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Impact of Polyethylene Glycol 6000 and Sodium Chloride Stresses Combined and Single on Morpho-Physiological Traits and Expression of *CAT* Gene in Wheat (*Triticum aestivum* L)

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Abstract: In some arid and semiarid places like Iraq, wheat growing may experience challenges with both drought and soil salinity. The goal of the present study was aimed to evaluate how drought and salt stress alone and combined affects growth and physiological characteristics and fresh weights of wheat plants to identify their response mechanisms. Two wheat genotypes (Latifya and Iba99) were treated with drought stress of-0.6*Mpa* (induced by PEG6000 solutions)), Salt stress of -0.6*Mpa* (induced by NaCl solutions) and combined stress (0, -0.5, -1.0, -1.5, -2.0*Mpa*). Results revealed there was a marked variation in studied traits in these two genotypes under single and combined (D+S) stress. Stress treatment significantly decrease fresh weights of shoot, root and whole plant, leaf water content (LWC), relative water content(RWC),, chlorophyll (a), chlorophyll (b) , total chlorophyll, and Carotenoids ,but it increase water saturation deficit (WSD).electrolyte leakage(EL) , expression of *CAT* gene leaf content of Na^+ and Cl^- . Interestingly, increased K^+ at most stress treatments. Together, the findings showed that the combined effects of salt and drought stress could result in increased WSD, Na^+ and Cl- buildup, and membrane degradation. It was concluded the Latifya genotype demonstrated excellent performance for all examined traits. It is clear that the Latifya genotype is more tolerant to stress than the Iba99 genotype.

Keywords: Combined stress(D+S), Water relations, EL, Pigments content, *CAT* gene, Gene expression.

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Introduction

Because of changing climatic conditions, plants typically experience changes in their water supply throughout their life cycle. Drought and salt are likely the abiotic stresses that have the greatest impact on development and production among all those that plants may experience in both natural and agricultural environments (1). Germination and seedling growth are crucial stages in a plant's life cycle. Salinity is a significant impediment in Iraq's central and southern areas. According to the results of a variance analysis, increasing salinity levels resulted in a decrease in fresh weight, dry weight, plant area, and plant height (2,3). This stress causes an important rise in the concentrations of Na^+ and Cl^- in leaves, which can significantly slow down seedling growth (4,5).

Polyethylene glycol (PEG) is a substance that can cause physiological dehydration under conditions of salt and water scarcity, as evidenced by earlier investigations (6). Several studies evaluated wheat genotypes using levels of PEG-6000 and they noted significant reduction in germination percentage, germination rate index, shoot and root length, fresh and dry weights of shoot and root, and root/shoot ratio in all treatments of PEG except control (7).

According to published research, some species are impacted by either PEG or NaCl, while others are impacted by both (8). The results of a study conducted by AL-Jobori and AL-Tamemy (9) on the response of four bread wheat genotypes to drought tolerance induced by PEG 6000 can be utilised as a database for Iraqi wheat genotypes to be used in the development of new varieties in breeding programmers.

One of the primary chloroplast components for photosynthesis is chlorophyll, and the association between relative chlorophyll content and photosynthetic rate is positive. According to a model score of oxidative stress, the decrease in chlorophyll concentration under stress is likely the result of pigment photo-oxidation and chlorophyll degradation, which has an impact on photosynthesis and plant growth(10). According to the vast majority of research, plants lose most of their chlorophyll when there is a water shortage in the mesophyll cells and very little from the bundle sheath cells(11). Chlorophyll concentration can serve as a helpful signal for calculating photosynthesis. According to several studies (7, 12), drought and salinity can reduce photosynthesis efficiency, deteriorate the photosynthetic apparatus, and lower chlorophyll a, chlorophyll b, and total chlorophyll content. Anthocyanin, chlorophyll fluorescence, and chlorophyll pigment levels were significantly decreased as a result of combined drought and salinity treatment(1). The amount of chlorophyll (a), chlorophyll (b), and total chlorophyll was dramatically reduced when Othmani *et al*. (7) employed PEG to create dryness.

Due to evaporation and temperature rise brought on by climatic change, water demand will rise even further .According to Kizilgeci *et al*. (6), maintaining turgor through osmotic adjustment is crucial for a plant's ability to withstand salinity and drought. Relative water content (RWC) decreased under conditions of combined salinity and drought compared to the control, but the reductions varied depending on the variety (1). RWC is higher in the early stages of leaf growth and declines as the leaf ages and accumulates dry matter. RWC is connected to both water uptakes by the roots and water loss through transpiration. Several authors have noted a decrease in RWC in a wide range of plants in response to drought stress (6, 7). Results by Muhu-Din Ahmed *et al*. (13) demonstrated that selection based on leaf water content and RWC at the seedling stage will genetically increase salinity tolerance. They showed that these two traits were connected with one another. The initial plant response to drought stress resulted in a striking alteration in the expression of *MAPK6* and *CAT* as well as both the *P5CS* and *P5CR* genes (14).The lack of soil moisture under persistent drought stress reduced the amount of hydration that the leaves received, which resulted in a significant rise in Water Saturation Deficit (WSD). Numerous research have noted this trend (15, 16). Therefore, this study was conducted to identify the relative importance of morpho-physiological traits associated with the combined and single salt and drought stresses.

Material and methods

Plant materials

Two genotypes were chosen phenotypically from an earlier experiment screening 16 genotypes to see if they are tolerat or sensitive to the combined and single effects of salt and drought. One genotype (Latifya) was chosen for tolerance, and another (Iba99) was chosen for susceptibility.

Treatments and experimental design

In plastic containers with 5 kg of soil, seeds were grown.Completely Randomised Block Design (CRBD) with three replications was used to set up the factorial experiment. Every replication included a control treatment (irrigate with fresh water), combined PEG 6000 and NaCl to induce stress conditions (-0.5, -1.0, -1.5, and 2.0 *Mpa*), as well as to single stresses of drought treatment- 0.6 *Mpa* (PEG-6000) and salt treatment-0.6 *Mpa* (NaCl). These treatments were made according to Michel and Kaufmann (17).

$\Psi s = - (1.18x10^{-2}) C - (1.18 x10^{-4}) C^2 + (2.67x10^{-4})CT + (8.39X 10^{-7}) C^2T$

Where:

Ψs = osmotic potential (*MPa*)

C= osmotic agent concentration (grams of PEG 6000/liter of water)

T= temperature (^oC).

Salt stress, with the same osmotic potentials (from -0.5 to -2.0 *MPa*), was modulated using NaCl, according to Fellahi *et al*. (18) in order to get the optimal concentration, distilled water used as a control. The crop was managed according to the recommended conventional agronomical practices. The experiment lasted for 60 days (from 15 November 2022 to 15 January 2023). Plants are watered when needed.

Table (1): Amount of sodium chloride and polyethylene glygol in different levels of water deficit

Ψo the level of *Mpa*	NaCl(g/l distilled water)	PEG(g/l distilled water)
0	0	0
-0.5	5.844	45
-1.0	11.718	61
-1.5	17.735	73
-2.0	23.647	84
Drought(-0.6)	----------	48.00
Salinity(-0.6)	9.00	------

Gene expression

RNA isolation and qRT-PCR analysis after 15 days of germination. Total RNA from wheat leaf seedlings was extracted using TransZol Up Plus RNA Kit (TransGen biotech, China) reagent according to the manufacturer's instructions. First-strand cDNA synthesis was performed according to the *TransStart*® Top Green qPCR Super Mix Kit protocol (TransGen biotech, China). Gene expression patterns of *CAT* was determined by qRT-PCR analysis using the primers: Forward primer:5'-CACCTGGTGGAGAAGA TCGC-'3, and reverse primer:5'-TCACCTCGAAGAAGCCCTTG-'3 (14). *TaActin* : Forward primer: 5'-CTTGTATGCCA GCGGT CGA ACA-'3 and reverse primer 5'-CTCATAAT CAAGGGCC ACGTA-'3 was used as the internal reference gene when examining gene expression of *CAT* gene. The qRT-PCR reaction in a final volume of 20µL contained 2µl of cDNA template, 2µl of gene-specific primers (20µM), 10µl *2xTransStart*® Top Green qPCR Super Mix, and 6µl ddH2O. The thermal cycles were 94 ◦C for 30 s, 40 cycles of 94 ◦C for 5 s and 58 ◦C for 15 s ending with a final extension of 72°C for20 s. Each sample was repeated three times as technical repeats. The cycle threshold (CT) value of the real-time PCR was further analyzed using the $2^{-\Delta\Delta Ct}$ method for calculating the relative expression levels (19).

Physiological, biochemical and growth traits estimation

After 30 days of growth under stress, wheat leaves were randomly selected with the same morphological characteristics and used for further investigation. The leaves were placed in labeled plastic sacks and transferred to the laboratory for examination of

physiological and biochemical properties. Additionally, plants were taken after 60 days of stress-induced development, washed with distilled water, and promptly separated into their root and shoot parts at the crown level. Following this, the fresh weights and the plant's Na^+, Cl^-, and K^+ contents in the leaves were recorded and measured as follows:

Water relations

The leaf water content(LWC) of the leaves was calculated the cut off eaves and weighed to obtain the fresh weight . Then, they were put into an oven at 105 ◦C for 30 min and then dried at 80 ◦C to obtain the dry weight. After then calculated by the following equation(20):WC=[(FW-DW /FW)*100 Where: FW = Fresh Weight (g), DW = Dry Weight (g). Relative water content(RWC) determine using 1 cm^2 segments of leaf tissue, The fresh weight (FW) of the leaf disks determine , and the disks floated in distilled water for 24 h. The turgid weight (TW) then recording, then oven-dry at 70°C for 48 h to measure the dry weight (DW). RWC is calculate using the following formula according to Barrs and Weatherly (21): RWC= (FW – DW)/ (TW – DW)*100 Where: TW = Turgor weight (g). Water Saturation Deficit (WSD %): It calculate as mentioned by Müller *et al*. (22): WSD (%) = 100 – RWC.

Estimation of electrolyte leakage (EL)

The injuries in the cell membrane were assessed by estimating the electrolyte leakage using an electrical conductivity meter. A cork cutter of the diameter of 4 mm was used to cut discs of wheat plant leaves were washed thoroughly in tap water followed by washing in distilled water. The membrane stability index was calculated using of 0.5 g leaf tissue in 25ml of deionized distill water (DDW) in two sets. One set was heated in a water bath at 40°C for 30 minutes, and the electrical conductivity (EC1) was measured using a conductivity meter. The second set was boiled for 10 minutes at 100°C in a water bath, and its electrical conductivity(EC2) was also measured using the conductivity meter(23). MSI was estimated with the following formula: EL % = [1- (EC1/ EC2)] × 100.

Pigments content

The leaves 30 days ages were collected randomly with the same morphological characteristics and placed in labeled plastic sacks and transported to the laboratory for analysis of chlorophyll and carotenoids. These pigments content were determined according to Goodwin (24) with some modification: A total of 500 mg of fresh leaves were homogenized in 10 ml of dimethylsulphooxidase (DMSO). The homogenized leaves were centrifuged at 3000 rpm for 15 min. after that the pigments content was determined spectrophotometrically at 663 nm, 645 nm,and 470 nm. pigments content were calculated on a fresh weight following the equations (24):

Chlorophyll a (Ca) (mg/L)= 12.7 $D_{(663)}$ – 2.69 $D_{(645)}$

Chlorophyll b (Cb) (mg/L) =22.9 $D_{(645)}$ – 4.68 $D_{(663)}$

Total Chlorophyll (mg/L) = 20.2 $D_{(645)}$ + 8.02 $D_{(663)}$

Carotene + Xanthophyll (C+X) = (1000 $D_{(470)}$ – 1.82Ca – 85.02Cb) / 198

Where: $D_{(663)}$ is the absorbance at 663 nm., $D_{(645)}$ is the absorbance at 645 nm., $D_{(470)}$ is the absorbance at 470 nm, Ca= Chlorophyll-a, Cb= Chlorophyll-b, C = Carotine, X= Xanthophyll

Fresh weights

After 60 days, the plants were removed, and the fresh masses of the roots, shoots, and whole plant were measured using a sensitive balance.

Determination of element concentration

Determination of K^+ , Na^+ and Cl^- ions was carried out according to the method of Munns *et al*. (25) by using Nitric acid 0.5 *M* (HNO_3) as an extraction solution. Briefly, dry plant tissues were grounded using mortar and pestle to increase the homogenization with extraction solution. Crude extraction solution was included 100 mg of plant tissue with 10 ml of extraction solution. Samples were shaked in shaker machine for 2 days at room temperature. Supernatant solutions were diluted to 1:100 in total volume of at least 10 ml for K^+ and Na^+ simultaneously. After using Flam photometer instrument, the results units of the machine were converted from micrograms per milliliter (µg/ml) into micromole per gram (µmol/g). Conversion equation for units is as below (25).

DW = (concentration in micrograms per milliliter × dilution factor, e.g. 100 for 1:100 dilution × volume of dilute acid extract, e.g. 10 ml) / (DW of tissue used in extraction ×MW of ion 39.098 for K^+ and 22.99 for Na^+). Cl^- can be measured in the same acid extract with a chloridometer or colorimetrically using a spectrophotometer.

Statistical analysis

The data obtained were subjected to ANOVA using the Statistical Analysis System- SAS program according to Randomized Completely Block Design-(RCBD) design with three replicates, and the Least Significant Difference (LSD) test was used at 0.05 probability level to compare the differences between treatment means.

Results and discussion

Gene expression

The results showed that the stress treatments increased the transcript levels of *CAT* gene in Latifya plants compared to the normal growing conditions, with difference in the response of *CAT* gene under all stress levels. Results of table 2 revealed that the expression of *CAT* gene was at high levels at -0.5 *Mpa* by 6.45 folds followed by -1.0 *Mpa* and salinity by 4.56 and 4.96folds , and at-1.5 and -2.0 *Mpa* by 3.29 and 3.25 folds, respectively than control, but exhibited slight rise at drought stress.In current study, *CAT* gene from leaves of Iba99 wheat plants exhibited decrease in its expression under all stress levels compared with control (Figure1).

Table (2): Fold of *CAT* gene expression depending on $2^{-\Delta\Delta Ct}$ Method.

Genotypes	Stress levels (*Mpa*)	*CAT* gene	*TaActin* gene	ΔCT	$2^{-\Delta\Delta Ct}$	Folding
Latifya	0	25.23	28.34	-3.11	8.63	1.00
	-0.5	22.03	27.83	-5.8	55.72	6.45
	-1.0	22.33	27.63	-5.3	39.40	4.56
	-1.5	23.01	27.84	-4.83	28.44	3.29
	-2.0	23.65	28.46	-4.81	28.05	3.25
	Drought(-0.6)	24.58	27.88	-3.3	9.85	1.14
	Salinity(-0.6)	22.81	28.23	-5.42	42.81	4.96
Iba99	0	24.47	27.8	-3.33	10.06	1.00
	-0.5	25.09	28.64	-3.55	11.71	1.16
	-1.0	25.22	28.06	-2.84	7.16	0.71
	-1.5	25.8	27.2	-1.4	2.64	0.26
	-2.0	25.02	27.55	-2.53	5.78	0.57
	Drought(-0.6)	32.53	28.14	4.39	0.05	0.01
	Salinity(-0.6)	25.74	27.88	-2.14	4.41	0.44

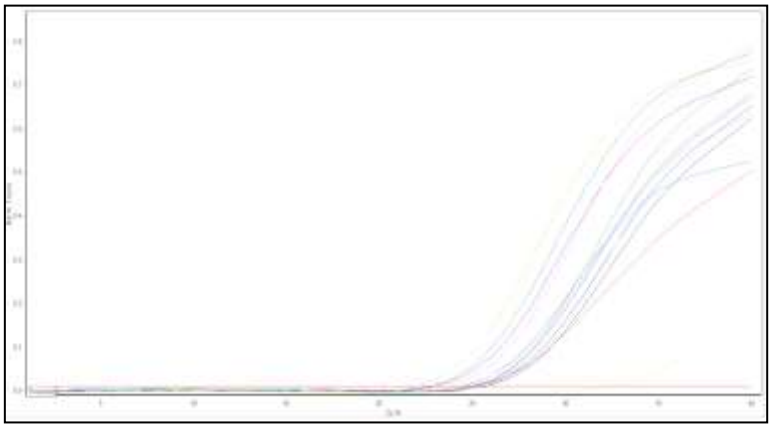

Figure (1): The amplification plots of *CAT* gene

Water relations

The findings demonstrated significant differences for water content, relative water content, water saturation deficit and electrolyte leakage among stress levels, genotypes, and stress levels x genotypes at 5% probability, indicating the existence of variation among various treatments. RWC gradually decreased with fall LWC values, As a result, changes in WSD and leakage had the opposite effect. The control condition had the highest values of 85.97and 84.48% for LWC and RWC, respectively, and the lowest values of 15.48% for WSD (Table 3). The values of LWC and RWC were decreased gradually with the increase in stress and reached to lowest values of 65.66 and60.81% at combined stress (-2*Mpa*), respectively, and increased to the highest values of 49.72% for WSD at salinity stress. The highest stress combined stress (-2*Mpa*) exhibited the highest electrolyte leakage reached to 72.68% In terms of single stresses, the salt stress had a bigger impact on the plant's water relations, significantly reducing RWC by 10.18% and increasing WSD by 13.39% stress. Additionally, it had an increase in electrolyte leakage by 12.71% compared to the drought (Table 3).

Table (3): Water relations and electrolyte leakage recorded after one month of exposed to single and combined drought and salinity stresses.

Stress (*Mpa*)	LW.C (%)	R.W.C (%)	W.S.D (%)	Electrolyte Leakage (%)
0	85.97	84.48	15.48	20.33
-0.5	80.77	78.38	21.91	58.61
-1.0	75.96	72.28	27.71	64.96
-1.5	71.37	66.50	33.49	69.45
-2	66.32	60.81	39.01	72.68
Drought(-0.6)	71.36	56.08	43.85	50.73
Salinity(-0.6)	73.27	50.37	49.72	57.18
LSD	2.57 *	1.104 *	1.18 *	1.29 *
* (P≤0.05).				

The two wheat genotypes' LWC and RWC differed as a result of different stress levels (Table 4). Under stressful circumstances, variations between genotypes were found to be significant at the 5% statistical level. The LWC and RWC of the Latifya genotype were higher than those of the Iba99 genotype by 3.85 and 8.14%, respectively. Electrolyte leakage in the Iba99 genotype increased under stressful circumstances and showed a 61.27% rise compared to a 51.28% for Latifya (Table 4).

Table (4): Water relations and electrolyte leakage recorded in studied wheat genotypes.

Genotypes	L.W.C (%)	R.W.C (%)	W.S.D (%)	Electrolyte Leakage (%)
Latifya	76.32	69.61	30.36	51.28
Iba99	73.49	64.37	35.69	61.27
LSD	1.38 *	0.590 *	0.631 *	0.694 *
*** (P≤0.05)**				

The interaction between wheat genotypes and stress levels showed that Latifya genotype had the highest LWC (86.73%) in the control condition (0 *Mpa*). However, the genotype Iba99 at -2*Mpa* level showed a minimum LWC of 63.44%. However, there was no marked difference between single stresses (drought and salinity) (Table 5).Under combined stress conditions, the RWC value dropped as the stress treatment rose. The percentage reduction in RWC was the lowest in Latifya which also had shown the highest value under control condition. However, Latifya maintained relatively higher RWC (64.2%) at −2.0 *MPa*, while in Iba99 it was 57.41%. WSD was gradually increased with fall LWC values in both genotypes with the increase in combined stress levels (Table5). However, Latifya maintained relatively lower WSD (35.58%) at −2.0 *MPa*, while in Iba99 it was 42.42% .LWC was decline with growing of combined stress levels. As a result, changes in EL had the opposite effect which increased in both genotypes with decreasing LWC (Table5). In Latifya, EL increased from 21.00% to 63.12%, while in Iba99, it increased from 19.66% to 82.24% at 0*Mpa* with significant differences between the two genotypes where Iba99 exhibited highest EL (Table5). Interestingly, the single stresses (drought or salinity) were more impact than the combined stresses levels on RWC, WSD and EL, and the effect of salinity more than drought ,also Iba99 showed more influence than Latifya.

Table (5): Water relations and electrolyte leakage recorded after one month of exposed to single and combined drought and salinity stresses in two wheat genotypes

Genotypes	Stress levels	LWC(%)	RWC(%)	WSD(%)	Electrolyte leakage (%)
Latifya	0	86.73	86.56	13.38	21.00
	-0. 5	80.15	80.12	19.87	52.50
	-1.0	76.39	75.26	24.74	58.47
	-1.5	72.25	70.7	29.30	60.40
	-2.0	69.20	64.2	35.58	63.12
	Drought (-0.6*Mpa*)	77.82	58.90	40.98	50.00
	Salinity (-0.6*Mpa*)	73.06	51.51	48.68	53.50
Iba 99	0	85.24	82.41	17.58	19.66
	-0. 5	81.39	76.64	23.96	64.73
	-1.0	75.55	69.30	30.69	71.45
	-1.5	70.49	62.30	37.69	78.5
	-2.0	63.44	57.41	42.42	82.24
	Drought (-0.6*Mpa*)	64.90	53.27	46.72	51.46
	Salinity (-0.6*Mpa*)	73.47	49.24	50.76	60.86
LSD		3.64 *	1.56 *	1.67 *	1.84 *
*** (P≤0.05).**					

Pigments content

The findings demonstrated a substantial impact on the amounts of leaf pigments. Under normal conditions, leaf chl.a, chl.b, total Chl. and carotenoids content were 2.51,1.00, 3.51 and 1.41mg g^{-1} FW, decreased gradually with the increase in combined stress reached to lower content to 0.706, 0.28, 0.99 and 0.256mg g^{-1} FW, respectively, at -2.0*Mpa* condition. When compared single with combined stress, the results revealed that drought stress was close to the high combined stress (-2.0Mpa). Whereas salinity stress was less effect which was close to -1.0 for Chl.a and total Chl., and to 0 for Chl.b, and to -0.5 for carotenoids (Table 6).

Table (6): Pigments content of wheat leaves expressed on a fresh weight (FW) basis recorded after one month of exposed to single and combined drought and salinity stresses

Stress (*Mpa*)	Chl.a(mg g^{-1} FW)	Chl.b(mg g^{-1} FW)	Total Chl. (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)
0	2.51	1.00	3.51	1.41
-0.5	2.16	0.84	2.88	1.22
-1.0	1.87	0.69	2.40	0.718
-1.5	1.01	0.56	1.57	0.411
-2	0.706	0.28	0.99	0.256
Drought(-0.6)	0.753	0.28	1.02	0.250
Salinity(-0.6)	1.44	0.97	2.40	1.013
LSD	0.241 *	0.060 *	0.337 *	0.058 *
*** (P≤0.05).**				

The studied genotypes showed no significant differences in chlorophylls content (Chl.a, CHl.b and total Chl.), but showed a statistically significant difference in the content of carotenoids between the two studied genotypes that had undergone single stresses (drought or salinity stress) and combined stress treatments, with Latifya being superior (0.80mg g^{-1} FW) on Iba99 (0.71mg g^{-1} FW) (Table 7).

Table (7): Pigments content of wheat leaves expressed on a fresh weight (FW) basis recorded in studied wheat genotypes

Genotypes	Chl.a (mg g^{-1} FW)	Chl.b (mg g^{-1} FW)	Total (mg g^{-1} FW) Chl.	Carotenoids (mg g^{-1} FW)
Latifya	1.56	0.669	2.23	0.80
Iba99	1.43	0.651	2.08	0.71
LSD	N.S	N.S	N.S	0.0315 *
*** (P≤0.05)**				

Significant differences in genotype × stress levels interactions were identified at 5%statistical level (Table 8). Both genotypes showed a considerable decrease in pigments content as a result of combined or single drought and salt stressors. For combined stress, the chl.a and carotenoid content were higher at control treatment (2.55 and1.49 mg.g^{-1} FW) in Latifya genotype and lowest content (0.663 and 0.21mg.g^{-1} FW) in Iba99 genotype at -2.0*Mpa*, whereas the chl.b and total chl. content were higher at control treatment (1.08 and3.55 mg.g^{-1} FW) in Iba99 genotype, whilst lowest content (0.28 and 0.893 mg.g^{-1} FW) also in Iba99 genotype at -2.0*Mpa* (Table 8).

Table (8): Pigments content recorded after one month of exposed to single and combined drought and salinity stresses in two wheat genotypes

Genotypes	Stress levels	Chl.a (mg g^{-1} FW)	Chl.b (mg g^{-1} FW)	Total Chl. (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)
Latifya	0	2.55	0.92	3.47	1.49
	-0. 5	2.24	0.85	3.09	1.29
	-1.0	2.01	0.733	2.413	0.80
	-1.5	1.02	0.56	1.583	0.40
	-2.0	0.75	0.296	1.046	0.30
	Drought (-0.6*Mpa*)	0.853	0.30	1.153	0.26
	Salinity (-0.6*Mpa*)	1.47	1.026	2.50	1.04
Iba 99	0	2.476	1.08	3.55	1.32
	-0. 5	2.08	0.83	2.66	1.16
	-1.0	1.743	0.65	2.39	0.63
	-1.5	1	0.56	1.56	0.42
	-2.0	0.663	0.28	0.946	0.21
	Drought (-0.6*Mpa*)	0.653	0.25	0.893	0.24
	Salinity (-0.6*Mpa*)	1.40	0.91	2.31	0. 89
LSD		0.341 *	0.085 *	0.476 *	0.083 *
* (P≤0.05).					

Fresh weights

The growth of wheat was inhibited as a result of the study's lowering trends in the mean values of numerous parameters, such as shoot, root, and Plant fresh weight, which were decreased by increasing combined stress levels over -0.5*Mpa* or by single stresses brought on by PEG and NaCl levels. Salt had a greater impact on growth inhibition than dryness (Table 9). Stress levels of -1.0, -1.5 and -2.0*Mpa* caused significant *(P≤0.05)* reductions by 26.96, 36.52, and 26,96% for shoot fresh weight, 30.09, 47.36, and 66.70% for root fresh weight, and 27.62, 38.25, and 34.65% for plant fresh weight compared with control (Table 9).

Table (9): Fresh weights and Na,Cl and K content recorded after two month of exposed to single and combined drought and salinity.

Stress (*Mpa*)	Shoot fresh weight (g)	Root fresh weight (g)	Plant fresh Weight (g)	Na(%)	Cl (%)	K (%)
0	4.71	1.12	5.83	0.478	25.01	2.45
-0.5	4.33	0.968	5.27	0.800	36.92	4.31
-1.0	3.44	0.783	4.22	2.73	42.02	2.93
-1.5	2.99	0.612	3.60	3.69	47.20	2.46
-2	3.44	0.373	3.10	4.92	53.73	1.35
Drought (-0.6)	4.10	0.833	4.94	0.700	0.738	2.10
Salinity (-0.6)	3.47	0.618	4.09	0.998	29.92	2.97
LSD	0.840 *	0.177 *	0.806 *	0.103 *	0.514 *	0.457 *
* (P≤0.05).						

The findings for all weight traits are shown in Table 10, which also indicates highly significant genetic differences. The results showed that there were significant differences in plant fresh weight between genotypes. In terms of plant fresh weight, genotype Latifya had the highest value (5.01g), whereas genotype Iba99 had the lowest value (3.87g).

Table (10): Fresh weights and Na,Cl and K content in the leaves of studied wheat genotypes

Genotypes	Shoot fresh Weight (g)	Root fresh weight (g)	Plant fresh weight (g)	Na(%)	Cl (%)	K (%)
Latifya	4.25	0.761	5.01	1.79	31.94	3.07
Iba99	3.03	0.753	3.87	2.29	35.36	2.24
LSD	0.449 *	N.S	0.431 *	0.055 *	0.275 *	0.244 *
*** (P≤0.05)**						

According to the interaction between genotypes and stress levels, maximum shoot and plant fresh weight (5.40 and 6.42 g) were seen in genotype Latifya under control conditions (0*Mpa*). The genotype Iba99 at -2.0*Mpa* condition, however, displayed the smallest fresh weights for the shoot and plant (1.43 and 2.34g) .But the results showed that Iba99 produced both the highest root fresh weight (1.21g) under control and the lowest weight (0.336g) at -2.0*Mpa* (Table11).

Table (11): Fresh weights and Na, Cl and K content recorded after exposed to single and combined drought and salinity stresses in two wheat genotypes

Genotypes	Stress levels	Shoot fresh weight (g)	Root fresh weight (g)	Plant fresh weight (g)	Na (%)	Cl (%)	K (%)
Latifya	0	5.40	1.02	6.42	0.41	24.1	2.88
	-0. 5	4.78	0.920	5.64	0.53	35.32	4.86
	-1.0	4.21	0.850	5.06	2.51	40.75	3.42
	-1.5	3.70	0.573	4.27	3.26	46.14	2.90
	-2.0	3.45	0.410	3.86	4.43	51.52	1.81
	Drought (-0.6*Mpa*)	4.29	0.906	5.19	0.54	0.63	2.11
	Salinity (-0.6*Mpa*)	3.94	0.653	4.59	0.70	24.38	3.50
Iba 99	0	4.02	1.21	5.24	0.54	25.22	2.03
	-0. 5	3.89	1.01	4.90	1.06	38.51	3.76
	-1.0	2.66	0.716	3.38	2.94	43.13	2.42
	-1.5	2.29	0.650	2.94	4.12	48.25	2.03
	-2.0	1.43	0.336	2.34	5.25	56.10	0.90
	Drought (-0.6*Mpa*)	3.92	0.760	4.68	0.86	0.84	2.08
	Salinity (-0.6*Mpa*)	3.01	0.583	3.59	1.36	33.82	2.45
LSD		1.188 *	0.250 *	1.140 *	0.146 *	0.727 *	0.647 *

Determination of element concentration

The contents of Na+, Cl-, and K+ in the wheat leaves were significantly impacted by both combined and individual stresses (drought or salt) (Table 9). Na+ content dramatically rose when the stress level The contents of Na+, Cl-, and K+ in the wheat leaves were significantly impacted by both combined and individual stresses (drought or salt) . Na+ content dramatically rose when the stress level rose to -1.0, -1.5, and -2.0*Mpa* by 471.13, 671.97, and 929.29%, respectively. Also increased the accumulation of Cl- ions by 47.62, 68.01, 88.72, and 114.83% at -0.5, -1.0, -1.5, and -2.0*Mp*a, respectively, compared to the control condition. It should be noted that the K+ content in the leaves significantly decreased under

stress of -2.0*Mpa*, but remained unchanged at -1.5*Mpa*. It's interesting to note that a stress of -0.5 *Mpa* induced a 2-fold rise in leaf K+ concentration and a considerable increase at -1.0 *Mpa* of 19.59% compared to control treatment. The balance of the decreased leaf K and the sharp increase in Na+ and Cl-content at -2.0*Mpa* stress results in a decreased K+ content (Table 9) raised to -1.0, -1.5, and -2.0*Mpa* by 471.13, 671.97, and 929.29%, respectively. Also increased the accumulation of Cl- ions by 47.62, 68.01, 88.72, and 114.83% at -0.5, -1.0, -1.5, and -2.0*Mpa*, respectively, compared to the control condition. It should be noted that the K+ content in the leaves significantly decreased under stress of -2.0*Mpa*, but remained unchanged at -1.5*Mpa*. It's interesting to note that a stress of -0.5 *Mpa* induced a 2-fold rise in leaf K+ concentration and a considerable increase at -1.0 *Mpa* of 19.59% compared to control treatment. The balance of the decreased leaf K and the sharp increase in Na+ and Cl-content at -2.0*Mpa* stress results in a decreased K+ content (Table 9).

Additionally, the tested wheat genotypes revealed notable variations. Iba99 had enhanced the Na^+ and Cl^- concentration in the leaves by 27.93 and 10.71% in comparison to Latifya. However, when compared to Latifya genotype, which accumulated the largest mean K^+ (3.07%), genotype Iba99 accumulated the least K^+ by 27.04% (Table 10).

The results displayed that combined and single stresses (drought or salinity) were statistically significant *(P≤0.05)* for accumulation of Na^+, Cl^- and K^+ ions in the leaves of two wheat genotypes (Table11). A distinct genotypic difference was found. In comparison to Latifya, Iba99 had increased Na^+ and Cl^- concentration in the leaves during combined and single stresses. In comparison to the control, Na^+ and Cl^- concentration increased by 980.49 and 113.78% in the leaves of Latifya and 872.22 and 122.44% in the leaves of Iba99 at -2.0*Mpa*, respectively. However, Iba99 showed higher accumulation by 18.51 and 8.9% over Latifya at the same stress (Table11). For the investigated wheat genotypes, the studied genotypes showed a significant range of variability ranging for K^+ from 4.86 to 1.81for Latifya and 2.03 to 0.90 for Iba99. The K^+ content in Ltifya leaves was greater at -0.5, -1.0, -1.5, and -2.0*Mpa* than in Iba99 leaves by 29.25, 41.32, 42.86, and 101.11%, respectively. The K^+ concentration in leaves for the two genotypes increased considerably at -0.5 and -1.0*Mpa*. It's interesting really that both genotypes had more K^+ than Na^+ deposited in their leaves, which may be a sign of tolerance mechanism (Table11).

Gene expression

Because of its capability to efficiently scavenge H_2O_2 and prevent its accumulation to hazardous levels, *CAT* has emerged as a crucial enzyme in the antioxidant system according to recent studies on the system. *CAT* does not need a reductant to scavenge H_2O_2, making it reducing power-free. The elimination of H_2O_2 produced under oxidative stress is one function of the proteins expressed by this gene, which may assist to explain how the expression of *CAT* is regulated. Bian and Jiang (34) assert that *CAT* may enhance the efficiency of H_2O_2 scavenging in leaf cells. The results showed that the genotype Latifya has a better antioxidant capacity than the genotype Iba99 under stress conditions. As a result, for the entire stress period in Latifya, enhanced *CAT* expression was accompanied by higher catalase activity

(Table 2). These results are in line with those of Wang *et al.* (35), who discovered a relationship between higher *CAT* expression and increased *CAT* activity in wheat that had undergone pre-acclimation to stress. Because it increases under drought stress circumstances, the *CAT* gene can be used as a potential gene to improve drought tolerance in wheat (14). The results of current study showed that *CAT* significantly enhanced wheat plants' ability to withstand stress. Antioxidant enzymes were activated by *CAT* to neutralize ROS.

Water relations

A drop in water content, turgor, total water potential, wilting, stomatal closure, and cell expansion and growth are all signs of water stress, the study's findings showed that both drought and salinity stresses (individually and together) significantly impacted the water content of both genotypes, though drought had a greater impact than salinity (Table 2). These findings are consistent with those of other authors (1, 20), who also found that both drought and salinity stresses (individually and together) significantly impacted the water content, though dryness had a greater impact than salinity. Furthermore, according to Rostami *et al*. (26), water content features decreased in conditions of salinity brought on by increased NaCl concentration as well as drought.

However, these results are at odds with another study(27) which showed that salinity stress cannot alter the water content of plants. Current study found that LWC decreased during stress and decreased rapidly when relative soil moisture was -0.5 *Mpa* or lower. This implies that, contrary to what Chen *et al*.(20) claimed, LWC would not drop when a drought struck and would only drop quickly when relative soil moisture was lower than 45.3%, where they suggested that LWC is not a sensitive water physiological index for spring wheat to respond to water deficit.

The relative water content significantly decreased as osmotic tension increased. The relative water content was similar, according to the results of numerous researchers (13, 16, 28). Research suggests that the fall in RWC during drought stress may be due to decreased plant vigour(29). The primary factor affecting a plant's ability to grow and develop is the water status of its cells., although NaCl significantly affected seedling RWC compared to normal irrigation (Table2). The beginning of stressful conditions may be associated with a decline in relative water content (RWC). Therefore, based on these findings, RWC may be a useful method for assessing water deficiency tolerance. A disturbed equilibrium between water uptake and loss by evapotranspiration can be used to explain the decline in RWC in Iba99. Plants with enhanced RWC retention are drought-resistant.

The WSD, which displays how much water has been diverted from the saturated leaf, provides an accurate estimate of how much water is in the leaf. According to Prathyusha and Chaitanya (15), it has been demonstrated the exact amount of water present in the saturated leaf by demonstrating how much of it has evaporated. WSD executed the RWC's reverse trend. The amount of water a plant is lacking is referred to as WSD. The results of the present investigation revealed that the lack of soil moisture under persistent stress decreased the quantity of moisture delivered to the leaves, resulting in a discernible increase in WSD (Table 2). The WSD was shown to have significantly increased in many research, including

those conducted Munsif *et al*.(16).When compared to Iba99, Latifya typically display less WSD values (Table3) , this study demonstrates that Latifya plants fared better than Iba99 under stressful water deprivation conditions.

In order to test the tolerance of the plants, it is crucial to determine the integrity of the cell membrane during salt or drought stress. The integrity of the wheat cell membrane under combined or single stress (drought or salt) was examined in this study by measuring the El of the leaf cells. Wheat leaves' EL increased when LWPs decreased (Table 2). Increased ROS generation due to drought leads to more rapid membrane damage brought on by the peroxidation of membrane lipids(30). Plants under salinity stress in the current study exhibited substantial differences from drought conditions (Table 2). This characteristic is frequently impacted by malondialdehyde (MDA), which is formed as a result of lipid peroxidation induced by ROS under salinity stress conditions . Current research shows that El in Iba99 leaves increased. In Latifya, in comparison, caused less damage to the cell membrane (Table 3). According to earlier studies (15, 27, 31), El rose differentially in the genotypes' leaves when there was a water shortage. Genotypes and stress levels have a considerable impact on electrolyte leakage (EL). EL rose in response to stress, however it depends on the genotypes and stress levels .In Latifya, El increased from 21 to 63.12%, while in Iba99, it increased from 19.66 to 82.24% (Table4). Several previous studies (22, 26) demonstrated that increased combined and single stresses (drought or salt)lead to increase El, these findings are consistent with current results.

Pigments content

Chlorophyll concentration is recognized as a significant indicator of plant productivity since it is directly related to the rate at which plants produce biomass through photosynthetic activities. The decrease in chlorophyll concentration in stressed plants may be caused by an increase in the activity of the enzyme chlorophyllase, which breaks down chlorophyll . In comparison to the control, the study's findings, revealed that both salt and drought stress (individually and together) had a significant negative influence on plant pigments (chl. a, b, total chl., and carotenoid content), but that drought stress had a greater negative impact than salinity stress (Table 5). The results of this study corroborated earlier research on abiotic stresses by other authors (13,16), which demonstrated significant decreases in chl.a, b, total chl., and carotenoids under stress. Hasson *et al*. (32) hypothesised that under salt stress circumstances, a decrease in de novo chlorophyll synthesis or an increase in chlorophyllase activity may be responsible for the decline in photosynthetic pigments.

In terms of chlorophyll content, the investigated wheat genotypes Latifya and Iba99 did not significantly differ from one another, while Latifya had the highest carotenoid content (Table 6).During times of stress, carotenoid acts as an antioxidant in plants to prevent oxidative damage to chlorophyll . This helps to maintain the chlorophyll level. Latifya therefore produces more chlorophylls than Iba99, though not to a significant level. Salt, drought, or a combination of the two stresses significantly reduced the levels of chl. a, chl. b, total chl., and carotenoid in the studied wheat genotypes. Chlorophyll and carotenoid

properties decreased significantly during drought stress than salt stress (Table 7). These results support the findings of Dugasa *et al*. (12), who found that salt, drought, or a combination of the two stresses significantly reduced the chl. a and chl. b levels of two wheat genotypes. By keeping these pigments in their thylakoid membranes, the stress-tolerance genotypes are able to endure stress. As a result, Latifya retains more pigments than Iba99 and keeps its chlorophyll content close to the control treatment (0 *Mpa*) at a stress level of -0.5 *Mpa* (Table 7).

Fresh weights

As the level of stress increased, there was a noticeable difference in the fresh weights. Wheat plants' fresh weights of shoot, root, and entire plant declined with an increase in stress levels when exposed to NaCl and PEG alone or in combination (Table 8). The primary factor affecting a plant's growth and development is its water status; inadequate cell division brought on by drought stress lowers root and shoot fresh weight through changing the cell's osmotic potential (13). Salinity causes low osmotic potentials, which limit the availability of water, as well as nutritional imbalance and ion toxicity due to severe ionic ratios. As a result, a decline in plant development in saline environments could either be brought on by a shortage of water or by sodium chloride's direct toxicity(33). Earlier studies (1, 27, 28), have reported that drought and salinity stresses had a significant impact on fresh weight of shoot, root and whole plant.

The results of this study, however, indicate in this research that there was a significant degree of genetic variability and that the analysed genotypes behaved differently depending on their environment at the seedling stage and during vegetative growth. In comparison to the other genotype, Iba99, the Latifya genotype had the largest fresh biomasses of the root, shoot, and entire plant (Table 9). Similar differences between genotypes in fresh weight production have been observed by numerous other researchers (1, 7, 12, 13). The genotypes both displayed observable growth retardations under stress; however the declines were not uniform. Additionally, all treatments resulted in noticeably decreased levels of fresh weight of shoot, root, and entire plant when compared to control plants. Under stress conditions. The genotype Iba99 showed more decline in fresh weight for the shoot, root, and whole plant than Latifya genotype (Table 10). Reduced root and shoot development might be a result of the negative impacts of the higher levels of NaCl and the seedlings' unbalanced nutrient uptake (18). According to AL-Jobori and AL-Waiely (2), increasing salt levels led to a drop in fresh weight and K^+ content but an increase in Na^+ content of wheat seedlings.PEG can cause physiological drought in both salt and drought environments (6), altering osmotic potential and interfering with nutrient uptake, which lowers biomass production.

Determination of element concentration

As the amount of combined stress increased, leaves' Na^+ and Cl^- content significantly increased (Table 8). One of the primary defence strategies is to manage the ratio of Na^+ and K^+ buildup in different plant tissues. Therefore, it is essential to assess Na^+ and K^+ buildup in root and shoot in order to identify the mechanisms behind salt tolerance . Interestingly, combined and single stresses treatments caused greater K^+ increase than Na^+, It was

found that all stress treatments increased the quantity of K^+ present in this study's findings, with the exception of those at -1.5 and -2.0*Mpa* (Table 8). The fact that the results of this study agree with those of Ahmadi *et al.* (33) suggests that high K^+/ Na^+ ratios and ion concentrations may be useful selection indicators for salt tolerance. Based on genotypic means, Iba99 was determined to have the highest Na^+ and Cl^- levels under stress, whereas Latifya showed the strongest capacity for K^+ ion transport from root to leaves and minimizing the impacts of salt stress (Table 9). Similar findings were made by Pour-Aboghadareh *et al*. (27) who discovered that variety G2 under stress showed the strongest ability for K^+ ion transport from root to shoot and inhibiting the effects of salt stress. According to research by Mahlooji *et al*. (31), tolerance in barley depends not only on a higher K^+: Na^+ ratio but also on avoiding Na^+ accumulation in above-ground plant sections.

The elements Na^+, Cl^-, and K^+ displayed different accumulation behaviours in the leaves of both genotypes because Na^+ and K^+ compete with one another for absorption into cells (Table10). The results show that under combined and single (drought or salt) conditions, the Iba99 genotype accumulated more Na^+ and Cl^- in the leaves, resulting in higher Na^+/ K^+ ratios in the leaves. As a result, there was a significant decrease in K^+ content and an increase in the Na^+/K+ ratio in plant tissue (Table10). In accordance with Müller *et al*. (21)'s findings, when Na accumulated, the Na^+/ K^+ ratio likewise declined in parallel with a noticeable drop in the concentration of the majority of other elements (Ca^{++}, K^+). As a result, increasing Na^+ levels in plants typically result in decreasing K^+ levels. The K^+ content was noticeably higher than Na^+ in the Latifya, however the Na^+/ K^+ ratio drastically dropped (Table10), making this genotype a tolerant one. Dugasa *et al*. (12) discovered that the Jimai22 tolerant genotype had significantly lower Na^+/ K^+ ratios than the Yangmai20 genotype did, even while the Jimai20 genotype had bigger Na^+/ K^+ ratios. According to their findings, AL-Jobori and AL-Waiely (2) suggested that K^+ and Na^+ contents as well as the K^+/Na^+ ratio determined at the seedling stage can be taken into consideration for screening wheat genotypes at high salinity concentrations.

Conclusion

According the above mentioned data it could be concluded that stress has a negative impact on growth, water relations ,pigments content and nutrients uptake, however, the overall results show that Latifya has a stronger stress tolerance than Iba99 since it maintained lower decreases in water relations, less Na^+ and high K^+ accumulation and more *CAT* expression and fresh weight buildup than Iba99 at all stress levels. Therefore, it is believed that Latifya can be grows in locations that are subject to this combined stress. The findings indicated that stress tolerance could be genetically improved by selection based on LWC, RWC, and WSD.

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