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Morphological, Biochemical and Proline-Related Genes Analyses in Resistant and Susceptible Wheat Cultivars in Iraq

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Abstract: A high salinity level negatively affects the morpho-physiological parameters of plants. As a result of salinity, these effects are considered crucial signs of plant damage. In this study, ten Iraqi wheat cultivars were examined against two salinity levels (3 and 15 ds m^{-1}) and some morphological, biochemical properties were measured. Also proline-related genes were analyzed using Ilumina RNA sequences and bioinformatics analyses. All cultivars demonstrated a decrease in the studied parameters with an increase saliness. 'Dijlah' cultivar showed best performance salinity stress, while 'Ibaa 99' was sensitive based on morphological and biochemical parameters. The competition was in favor of the sodium ion at the expense of the potassium ion in high salinity conditions. Proline accumulation in wheat blade leaves was about 2.5 times higher at the peak salt level. The transcriptomic analysis was done and the transcripts per million (TPM) values were estimated for some proline genes. The genes of probable proline transporter 2, proline dehydrogenase 2, and GSK-like kinase 1A obtained the higher TPM values in cultivar 'Dijlah' cultivar than in 'Ibaa 99' cultivar. It can be concluded that 'Dijlah' cultivar is a salt tolerant cultivar as compare with the susceptible 'Ibaa 99' cultivar, and their proline accumulation was increased with salinity stress and was related with TPM values. Morphological, biochemical and TPM values would offer a good combined- criteria for recognition the tolerant genotype.

Keywords: Ilumina, Proline, Salinity, Sequencing, TPM, Wheat.

Introduction

Abiotic stressors are the main causes of a plant's physiological, morphological, molecular, and biochemical characteristics being affected, which impairs the plant's capacity to operate normally and, in turn, significantly lowers crop output. A wide cultivar of responses can be triggered or generated by plants, notably altered metabolism, diminished growth, and development limitations (Shabbir *et al*., 2022). Salt stress, which is climate change-related, is one of the most important environmental factors that impacts agricultural output globally (Abboodi *et al*., 2021). Around 830 million hectares of land (6–7% of all arable land) are thought to be affected by soil salinity, The global need for wheat is constantly increasing, and it is expected that the demand of wheat production will increase dramatically, because the world population is expected to reach 9.7 billion people by the next two decades (Barnes, 2022). Soil salinization occurs through natural or human-induced processes resulting in the accumulation of soluble salts in soil layers. Soil salinity is described and characterized in terms of the concentration and composition of the soluble salts, the accumulation of salt has a significant negative impact on seed germination, reduces water retention, and creates an ion imbalance that can be poisonous and cause osmotic imbalance (Khan & Panda, 2008). Hussain *et al*. (2023) mentioned that a biotic stress has a major negative impact on seed quality in wheat.

Abiotic stresses are the main factors influencing wheat (*Triticum aestivum* L.) productivity globally. Wheat is a substantial crop and a staple meal in many countries throughout the world. However, wheat production is low in many countries. Nearly 55% of the world's total consumption of carbs and 20% of all food calories come from wheat, which is the most produced grain in the world. In Iraq, salt represents a serious problem to the production of wheat that considered as moderately salt tolerant crop (Munns *et al*., 2006). There is tremendous genetic diversity to help this crop to develop salt tolerance (Shavrukov *et al*., 2011). However, wheat tolerance can change depending on the stage of growth (El-Hendawy *et al*., 2005). Knowledge of diversity patterns allows plant breeders to better understand the evolutionary relationships among accessions, to sample germplasm to develop strategies to incorporate useful diversity in their breeding programs (Puhakka, *et.al*., 2011).

Understand some physiological and biochemical responses of some wheat cultivars grown under two levels of salt, which in turn affects growth and yield, was the goal of this study. The parameters exhibiting distinct patterns of responses to salinity between salt-tolerant and salt-sensitive cultivars may be the potential objectives for the improvement of salt-tolerant wheat by conventional and transgenic methods.

Materials & Methods

Field experiment

The present study was carried out at Institution of Genetic Engineering and Bio- technique for postgraduate Studies university of Baghdad during 2021- 2022 season. Ten cultivars of wheat were obtained from the seed testing and certification office, Ministry of Agriculture, Iraq. The current study was carried out in the plastic pots filled with 7 kg of sandy loam sieve soil. Physical and chemical properties of the soil samples were measured (Table 1). Seeds from each cultivar were placed in plastic seedling plate suggested for nurseries then transferred to the field pots. Fertilizers used were urea (46% N) at 180 kg N. ha^{-1} (Al-Malaky & Abdulkareem 2019). Super phosphate (46% P_2O_5) applied at 90 P kg ha^{-1} (Alsulaiman & Al-Ansari, 2023). The crop was managed according to the recommended conventional agronomical practices. The field equipped with rain fall shelter to avoid rain. The configuration of the experiment was Completely Randomized Design (CRD) with factorial arrangement, two treatments of NaCl, i.e. 3 and 15 dSm^{-1}were added to the water irrigation.

The data were recorded at elongation stage for six empirical plants were chosen randomly from each replication on morphological parameters such as root weight, plant height, plant biomass, tillers number, and biochemical parameters such as K/Na ratio, chlorophyll content, relative water content, proline content, amino acid determination and total protein. Analysis of variance was used to statistically analyze data, and least significant Difference

(LSD) test at $P_{\leq 0.05}$ was used to differentiate between treatments average, some traits have analyzed according to T-test.

Sequencing Experiment

RNA extraction

Two leaf samples of tolerant Dijlah, and susceptible Ibaa99 wheat cultivars were collected for RNA extractions. To extract RNA, the leaf sample was put in an Eppendorf tube and immersed in 5x volume RNA Later solution, and sent to DNA-link Company, Republic of Korea. The total RNA was extracted following the company instruction using the RNeasy® Plant Mini Kit (QIAGEN, Hilden, Germany).

Transcriptomic analysis

The Next Generation Sequencing library preparation was attempted in the company, using TruSeq total RNA library prep kit for RNA sequencing. The extracted RNA sample was checked with a 2100 Expert Bioanalyzer (Agilent), and then sequenced using NovaSeq6000, 2×101PE to obtain the total RNAseq.

Map to reference

The forward and reversed reads of RNA Sequence data were paired and then used in map to reference runs with Geneious RNA (Sensitivity: Medium-Low Sensitivity) to the reference gene sequences using Geneious prime version 11 (Kearse *et al*., 2012). Nine genes sequences of proline were examined against the whole transcript reads, and the higher assembled sequences were chosen, and the consensus sequence was extracted, while the other lower mapped sequences were discarded (Table 1). The analysis of the RNA reads was computed based on the transcripts per million (TPM) value of the reference sequence as the read count per kilobase of sequence (RPK) divided by a million (Bester *et al*., 2021).

Table (1): The accession numbers of proline gene sequences and the examined length of each.

Gene ID	Sequence
LOC100136980	871
LOC123038303	4101
LOC123045169	3763
LOC123048297	1834
LOC123051674	2591
LOC123091745	17610
LOC123119063	8886
LOC123112544	8140
LOC123088555	12307
KM523670	2407
DQ678922 (GSK1A)	3379

Results

The mean squares of source of variance. Obviously, genotypes, salt stress and some of their interaction own highly significant effects on the studied traits. Except of root characters, no interaction has happened between genotypes and salt treatments, and this is due to the low percentage of the variance of this source out of the total variance (Table 2).

Morphological parameters

Root weight and length

High NaCl concentrations had a detrimental impact on root dry weight (Table 3).

Table (2): Mean squares of source of variation for the studied characters.

Cultivars	Salinity levels		mean	Depression%
	(control) 3 ds m^{-1}	15 ds m^{-1}		
Ibaa 99	15.60 e	8.63 a	12.12 AB	44
Furat	28.40 ij	17.43 fg	22.92 F	38
Baghdad	21.87 h	15.43 e	18.65 E	29
Bohooth 22	14.57 e	9.50 ab	12.03 A	34
Abo Ghareeb	19.97 gh	14.33 d	17.15 DE	28
Uruk	15.27 ef	11.57 bcd	13.42 A	24
Adana	16.60 e	11.50 bc	14.05 BC	30
Saber Bek	29.17 j	19.53 gh	24.35 F	33
Baraka	17.67 f	14.17 cde	15.92 CD	19
Dijlah	25.90 i	20.53 h	23.22 F	20
mean	20.50 A	14.26 B		

Uppercase letters indicate significant differences for testing the individual effect of salinity and individual effect of wheat cultivars Lowercase letters indicate significant differences for testing the effect of wheat cultivars and salinity interaction following LSD test at $P_{\leq 0.05}$.

Table (3): Effect of varieties, NaCl and there interaction on root dry weight (g).

S.O.V	d.f.	Root dry Weight	Root length	shoot weight	shoot length	Tillers plant^{-1}	K/Na ratio	RWC ratio	Total Chlorophyll
Cultivars	9	133.46**	1167.21**	206.47**	661.47**	59.37 **	142.46	126.86**	23.08**
Salinity	1	583.44**	523.92**	1139.70**	280.80**	0.15 ns	26534.81**	4165.00**	2643.8**
Cultivars x Salinity	9	8.69**	36.72**	10.41ns	2.15ns	0.40 ns	112.44 ns	6.85ns	12.37**
Error	40	2.84	7.55	0.94	4.82	0.91	57.57	0.45	0.57**

The highest root dry weight was recorded under control conditions by 25.90 g for Dijlah cultivar while the lowest dry weight was noted at the high salinity level of 15 dSm^{-1} by 8.63 g for Ibaa 99 cultivar in terms of cultivars, 'Saber Bek' and 'Dijlah' had the highest root dry weight, whilst 'Bohooth 22', 'Ibaa 99', and 'Furat' had the lowest value (Table 3).

According to research by Kalhoro *et al.* (2016), the salinity had a considerable negative impact on the dry weight of the roots. The cultivars most affected by salinity were Ibaa99 and Furat, with root weight depressions of 44 and 38%, respectively, in response to an increase in salt from 3 to $15dSm^{-1}$, whereas Baraka and Dijlah showed least depression by 19 and 20%. Similar to root weight, root length was also impacted in similar way, however most cultivars showed a minor reduction as a result of the rise in salinity from the low to the high level except Baraka (Table 4).

Shoot weight & length

Wheat genotypes were strongly influenced by salinity level in terms of plant weight and length .Which were higher at the control treatment by 48.8 g and 77.8 cm, respectively. In both characters, the cultivars "Dijlah" and "Saber Bek" performed better than "Uruk," which performed poorly in both characters. Also, "Dijlah" and displayed the lowest percentage of depression by 10% and 12%.respectively, while "Uruk" and "Abo Ghareeb" both displayed the greatest rate of depression by 30 and 24% respectively. (Tables 5 & 6).

Table (4): Effect of varieties, NaCl and there interaction on root length

Cultivars	Salinity levels		mean	Depression %
	(control) 3 ds m^{-1}	15 ds m^{-1}		
Ibaa 99	42.43 bc	38.10 a	40.27 B	10
Furat	47.23 de	38.40 ab	42.82 BC	18
Baghdad	80.17 i	76.37 i	78.27 H	04
Bohooth 22	37.67 a	34.20 a	35.93 A	09
Abo Ghareeb	61.03 g	52.17 f	56.60 E	14
Uruk	36.33 a	35.23 a	35.78 A	03
Adana	45.43 c	43.97 cd	44.70 C	03
Saber Bek	48.93 ef	45.60 c	47.27 D	06
Baraka	69.67 h	51.90 f	60.78 F	25
Dijlah	67.73 h	61.60 g	64.67 G	09
mean	53.66 A	47.75 B		

Uppercase letters indicate significant differences for testing the individual effect of salinity and individual effect of wheat cultivars Lowercase letters indicate significant differences for testing the effect of wheat cultivars and salinity interaction following LSD test at $P_{\leq 0.05}$.

Table (5): Effect of varieties, NaCl and there interaction on shoot weight.

Cultivars	Salinity levels		mean	Depression%
	(control) 3 dsm^{-1}	15 ds m^{-1}		
Ibaa 99	47.27 a	39.03 a	43.15 BCD	17
Furat	50.43 a	43.10 a	46.77 DE	14
Baghdad	43.20 a	35.40 a	39.30 AB	18
Bohooth 22	45.53 a	36.67 a	41.10 ABC	19
Abo Ghareeb	54.37 a	41.10 a	47.73 EF	24
Uruk	44.70 a	31.07 a	37.88 A	30
Adana	48.90 a	40.83 a	44.87 CDE	16
Saber Bek	53.93 a	47.43 a	50.68 F	12
Baraka	41.63 a	34.07 a	37.85 A	18
Dijlah	58.73 a	52.83 a	55.78 G	10
mean	48.87 A	40.15 B		

Uppercase letters indicate significant differences for testing the individual effect of salinity and individual effect of wheat cultivars Lowercase letters indicate significant differences for testing the effect of wheat cultivars and salinity interaction following LSD test at $P_{\leq 0.05}$.

Table (6) Effect of varieties, NaCl and there interaction on shoot length.

Cultivars	Salinity levels		mean	Depression%
	(control) 3ds. m^{-1}	15 ds m^{-1}		
Ibaa 99	72.93a	69.00a	70.97B	05
Furat	76.67 a	73.00a	74.83C	05
Baghdad	86.67 a	82.00 a	84.33D	05
Bohooth 22	77.33 a	73.33 a	75.33C	05
Abo Ghareeb	79.33 a	73.00a	76.17C	08
Uruk	55.00 a	51.33a	53.17A	07
Adana	74.00 a	69.00a	71.50B	07
Saber Bek	95.67 a	89.67a	92.67E	06
Baraka	75.67 a	72.00a	73.83BC	05
Dijlah	85.67 a	83.33 a	84.50D	02
mean	77.89 A	73.57 B		

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Tillers number per plant

High salinity had not considerably effect on tillers number per plant in average (Table 7). In this attribute, "Dijlah" and "Saber Bek" cultivars were once more significantly dominating, while "Uruk", "Baraka" and "Baghdad" were the lowest. It seems that tiller number difficultly affected by salinity stress. (There is no significant interaction between cultivars and Salinity).

Table (7): Effect of varieties, NaCl and there interaction on tillers $plant^{-1}$

Cultivars	Salinity levels		mean
	(control) 3 ds m^{-1}	15 ds m^{-1}	
Ibaa 99	2.0a	3.0 a	2.5 A
Furat	3.7 a	3.7 a	3.7 B
Baghdad	2.0 a	2.0 a	2.0 A
Bohooth 22	5.0 a	5.0 a	5.0 C
Abo Ghareeb	8.0 a	7.3 a	7.7 D
Uruk	2.0 a	2.3 a	2.1 A
Adana	5.3 a	5.7 a	5.5 C
Saber Bek	9.0 a	8.3 a	8.7 D
Baraka	1.7 a	1.7 a	1.7 A
Dijlah	10.3 a	11.0 a	10.7 E
mean	4.9 A	5.0 A	

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Biochemical parameters

Chlorophyll content

All wheat cultivars' total chlorophyll content drastically dropped as NaCl concentration rose. The control condition produced the greatest total chlorophyll by 26.23 μg.ml^{-1} "Saber Bek", while 15 dSm^{-1} produced the lowest average by 6.82 μg.ml^{-1} "Ibaa 99". The two cultivars “Furat” and "Saber Bek" gave the highest total chlorophyll by 24.69 and 26.23 μg.ml^{-1} respectively, whereas "Ibaa 99" and “Abo Ghareeb" had the lowest total chlorophyll by 6.82 and 6.94μg.ml^{-1} respectively (Table 8). According to some studies (Ashraf *et al*. 2005; Hasan *et al*. 2015), a significant decrease in total chlorophyll content and a subsequent decrease in photosynthetic efficiency may be caused by the chloroplastids' cell membrane degeneration under salt stress. Chlorophyll has also been mentioned as a potential critical characteristic for determining whether crop plants are sensitive or tolerant of salt. It is worth noting that the studied cultivars did not differ in the percentage of depression of total chlorophyll between control and salt stress treatment.

Table (8): Effect of varieties, NaCl and there interaction on chlorophyll content (μg. ml^{-1})

Cultivars	Salinity levels		mean	Depression%
	(control) 3 ds m^{-1}	15 ds m^{-1}		
Ibaa 99	16.19 c	6.82 a	11.50 A	58
Furat	24.69 f	8.30 b	16.49 FG	66
Baghdad	21.53 e	8.37 b	14.95 E	61
Bohooth 22	20.05 e	7.24 ab	13.65 CD	64
Abo Ghareeb	21.11 e	6.94 a	14.02 DE	67
Uruk	18.01 d	7.66 ab	12.83 BC	57
Adana	18.30 d	7.31 ab	12.90 BC	60
Saber Bek	26.23 g	8.41 b	17.32 G	68
Baraka	18.14 d	6.85 a	12.50 B	62
Dijlah	24.59 f	8.18 b	16.38 F	67
mean	20.88 A	7.61 B	14.25	46

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K/Na Ratio

Salinity has an impact on the concentration of mineral ions in the leaf blade of wheat genotypes. The competition was in favor of the sodium ion at the expense of the potassium ion in high salinity conditions, as shown by the fact that 3 dSm^{-1}treatment outperformed 15 dSm^{-1} in K/Na ratio by 420%. This competition may be at the transport level or the uptake level. Cuin *et al*. (2008) pointed that maintenance of a high K/Na ratio is a crucial aspect of plant salt tolerance. The salt-tolerant cultivars "Dijlah" and "Saber Bek" were found to have higher ratios of K/Na under control treatment 3dSm^{-1}salinity levels by (63.83) % and (66.11) % respectively. The ' Ibaa 99' cultivar, on the other hand, has lower K/Na under both concentrations, which mean it is sensitive cultivar by (16.57) %.

At 15 dSm^{-1}salinity level 'Furat' and 'Saber Bek' cultivars were the best in this attribute. With a high depression due to high salinity (15 dSm^{-1}), the cultivars exhibited the same trend in reduction of K/Na ratio with considerable differences from each other, (There is no significant interaction between cultivars and Salinity, see table 9).

Table (9): Effect of varieties, NaCl and there interaction on K/Na ratio (%).

Cultivars	Salinity levels		mean	Depression%
	(control) 3 ds m^{-1}	15 ds m^{-1}		
Ibaa 99	47.90 a	13.11 a	30.50 A	72
Furat	58.95 a	12.67 a	35.81 A	78
Baghdad	49.45 a	9.67 a	29.56 A	80
Bohooth 22	50.98 a	11.69 a	31.34 A	77
Abo Ghareeb	52.19 a	10.21 a	31.20 A	80
Uruk	35.73 a	8.03 a	21.88 A	77
Adana	56.14 a	8.77 a	32.45 A	84
Saber Bek	66.11 a	9.06 a	37.58 A	86
Baraka	45.21 a	10.08 a	27.65 A	77
Dijlah	63.83 a	12.60 a	38.21 A	80
mean	52.65 A	10.58 B		

Uppercase letters indicate significant differences for testing the individual effect of salinity and individual effect of wheat cultivars Lowercase letters indicate significant differences for testing the effect of wheat cultivars and salinity interaction following LSD test at $P_{\leq 0.05}$.

Relative Water Content (RWC)

The findings on relative water content (RWC) showed that, wheat cultivars significantly responded to salinity levels. Additionally, the interaction between cultivars and NaCl concentrations was also significant (Table 10.). Under 3 dsm^{-1}, the highest RWC was noted by (66.11) % in cultivar" Bohooth 22", while under 15 dSm^{-1} conditions, the lowest (RWC) was obtained by (41.37) % in "Saber Bek" cultivar. Due to the shoots greatly reduced salt-water content (Bajji *et al.,* 2000), salt stress conditions cause drought stress in the roots because roots are unable to absorb as much water. For both concentrations, "Furat," "Bohooth 22," and "Dijlah" were reported to have the highest relative water content (RWC), while "Baghdad" cultivars had the lowest RWC values. Cultivar ' Saber Bek' had the lowest percentage of depression of RWC, while ' Baghdad' showed the highest percentage, (There is no significant interaction between cultivars and salinity).

Proline

Proline accumulation in wheat flag leaves was measured at both 3 and 15 dsm^{-1}. The accumulation at high salt level, was about 2.5 times higher than lowest level (Table 11). The rapid synthesis of proline during stress in the leaves may be due to Proline-rich extension-like receptor kinases are a class of receptor kinases implicated in multiple cellular processes in plants, several cis-acting regulatory elements, essential for plant growth and development and the response to light, phytohormones, and diverse biotic and abiotic stresses (Kesawat *et al*., 2022). Proline has also been referred to as a physiological adaptation that help plants to survive under salt stress (Al–Saadi & Kubba, 2015). Proline plays a major role in maintaining membrane stability and subsequently reducing the leakage of nutrients and the loss of water in cells grown under drought stress (Saad *et al*., 2021) under 3 and 15 $ds.m^{-1}$, different wheat cultivars showed varying degrees of proline accumulation.

Table (10): Effect of varieties, NaCl and there interaction on RWC ratio (%).

Cultivars	Salinity levels		mean	Depression%
	(control) 3 ds m^{-1}	15 ds m^{-1}		
Ibaa 99	61.20 a	43.03 a	52.12 B	29
Furat	68.17 a	49.03 a	58.60 D	28
Baghdad	50.80 a	34.03 a	42.42 A	33
Bohooth 22	66.07 a	47.37 a	56.72 D	28
Abo Ghareeb	58.57 a	43.47 a	51.02 B	25
Uruk	60.77 a	42.40 a	51.58 B	30
Adana	57.93 a	42.87 a	50.40 BC	26
Saber Bek	54.27 a	41.37 a	47.82 B	23
Baraka	56.30 a	41.83 a	49.07 B	25
Dijlah	63.47 a	45.50 a	54.48 CD	28
mean	59.75 A	43.09 B		

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Table (11): Effect of varieties, NaCl and there interaction on proline content (ppm).

Cultivars	Salinity levels		mean
	(control)	15 ds. m^{-1}	
Ibaa 99	36.8a	83.2b	60
Furat	39.5a	87.5b	64
Baghdad	33.4a	77.7b	56
Bohooth 22	36.9a	83.0b	60
Abo Ghareeb	42.1a	77.8b	60
Uruk	32.1a	84.4b	58
Adana	38.2a	78.1b	58
Saber Bek	42.1a	73.4b	58
Baraka	36.4a	79.0b	58
Dijlah	40.4a	88.3b	64
mean	A37.786	81.261B	59.5

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Furat and Dijlah cultivars had the maximum proline accumulation at 15dSm-1. The lowest level was obtained at 3 dSm^{-1} in Uruk and Baghdad by 32.1 ppm and 33.4 ppm respectively. Both salt stress levels resulted in considerable differences between the cultivars.

Transcriptomic analysis

After trimming, the total RNA reads were 46,257,366 for Dijlah, the tolerant cultivar and 55,372,464 for Ibaa99, the susceptible cultivar. The reads were successfully mapped against the proline gene sequences. The accession numbers of, LOC123051674, LOC123091745, KM523670 and DQ678922 were obtained the highest number of assembled reads 10,582 and 15,499, 10,739 and 6,725 reads for Dijlah, and 4,409, 4,813, 13,061 and 4,072 reads for Ibaa99 respectively.

The transcripts per million (TPM) values of LOC123051674 and LOC123091745, KM523670 and DQ678922 were 371,272, 80,000, 406,727 and 181,363 for Dijlah respectively. The TPM values of LOC123051674 and LOC123091745, KM523670 and DQ678922 were 212,625, 34,125, 680,250 and 151,000 for Ibaa99 respectively (Table 12).

Table (12): The assembled reads and TPM values of proline genes, LOC123051674 and LOC123091745, KM523670 and DQ678922 in Dijlah and Ibaa99 verities.

Gene ID	Sequence length	TR assembled	Dijlah TPM	SR assembled	Ibaa99 TPM	Proline
LOC123051674	2,591	10,582	371,272	4,409	212,625	proline dehydrogenase 2,
LOC123091745	17,610	15,499	80,000	4,813	34,125	probable proline transporter 2
KM523670	2,407	10,739	406,727	13,061	680,250	delta1-pyrroline-5-carboxylate
DQ678922 (GSK1A)	3,379	6,725	181,363	4,072	151,000	GSK-like kinase 1A

Discussion

When plants are exposed to high salinity levels, they experience various detrimental effects on their morpho-physiological parameters. (Sinthumule *et al*., 2021). These effects are considered crucial signs of plant damage caused by salinity. The inhibition of these parameters serves as an indicator of the harmful impact of salt stress on plants. Morpho-physiological traits refer to the physical and functional characteristics of plants. (Saleem *et al*., 2021). These parameters include plant growth, leaf area, root development, and the ability to absorb nutrients. Root/shoot ratio is an important indicator for tolerant genotypes for salinity (Mohi-Ud-Din *et al*., 2021). In spite of Furat, Saber Bek and Baghdad cultivars showed maximum root/shoot ratio (47-49%), but Abo Ghareeb, Uruk and Dijlah showed the least percentage of depression (5-11%) while Ibaa 99 showed the highest percentage of depression (33%) between 3 and 15 EC treatments. Al-Temimi *et al*. (2013) mentioned at higher water deficit stress Ibaa 99 was drought tolerance cultivar.

Salinity disrupts these parameters due to its effect on soils water potential, i.e. less water is available to plant roots, and an imbalanced nutrients leading to stunted growth, reduced leaf area, and impaired root development. Consequently, plants become less efficient in absorbing essential nutrients required for their survival and growth (Rasmuson & Anderson 2002). The averages of all examined studied parameters have decreased ($P \leq 0.05$) with high salinity level, except for the proline percentage. These results are compatible with the results of Zhou *et al* (2018) and Raza *et al* (2023). This reduction was anticipated because wheat's growth and productivity are restricted by a decrease in photosynthetic pigments and changes to the ionic balance (Mahboob *et al*., 2016).

Also, salt buildup adversely impacts on reduces water retention, and causes an ion imbalance that result in ion toxicity and an osmotic imbalance (Khan & Panda, 2008). The Dijlah cultivar is distinguished by the characteristics of length and weight of the root, number of tillers, K/Na ratio, chlorophyll and proline, and RWC, making it a distinct cultivar in tolerating salt stress. Saberbek cultivar also excelled in most of the studied traits, but it did not outperform the Dijlah cultivar. The combination of some of phenotypic and physiological mechanisms can improve the salt stress tolerance of the genotypes and can therefore be adopted as selection criteria. Therefore, relying on a single parameter is considered inefficient in the selection process. For example, it is noted that the Furat cultivar has a high percentage of proline, relative water content, and chlorophyll, but it has a low root weight and length. However, this means that Furat cultivar possesses physiological mechanisms suitable for salt tolerance.

Proline is one of the essential osmolytes involved in osmotic adjustment (Soudry *et al*., 2005; Iqbal *et al*., 2008), and plants have long used it as an adaptation to salt stress (Parida & Das, 2005; Ashraf & Foolad, 2007; Kubba *et al*., 2015).

Salinity stress results in the accumulation of intracellular proline, which not only increases resistance to stress but also acts as an organic nitrogen reserve during stress recovery. Despite the high percentage of proline in the Dijlah cultivar under 3 dsm^{-1}, it was not the highest one, as the Saberbeg and Abu Ghareeb cultivars excelled by giving 42.1 ppm. However, under 15 dSm^{-1}, Dijlah and Furat cultivars were superior, and this led to the superiority of these two cultivars in the overall mean. It is interesting that the Saberbek cultivar gave the lowest rate of proline under salinity conditions, which is an indicator that may not be in favor of this cultivar if we know that proline concentration rises in response to salinity stress (El-Shintinawy & El-Shourbagy, 2001) and consider as a physiological marker of tolerant genotype (Hasan *et al*., 2015). However, some researchers hypothesized that a significant buildup of proline was a sign of a salt-stress damage that resulted in proteolysis and senescence (Lutts *et al*,. 1999) Therefore, in this regard, Saber Beg's low proline.

Percentage may be a sign of salinity tolerance. Anyway, a reasonable measurement to identify the tolerant cultivar would be grain yield, which has not been researched. In order to identify salt-tolerant genotypes, it is not possible to depend exclusively on averages of the high averages of traits at salinity condition unless the performance of these cultivars is compared under normal conditions.

The genotypes that show the least depression in their traits between saline and normal condition can be distinguished as genotypes with a genetic structure suitable for growth in saline conditions. Some genotypes show worth means of their characteristics under saline conditions, but the percentage of decline in their traits as compare to normal condition appear large and this is a negative indicator. In contrast, genotypes that show stability or a slight decline in their traits, when comparing their growth between the normal and saline condition, represent tolerant genotypes and this is due to their possession of appropriate physiological mechanisms. In general, this situation is complex and not understandable, as some genotypes had high traits under normal conditions while other genotypes had low traits under the same conditions, and both of their rates decreased under salt conditions, as in theFurat and Ibaa99 cultivar for root weight trait. This is due to own Furat cultivar favorable genes for root weight that express under normal conditions, while Ibaa99 cultivar has not possess them . However, both of them do not possess the physiological mechanisms suitable for tolerating high salinity. As for the genotypes that showed stability or a slight decline in their traits when comparing their growth between the normal and saline condition, they are often elite genotypes and requested by plant breeders because they possess genes that are favorable for that trait under normal conditions and possess appropriate mechanisms for tolerating salinity, as in Dijlah cultivar, which had low percentage of decline in its traits

The transcriptomic analyses showed that the accession numbers of proline dehydrogenase 2, mitochondrial-like (LOC123051674), probable proline transporter 2

(LOC123091745), delta1-pyrroline-5-carboxylate synthetase (KM523670) and GSK-like kinase 1A (DQ678922) obtained high number of mapped transcripts among all the examined gene sequences. Except of delta1-pyrroline-5-carboxylate synthetase, the genes of probable proline transporter 2, proline dehydrogenase 2, and GSK-like kinase 1A obtained the higher TPM values in the tolerant cultivar Dijlah than the susceptible cultivar Iba99. Aycan *et al*., (2022) showed that all genotypes increased proline content under salt stress, but salt-tolerant genotypes showed a greater increase than moderate and sensitive genotypes. Salt-tolerant and moderate genotypes showed an increase in gene expression levels under salinity stress. However, Tavakoli *et al*. (2016) found that proline was highly degraded in the fourth leaf of the tolerant cultivars and that was probably due to the provision of a source for energy or nitrogen, which helped to enhance the yield of these cultivars under salinity stress

Conclusion

The expression of the investigated genes in this work along with the content of proline in the most tolerant cultivars was extremely higher than that of the sensitive ones. These genes can be salinity stress-induced and could be used as indicators for improvement tolerance characters along with other morph-physiological parameters.

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Contributions of authors

A.A.R.A: Collection of specimens, Laboratory techniques, wrote and revised the manuscript.

A.H.A: Suggestion the proposal of the article, wrote and revised the manuscript.

H.A.S: Wrote and revised the manuscript.

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Conflicts of interest

The authors declare that they have no conflict of interests.

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التحليل المظهري، الكيموحيوي و جينات البرولين في أصناف القمح المقاومة والمتحسسة في العراق

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المستخلص: يؤثر مستوى الملوحة المرتفع تأثيرا سلبيا على النبات مظهريا و فسلجيا. نتيجة للتملح , تعتبرهذه التاثيرات دليل على وجود ضرر جسيم في النبات .في هذه الدراسه , تم اختبار عشرة اصناف من الحنطة العراقية على مستويان ملحيان وتم تقدير بعض المؤشرات المظهريه والكيمائيه الحيوية . كذلك تحليل او تشخيص الجينات المرتبطة بالبرولين باستخدام نظام (تسلسل الحمض النووي الرايبوزي اللومينا) و بالتحليل بواسطة المعلوماتية الحيوية .جميع الاصناف اظهرت انخفاضا في المؤشرات المدروسة عند زيادة الاجهاد الملحي .الصنف دجلة اظهر افضل اداء عند تعرضه للاجهاد الملحي ,بينما الصنف اباء99 كان متحسس بالنسبة للمؤشرات المظهرية والكيميائية الحيوية. التنافس كان لصالح ايون الصوديوم على حساب ايون البوتاسيوم عند ظروف الملوحة الشديدة . تراكم البرولين في نصل الوراق كان اعلى بحوالي 2,5 مرة عند اعلى نقطة بالمستوى الملحي . تم تحليل بيانات الاستنساخ و تم تقدير قيمة التعبير الجيني على اساس عدد النسخ لكل مليون قراءة لبعض جينات البرولين Probable proline transporter 2, proline dehydrogenase 2, and GSK-like kinase 1A. تم الحصول على قيم تعبير جيني اعلى في الصنف دجلة اكثر من تعبيرها الجيني في الصنف اباء99 . تم الاستنتاج ان الصنف دجلة هو صنف متحمل للملح مقارنة بالصنف اباء99 المتحسس . و ان تركيز البرولين ازداد بزيادة الاجهاد الملحي وكان هذا متطابقا مع قيم التعبير الجيني (المؤشرات المظهريه والكيمائيه الحيوية والتعبير الجيني على اساس عدد النسخ) نعتبر معايير مشتركة مهمة لتمييز التركيب الوراثي.

الكلمات المفتاحية : البرولين، التعبير الجيني، القمح، اللومينا، الملوحة.