The results were analyzed statistically and the significant difference between the averages were tested according to the least significant difference test (LSD) at the probability level (0.05) (21).

Results

Vegetative growth characteristics

Plant height (cm)

The results presented in Table 3 indicate that increasing water stress from S0 to S1 led to a significant decrease in plant height by 6.22%. The Adna 99 variety was significantly taller than the other varieties reaching a height of 36 cm, while the Wafia variety gave the lowest value of 23.06 cm. As for the bilateral overlap between varieties and water stress levels, the results showed significant differences in plant height and the Adna 99 variety showed the highest height of 35 cm when applying the S2 stress treatment compared to the other varieties. Conversely, plant height in the Wafia variety decreased to 20 cm under the same stress treatment.

Leaf area (cm^2)

The results presented in Table 4 demonstrate that there was no significant effect of the three water stress levels, S0, S1 and S2 on the leaf area. However, the varieties differed significantly, with the Adna 99 variety recording the highest average leaf area of 11.90 cm^2, which significantly exceeded the rest of the varieties. On the other hand, the Wafia variety gave the lowest value of 4.63 cm^2. The results showed a significantly strong interaction between water stress levels and varieties in terms of leaf area. The Adna 99 variety showed a higher area of 15.50 cm^2 at stress level S2 compared to the rest of the varieties, while the leaf area in Wafia variety decreased to 3.80 cm^2 for the same stress level.

Table 2. Primer sequence used in the study and diagnosis of genes under study in RT-qPCR

Primer	Sequence	Primer sequences 5'- - 3'	PCR product	Clarification
SOD	F	ACCGGGTATACCGAGGTGA	104 bp	
	R	GTAGAGTTGCAGCCGTTGGT		
BADH-1	F	ATCCCACAACGCCAACTCTT	185 bp	Primer design
	R	CAGTCGCGACCCCGATTC		
Actin (reference gene)	F	TGAAGAGTCGGTGAAGGGGACT	139 bp	Primer design
	R	GCTGAACCGAGACTGATTTTCCT		

Table 3. Effect of drought stress on plant height (cm) in wheat varieties under study

Variety	Stress levels (MPa)			Mean of V.
	S0	S1	S2	
Abba 99	25.50	23	24	24.16
Adna 99	37	36	35	36
Baraka	31	28.5	31	30.16
Bohooth 10	32	26	33	30.33
Bohooth 22	32.5	29.5	34.5	32.16
Bora	32.5	29.5	27.4	29.8
Jehan	30.5	28.5	28.5	29.16
Dijla	27.2	23.3	22.5	24.33
Sham 6	32.5	28.5	31	30.66
Wafia	25.2	24	20	23.06
Mean of C.	30.59	27.68	28.69	----
L.S.D..	**V. 4.08*,**	**Conc. 1.80*,**		**V×C. 1.5*.**

.(p≤0.05) *

Table 4. Effect of drought stress on leaf area (cm^2) in wheat varieties under study

Variety	Stress levels (MPa)			Mean of V.
	S0	S1	S2	
Abba 99	12.82	7.88	9.08	9.92
Adna 99	9.84	10.36	15.50	11.90
Baraka	9.12	7.12	8.50	8.24
Bohooth 10	8.59	8.73	10.34	9.22
Bohooth 22	11.35	7.12	10.50	9.65
Bora	11.11	10.02	9.02	10.05
Jehan	9.12	8.83	7.41	8.45
Dijla	6.12	5.12	4.94	5.39
Sham 6	14.44	7.22	10.83	10.83
Wafia	6.70	3.40	3.80	4.63
Mean of C.	9.21	7.58	8.99	----
L.S.D..	**V.3.33*,**	**Conc. 1.82* ,**		**V×C. 5.77*.**

.(p≤0.05) *

Stem diameter (mm)

The results of Table 5 showed a significant effect on the stem diameter due to the increase in the water stress levels. As the stress level increased from S0 to S2, stem diameter decreased by 21.71%. The varieties also differed morally among themselves, as the Adna 99 variety exceeded morally over the rest of the studied varieties. The Adna 99 gave the highest average in stem diameter of 1.68 mm, while the Dijla variety gave the lowest value of 0.70 mm.

As for the bilateral overlap between the varieties and stress coefficients, the results indicated that there are significant differences in the diameter of the stem, as the Adna 99 variety showed with the highest stem diameter of 1.80 mm at S2, while the Dijla and Wafia varieties had the lowest diameter of 0.60 mm.

Wet weight of leaves (g)

The results in Table 6 indicated a significant decrease in wet weight due to increasing water stress. Wet weight decreased by 30.34% as a result of increasing the stress level from S0 to S1. The results also showed a significant impact of variety on wet weight, with Adana 99 variety exhibiting significantly higher wet weight compared to the varieties, reaching 0.74 g, while the Sham 6 variety had the lowest wet weight of 0.26 g.

As for the bilateral overlap between the varieties and the stress coefficients, the results showed significant differences in the wet weight. The Adna 99 variety showed the highest wet weight of 0.80 g at the level of S2 compared to the rest of the varieties, while the wet weight in the Dijla and Wafia varieties decreased to 0.23 g for both.

Dry weight of leaves (g)

The results of Table 7 showed significant differences in dry weight due to the effect of water stress. Exposure to the high stress level S2 led to a significant decrease in dry weight compared to stress at the S1 level and the value of the decrease was 29.49%. The results indicated that the

Table 5. Effect of drought stress on stem diameter (mm) in wheat varieties under study

Variety	Stress levels (MPa)			Mean of V.
	S0	S1	S2	
Abba 99	1.29	0.96	1.00	1.08
Adna 99	1.65	1.60	1.80	1.68
Baraka	1.10	1.28	1.10	1.16
Bohooth 10	1.50	1.43	1.39	1.44
Bohooth 22	1.20	1.40	1.13	1.24
Bora	1.38	1.23	0.75	1.12
Jehan	1.68	1.45	0.68	1.27
Dijla	0.80	0.70	0.60	0.70
Sham 6	1.48	1.15	1.14	1.25
Wafia	0.90	0.83	0.60	0.77
Mean of C.	1.29	1.20	1.01	----
L.S.D..	**V. 0.38*,**	**Conc. 0.21* ,**		**V×C. 0.67*.**

.(p≤0.05) *

Table 6. Effect of drought stress on wet weight (g) in wheat varieties under study

Variety	Stress levels (MPa)			Mean of V.
	S0	S1	S2	
Abba 99	0.60	0.35	0.34	0.43
Adna 99	0.75	0.69	0.80	0.74
Baraka	0.45	0.33	0.42	0.40
Bohooth 10	0.64	0.66	0.67	0.65
Bohooth 22	0.60	0.50	0.45	0.51
Bora	0.73	0.55	0.69	0.65
Jehan	0.69	0.46	0.36	0.50
Dijla	0.45	0.26	0.23	0.31
Sham 6	0.61	0.44	0.57	0.26
Wafia	0.45	0.26	0.23	0.31
Mean of C.	0.59	**0.45**	**0.47**	----
L.S.D..	**V. 0.18*,**	**Conc. 0.10*,**		**V×C. 0.32*.**

.(p≤0.05) *

Table 7. Effect of drought stress on dry weight (g) in wheat varieties under study

Variety	Stress levels (MPa)			Mean of V.
	S0	S1	S2	
Abba 99	0.19	0.07	0.07	0.11
Adna 99	0.50	0.30	0.55	0.45
Baraka	0.08	0.06	0.07	0.07
Bohooth 10	0.40	0.30	0.30	0.33
Bohooth 22	0.20	0.13	0.10	0.14
Bora	0.45	0.22	0.30	0.32
Jehan	0.13	0.09	0.06	0.09
Dijla	0.08	0.04	0.04	0.05
Sham 6	0.27	0.09	0.12	0.16
Wafia	0.05	0.04	0.03	0.04
Mean of C.	0.78	0.56	0.55	----
L.S.D..	**V. 0.12*,**	**Conc. 0.06*,**		**V×C. 0.21*.**

.(p≤0.05) *

varieties differed significantly among themselves, as the Adna 99 variety exceeded significantly compared to the varieties under study, reaching 0.45 g, while the Wafia variety had the lowest dry weight at 0.04 g.

The results showed significant differences in dry weight resulting from the bilateral interference between the varieties and water stress, as the overlap between the Adna 99 variety and the stress at (S2) gave the highest dry weight of 0.55 g compared to the rest of the varieties, while the Wafia variety showed the lowest dry weight of 0.03 g.

Total chlorophyll index

The results of Table 8 indicate that there were no significant differences in the total chlorophyll index in leaves at 15 days of age due to the effect of water stress. Additionally, the varieties did not differ significantly among themselves. The Adna 99 variety gave the highest average of 42.73, while the Dijla variety gave the lowest value of 31.48. Regarding the interaction between varieties and water stress at 15 days, no significant differences were observed in chlorophyll content and the Adna 99 variety showed with the highest average of 45.85 at concentration (S2) compared to the rest of the varieties, while the Wafia variety gave a minimum value of 30.12.

For the chlorophyll index at 30 days of age, the results of Table 8 indicated significant differences due to the effect of water stress. Exposure to high S2 stress levels caused chlorophyll index to decrease by 17.06% compared to S1 stress level. The varieties also differed significantly among themselves. The Adna 99 variety exceeded significantly over the rest of the varieties and gave the highest average of 44.09, while the Wafia variety gave the lowest value of 29.08. As for the bilateral overlap between the varieties and the stress coefficients, the results showed significant differences in the chlorophyll content at the age of 30 days, as the Adna 99 variety showed with the highest content of 48.14 at concentration (S2) compared to the rest of the varieties, while the chlorophyll content in the Wafia variety decreased to 22.

Gene expression

The results in Fig. 1 showed significant differences in the gene expression of both genes among the varieties under study. The variety Adna 99 exhibited the highest gene expression levels of 8.574 and 8 for the SOD and BADH-1 genes, respectively, at the S2 treatment, ~8-fold higher compared to that in the S0 treatment. In contrast, the variety Baraka showed the lowest gene expression value of 0.001 in the SOD gene at the S2 treatment compared to the other varieties, while the variety Wafia showed the lowest gene expression value of 0.002 in the BADH-1 gene at the S2 treatment.

The heat map of gene expression for the SOD and BADH-1 genes demonstrated the superiority of the Adna 99 variety (Fig. 2, indicated by dark green, with higher expression levels of 8.5 and 8 under treatment S2). In contrast, other varieties showed expression lower levels as depicted by colours yellow and red. This enhanced gene expression correlates with Adna 99's superior phenotypic and physiological traits and its tolerance to water stress, attributed to its favourable genetic characteristics that promote vital activities for stress resistance and plant growth. Conversely, the Baraka and Wafia varieties exhibited significantly lower gene expression levels (0.001 and 0.002, respectively, under S2), as indicated by red on the heat map. The lack of gene expression superiority in

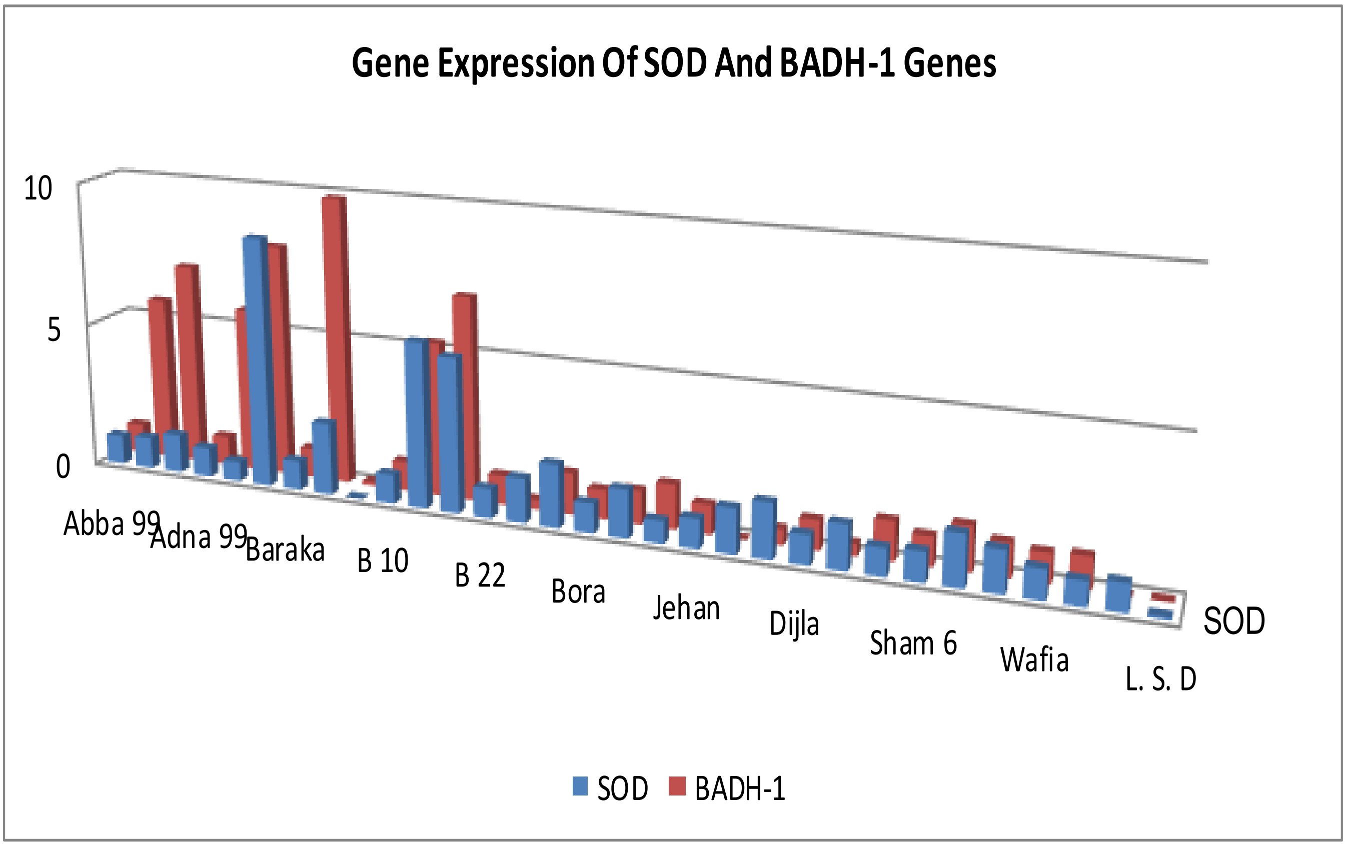

Fig. 1. Effect of drought on gene expression of SOD and BADH-1 genes.

Baraka and Wafia varieties accounts for their diminished phenotypic and physiological traits and their intolerance to water stress, resulting from less favourable genetic traits that negatively impact essential biological functions and lead to plant degradation.

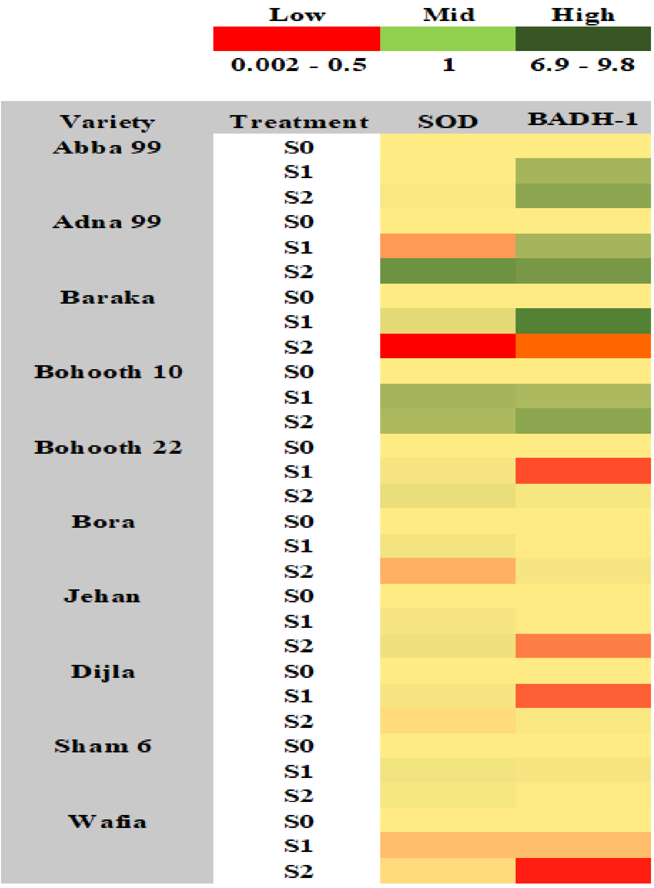

Fig. 2. The heat map of gene expression in wheat varieties.

Discussion

The growth of wheat varieties under drought stress revealed significant differences in their resistance, as seen in phenotypic traits like plant height, leaf area, stem diameter and wet and dry weight (Table 3–7). Resistant varieties, such as Adna 99, maintained the activity of antioxidant and osmotic adaptation genes, with higher expression of the SOD gene and the BADH-1 gene. This expression helps eliminate ROS induced by stress, protecting plant cells and sustaining growth. Significant differences in water stress tolerance among varieties have been reported in previous studies (22, 23). Drought stress negatively affects plant height, especially when using PEG6000, which creates a high osmotic environment that limits water availability, inhibits cell division and restricts growth (24, 25).

This highlights the need to select genetic models with high drought tolerance and desirable phenotypic traits for improved productivity under limited water conditions. The study found that drought stress significantly affected the leaf area of wheat varieties, with some excelling at the S2 stress level. Water stress led to reduced enzymatic activity, cell elongation and nutrient absorption, resulting in decreased foliar area due to diminished cell division and expansion (26).

The osmotic compounds like proline stabilize cellular proteins essential for photosynthesis and respiration, maintaining water balance, while glycine betaine protects membranes and enhances cell stability, preserving leaf area during drought (27–29). PEG6000 has a role in stimulating physiological, hormonal and enzymatic changes, leading to stomatal closure and accumulation of abscisic acid, proline and antioxidants (30). The increased stem diameter in resistant varieties is attributed to their high antioxidant content, such as SOD and glutathione peroxidase, which helps neutralize free radicals, protecting proteins, fats and nucleic acids, thereby maintaining cell membrane integrity and facilitating vital material transfer compared to non-resistant varieties, thus supporting overall plant vitality.

The vital mechanisms, such as enhanced antioxidant activity, osmotic regulation and efficient water retention, help plants resist the stress caused by lack of water, contributing to the maintenance of stem diameter. The BADH-1 gene plays a vital role in the plant's response to water stress caused by drought by regulating osmotic pressure and protecting the soft weight of plants (31). This gene encodes the enzyme, BADH-1, which senses stress via abscisic acid (ABA) receptors that inhibit the phosphatase protein (PP2Cs) and activate the kinase protein, SnRK2, which contributes to the synthesis of glycine betaine, an osmoprotectant compound. Glycine betaine is known to stabilize the tertiary structure of proteins and enzymes, maintaining their vital activity and preventing their degradation under drought conditions. The activity of the gene also contributes to protecting cell membranes and stimulating hormonal signals such as ABA, which regulate the process of opening and closing stomata, which reduces water loss and improves plant tolerance to drought (32, 33).

The results showed that the variety Adna 99 showed high tolerance to drought, attributed to the high concentration of osmotic compounds such as proline and glycine betaine in its fresh leaves. This led to enhancing its ability to retain water and maintain its physiological activity such as increasing photosynthesis and providing the necessary elements under drought conditions, thus increasing the manufacture and accumulation of carbohydrates in the plant, leading to dry weight gain. Furthermore, the role of the BADH-1 gene in enhancing the tolerance of plants to stress by regulating the accumulation of proline and increasing gene expression is higher in extreme conditions (34).

The results of Table 8 showed that there were no significant differences in the overlap between the soft wheat varieties under study and the drought stress coefficients, as the varieties did not differ significantly among themselves. The growth of wheat varieties in the middle of drought stress did not significantly affect the chlorophyll content at the age of 15 days. On the contrary, the growth of wheat varieties exposed to drought stress significantly affected the chlorophyll content at the age of 30 days, as some varieties showed significant superiority over others. The absence of significant differences in chlorophyll content between varieties at 15 days may be due to limited exposure to water stress. However, as plants are subjected to prolonged drought conditions, differences emerge by 30 days, likely due to variations in the expression of stress-resistance genes like SOD.

Increased ABA levels regulate stomatal closure to reduce water loss, help maintain water balance and chlorophyll content. This physiological adjustment enhances photosynthesis efficiency under water stress (35). The auxin, indole-acetic acid (IAA), influences the expression of proteins related to photosynthesis, improving efficiency, enhancing stress tolerance and increasing chlorophyll content. This aligns with Qadir et al. (36). The drought-resistant varieties accumulated higher levels of ABA, maintaining greater chlorophyll and photosynthesis efficiency under drought. ABA plays a key role in regulating ion concentrations in guard cells, causing stomatal closure by releasing potassium (K^+) and anions (Cl^-, NO_3^-), reducing water loss and improving leaf water balance. It also boosts the expression of antioxidant enzymes like SOD (37).

Exposing ten soft wheat varieties to water stress revealed significant differences in the gene expression of SOD and BADH-1. The variety, Adna 99, showed the highest gene expression, while Wafia had the lowest (Fig. 1). The high expression in superior varieties is due to their increased levels of compounds that regulate gene expression under stress. This boosts enzymatic antioxidant activity, helping the plant counteract the harmful effects of free radicals (-OH, H_2O_2, O_2^-, $^1O_2$), which are triggered by water stress and negatively impact growth and vital functions (38). Free radicals are concentrated within chloroplasts, mitochondria and cellular bodies, causing reactions between them that stimulate the plant to activate its enzymatic defences that act as anti-radicals (39), also known as free radical-sweeping enzymes, such as SOD, and increasing its gene expression (40).

Transcription factors such as Dehydration-responsive element-binding protein (DREB), Myeloblastosis proto-oncogene (MYB) and NAM, ATAF and CUC (NAC) transcription factor play an important role in promoting gene expression under stress conditions. The SOD gene exhibits relatively high gene expression in resistant varieties, which contributes to improving vegetative phenotypic traits of the plant such as increased longitudinal growth and leaf development. In contrast, low gene expression in sensitive varieties leads to the accumulation of ROS species, which negatively affects plant growth and causes deterioration in vegetative phenotypic traits (41, 42).

Previous research has suggested that the high level of gene expression of enzymes such as SOD helps reduce oxidative damage that can seriously disrupt plant metabolic processes by causing oxidative damage to protein lipids, dyes and nucleic acids, by converting harmful free radicals into less harmful substances, protecting plant cells and improving their ability to withstand water stress conditions (43).

SOD plays a crucial role in plants' response to environmental stress by converting superoxide anions into water and oxygen, reducing oxidative stress and protecting plant cells from damage caused by the accumulation of ROS and contributes to regulating the balance of nitric oxide (NO) and improving the stability of cell membranes, enhancing the plant's ability to adapt to various environmental stresses (44, 45).

Water stress indicated an increase in the activity of enzymatic antioxidants such as BADH-1 in resistant varieties, which is one of the defence methods used by the plant to counter free oxygen radicals, to reduce their damage to various plant vital activities and events, as a result of their accumulation inside the cell (46, 47).

The high gene expression in superior varieties, like Adna 99, is due to their elevated levels of compounds and hormonal signals that regulate gene expression, such as ABA and ethylene and enhance gene expression under water stress. The factors like osmotic stress, salinity and dehydration influence BADH-1 gene expression (48). Using qRT-PCR, studies have shown that BADH-1 expression is higher in drought-resistant wheat varieties, boosting the plant's ability to synthesize osmolytes like glycine betaine, which helps stabilize proteins and cell membranes under stress, improving vegetative traits (49, 50). In conclusion, BADH-1 expression is crucial for enhancing drought resistance in wheat, making it a key target for improving crop resilience through genetic manipulation and breeding strategies (50).

Conclusion

The results emphasize the critical importance of selecting drought-resistant wheat varieties to ensure sustainable production in arid regions, especially as climate change and diminishing water resources continue to pose challenges. Among the ten wheat varieties tested under different levels of water stress using polyethylene glycol (PEG 6000), Adna 99 exhibited remarkable resistance, outperforming in key phenotypic and physiological traits such as plant height, leaf area, stem diameter, fresh weight, dry weight and chlorophyll content. Additionally, Adna 99 showed a significant increase in the expression of drought-responsive genes (SOD and BADH-1), with an eightfold increase under severe water stress (-2.95 MPa). In contrast, the varieties Wafia and Baraka exhibited lower gene expression and weaker performance in phenotypic traits under similar conditions. These findings indicate that Adna 99 is a promising candidate for drought tolerance, making it a suitable variety for cultivation in water-scarce environments. The integration of phenotypic, physiological and genetic data in this study provides a solid scientific basis for the selection of drought-resistant wheat varieties and supports future breeding efforts to improve wheat's resilience to environmental stresses.

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Authors' Contributions

ATS conducted the experiments, selected the ten soft wheat varieties, applied drought stress using polyethylene glycol (PEG), evaluated the phenotypic and physiological traits and performed the gene expression experiment using RT-qPCR. AKA selected the genes responsible for drought stress resistance (SOD, BADH), designed the experiment, determined PEG6000 as the drought-inducing substance and identified the key traits to be studied, such as plant height, leaf area, stem diameter, fresh and dry weight and chlorophyl content.

Compliance with Ethical Standards

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

Ethical issues: None

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