Ibn Al-Haitham Journal for Pure and Applied Sciences
Journal homepage: jih.uobaghdad.edu.iq
PISSN: 1609-4042, EISSN: 2521-3407
IHJPAS. 2025, 38(3)

Screening of Wheat Genotypes under Drought and Salinity Stresses During Germination and Seedling Traits

Muhammaed Jawad Husain[1*] and Kamil M. AL-Jobori[2]
[1,2] Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Baghdad, Iraq.
*Corresponding Author.

Received: 20 September 2023   Accepted: 9 January 2024   Published: 20 July 2025
doi.org/10.30526/38.3.3743

Abstract
This study examines wheat genotypes under combined and single drought and salinity stresses, assessing germination and seedling growth traits, as environmental issues like high salinity and drought significantly impact plant life. The experiment was carried out to meet these goals. To identify the most and least tolerant genotypes, 16 genotypes were tested. Water potentials of 0. -0.5, -1.0, -1.5, and -2.0Mpa were used as treatments to create water stress by adding polyethylene glycol (PEG-6000) and sodium chloride together(D+S). After 15 days of putting seeds in Petri dishes, growth parameters were assessed, including germination percentage (%), speed of emergence (%), seedling vigour index, shoot length (cm), and fresh and dried weight of shoot, root, and whole seedling (g). Results showed that all genotypes demonstrated a decrease in germination and biomass with an increase in stress-induced water stress, where germination (%) and biomass are inversely correlated with combined stress. While the genotypes Babil, Hashimia, Baraka, Buhooth 158, Tamooz 3, and Uruk did not germinate at -2.0 MPa, Latyfia showed better germination (76.66%), higher speed of emergence (81.93%), seedling vigor index (5.70), seedling length (7.50 cm), root and seedling fresh weight (0.0450 and 0.0650 g) and root and seedling dry weight (0.0312 and 0.0411 g). Iba99 exhibited the lowest values in the majority of traits at -2.0 MPa. In conclusion, each genotype behaved differently in response to salinity, drought, or levels of combined stresses during germination. The genotype Latifya performed better in the study compared to the other genotypes because it was better able to endure the effects of these stresses. Consequently, it can be utilized as breeding stock to increase the wheat crop's tolerance to salinity and drought. The study's findings increase knowledge of how plants react to challenges like salt, drought, and both in combination.
Keywords: Combined stress(D+S), Germination traits, Seedling growth traits.

1. Introduction
Climate change, environmental constraints, especially drought, excessive soil salinity, temperature changes, and UV radiation are becoming more and more prevalent and are now the most important factors that significantly lower crop productivity. This presents a

significant challenge to the development and production of plants (1). Both stresses, drought and salinity, have the potential to influence morphological, physiological, molecular, biochemical, and metabolic processes through a variety of mechanisms, ultimately impacting plant growth, development, and production. The reactions of plants to these stresses are extremely complicated and influenced by a variety of other variables, including the species, genotype, age, size, and pace of development of the plant, in addition to the intensity and duration of the stresses. According to (2) and (3), these variables have a significant impact on plant response and control whether adaptation-related mitigation activities take place.

A series of biological and biochemical events are triggered by the seedling's germination, which is a physiological process that begins and develops the seedling (4). Rapid water absorption by seeds during the early stages of germination, known as imbibition, causes the seed coat to stretch and become flexible at the right temperatures. This internal activation triggers the seed to start breathing (5). Finally, the cracked seed coatings enable the development of radicles and plumula. Water participates in the subsequent germination metabolic stages (6), which include the restoration of vital activities like enzyme activation, transcription, translation, and DNA repair, followed by cell elongation and division (7, 8). According to (9), wheat is particularly susceptible to drought during the germination and early seedling phases, which lowers the germination percentage (10).

Germination, emergence, and early seedling growth are the three primary stages of wheat crop establishment that are most sensitive to salinity; for example, (11) reported that germination and seedling growth are crucial stages in a plant's life cycle. For crop plants to grow from seeds to seedlings, there must be a crucial precursor phase. Low moisture availability during seed germination of wheat crops is a problem in semiarid regions of the world (12). Excessive soil salinity can significantly inhibit seed germination and seedling growth due to the combined effects of high osmotic potential and specific ion toxicity (13), but mean germination time increased with lowering osmotic levels (14). Salinity is a significant impediment in Iraq's central and southern areas. In the seedling phase, combined stresses are more lethal than any type of single stress; according to several studies (15) and (16), seed germination and seedling establishment phases are susceptible to all types of stresses under either single or combined conditions. 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 (17). The goal of the current study was to test the seed viability of selected wheat genotypes in combined salt and drought stress conditions and determine the most tolerant and sensitive genotypes at the germination stage and early seedling growth.

2. Materials and Methods

2.1. Plant Material

Seeds of 16 genotypes of wheat were acquired from the Ministry of Agriculture/Directorate of Seed Examination and Certification–Iraq. The selected seeds carry official certificates, and they have adapted to the environmental conditions of Iraq for many years. They have been cultivated in both the central and southern parts of Iraq. The ability of these genotypes to tolerate combined drought and stress has not yet been tested. Therefore, the study utilized the maximum amount of germplasm to identify new sources of stress tolerance.

2.2. Sterilization

Seeds were prepared to obtain uniform growth. Surface sterilization of seeds was done by washing with 70% ethanol (v/v) for one minute, followed with sodium hypochlorite 3% (v/v) for five minutes, and washed twice with sterile distilled water (18). We thoroughly cleaned and sterilized the petri dishes in an oven at 70°C for 36 hours, and we sterilized the Whatman filter paper for 24 hours at the same temperature (19).

2.3. Experimental Design and Treatments

The experiment was a combination of 16 wheat genotypes and osmotic levels (combined NaCl and PEG) with five levels (0, -0.5, -1.0, -1.5, -2.0 MPa). The experiment was a factorial in completely randomized block design (RCBD). We replicated the experiment thrice, using 50 seeds per replicate for each treatment. Polyethylene glycol, with a molecular weight of 6000 (PEG-6000), induced drought stress. We prepared four levels, each with a different osmotic potential of -0.5, -1.0, -1.5, and -2.0 MPa, following the instructions from (20).

$\Psi s = -(1.18\text{x}10^{-2})\, C - (1.18\ \text{x}10^{-4})\, C^2 + (2.67\text{x}10^{-4})\, CT + (8.39\text{X}\ 10^{-7})\, C^2T$ Where:

Ψs = osmotic potential (*MPa*)

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

T= temperature (^{o}C).

Salt stress, with the same osmotic potentials (from -0.5 to -2.0 MPa), was modulated using NaCl, according to (15), in order to get the optimal concentration. Distilled water was used as a control (**Table 1**).

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

2.4. Growing Conditions

The screening of stress-tolerant wheat genotypes based on seed germination and seedling growth tests was carried out. We allowed 25 uniformly sized seeds from each of the 16 genotypes to germinate in 10 mm Petri dishes on filter paper, moistened with 10 ml of appropriate stress levels (combined NaCl and PEG-6000) and distilled water for the respective experimental and control treatments, respectively (21). The Petri dishes were labeled and were covered in order to prevent moisture loss by evaporation. The seeds were checked for germination every day, and the germination was continued until the 15th day. A seed is considered to have germinated when its radicle emerges to a length of 1-2 mm (9).

2.5. Sampling and Measurement

The number of germinated seeds was counted 15 days after sowing in each petri dish of the testing treatment, and the mean was expressed in percentage. The germination percentage is calculated using the following equation (22):

Germination Percentage (%)= (number of germinated seeds (n)/ total number of seeds (N))*100

The following formula was used for calculating the speed of emergence (23):

Speed of Emergence = ((number of seedlings emerged 5 days after sowing / number of seedlings emerged 15 days after sowing) × 100)

The Seedling Vigour Index was calculated by multiplying the totality of seedling dry matter (shoot and root dry matter) with germination percentage as below (24):

Seedling Vigour index = Germination (%) * Seedling dry matter (g).

After 15 days of putting seeds in the Petri dishes, the length of shoot was measured (the distances from crown to leaf tip) in each of the seedlings by a ruler, and the mean value was expressed in centimeters (cm). Fresh weight of shoot, root, and whole seedling were weighed immediately using a sensitive balance to obtain their wet weights from each Petri dish, and the average was recorded. These seedling parts were dried at 70°C for 48 h in an oven (to a constant weight), then they were cooled in a dry environment; after that, dry weights of shoot and root were measured in grams by using a sensitive balance, and the average was recorded (25, 26). The temperature was 25°C, and the moisture content was approximately 60–70%.

2.6. Statistical Analysis

The data obtained from the laboratory experiment were subjected to ANOVA using the Statistical Analysis System (SAS) program (27) according to the Randomized Completely Block Design (RCBD) with three replicates, and the Least Significant Difference (LSD) test was applied at 0.05 and 0.01 probability levels to compare the differences among treatment means.

3. Results

3.1. Germination traits

The performance of various germination parameters of wheat genotypes under drought and salt stress is representative of the stress tolerance of wheat genotypes at germination and seedling stages. The results in **Table (1)** show that there are big differences ($P \leq 0.01$) between the stress potentials of 0 to -2.0 MPa for all the traits that were studied when salt and PEG stress are added together. In this study, we observed a gradual decrease in the germination percentage of wheat, with a wide degree of variation from 90.04 to 20.18%, and a decrease in osmotic potentials starting with 0 to -2.0 MPa stress levels. We observed a marked decrease in the germination percent at the -1.0 and -1.5 MPa stress levels compared to the control treatment, which was 50.55 and 37.87%, respectively. We noted the lowest percent (20.18%) at the -2.0 MPa stress level (**Table 2**).

We verified that the emergence speed markedly decreased at the -1.0 and -1.5 MPa stress levels compared to the control treatment, with emergence speeds of 45.30% and 36.91%, respectively. Overall, emergence speed was lowest (24.95%) in the presence of the lowest osmotic potential (-2.0 MPa). The control treatment (0 MPa) recorded the highest emergence speed values (71.16%) (**Table 1**). There were significant differences ($P \leq 0.01$) between control and stress treatments, except for the osmotic potential of −0.5 MPa for the seedling vigor index. Compared with the control, the -1.0, -1.5, and -2.0 MPa treatments decreased the seedling vigor index by 57.31%, 77.57%, and 88.60%, respectively. The control (0 MPa) recorded a high seedling vigor index of 6.02, while the treatment -2.0 MPa recorded a low seedling vigor index of 0.686 (**Table 2**).

Table 2. Germination traits recorded after 15days of exposed to combined.

Stress(*Mpa*)	Germination traits		
	Germination (%)	**Speed of emergence (%)**	**Seedling vigor index**
0	90.04	71.16	6.02
- 0. 5	83.08	63.81	4.80
- 1.0	50.55	45.30	2.57
- 1.5	37.85	36.91	1.35
- 2.o	20.18	24.95	0.686
LSD	6.08 **	6.08 **	2.10 **

** ($P \leq 0.01$)

Among the 16 genotypes, Latyfia and Fatah resulted in better germination (79.00 and 72.67%) with significant performances than other genotypes. The genotypes Babil and Tamooz3 were more affected and exhibited the lowest germination percentages of 28.00 and

30.66%. On the basis of germination results, it can be divided into three categories: high germination, medium germination, and low germination (**Table 3**).

Results from this study showed significant differences among diverse wheat genotypes. The Fatah and Latyfia genotypes exhibited the most speed of emergence (64.26% and 71.07%), and the lowest speed of emergence was ranged between 25.84% and 37.34% for the Tamooz3, Babil, Uruk, Buhooth158, and Hashimia genotypes, whereas around 42.9% and 59.85% for the rest of the genotypes (**Table 2**). Seedling vigor index significantly differed among the genotypes and exhibited variation from 1.61 to 5.19. The Latyfia genotype of 5.19 exhibited a high vigor index. Other genotypes exhibited seedling vigor index ranges between 2.20 and 3.96. Whereas the lowest seedling vigor index of 1.61 and 1.90 was recorded by genotypes Babil and Tamooz3 (**Table 3**).

Table 3. Germination traits of studied wheat genotypes.

Genotypes	Germination traits		
	Germination (%)	**Speed of emergence**	**Seedling vigor index**
Babil	28.00	26.33	1.61
Hashimia	60.00	37.34	3.80
Iba 99	55.31	48.74	3.17
Baraka	49.27	42.90	3.08
Sham 6	62.62	51.24	3.28
Fatah	72.67	64.26	3.11
Dijlah	61.31	59.85	3.11
Furat	55.32	57.60	3.87
Abu Graib	64.80	59.49	3.75
Buhooth 22	57.84	49.70	2.20
Sabah	62.65	58.16	3.96
Latifya	79.00	71.07	5.19
Mexipak	62.63	51.30	2.07
Buhooth 158	46.00	36.33	2.09
Tamooz 3	30.66	25.84	1.90
Uruk	53.33	34.56	3.19
LSD	10.88**	10.84 **	0.881 **

** ($P \leq 0.01$)

It was observed that interactive effects of genotypes and stress (combined PEG-induced drought stress and salt) significantly *($P \leq 0.01$)* decreased germination percentage in all of the cultivars with stress increment. There were significant differences between genotypes for germination percentage. Germination percentage of all cultivars significantly decreased when they were subjected to stress of -1.0 *MPa* and below, but no significant changes were observed at the stress of -0.5 *MPa*. Retardations of germination percentage were clearer in all genotypes, especially at -2.0 *MPa*, with the exceptions of Abu Graib and Latyfia, which gave 66.67% and 76.66%, respectively, while the genotypes Babil, Hashimia, Baraka, Buhooth 158, Tamooz 3, and Uruk failed to germinate (**Figure 1**).

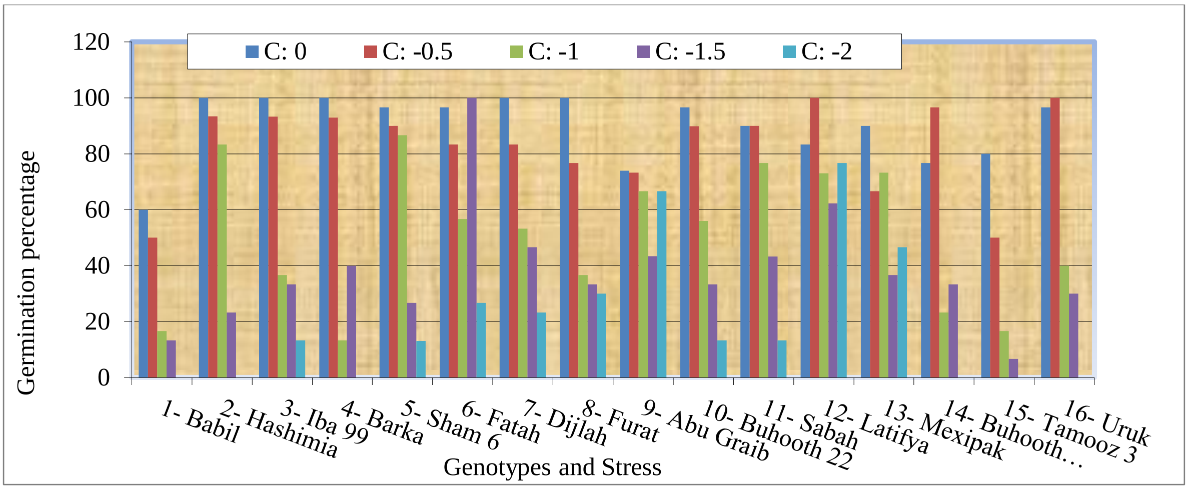

Figure 1. Effect of combined drought and salinity stresses on germination(%) of 16 wheat genotypes, LSD=24.34.

Except for Abu Graib (67.00%) and Latyfia (81.93%), which surprisingly showed an increase compared to all other treatments, the decrease and delay in speed of emergence increased at -1.0 MPa and reached their maximum at -2.0 MPa. There was also no speed of emergence for genotypes Babil, Hashimia, Baraka, Buhooth 158, Tamooz 3, and Uruk because they did not germinate at -2.0 MPa (**Figure 2**).

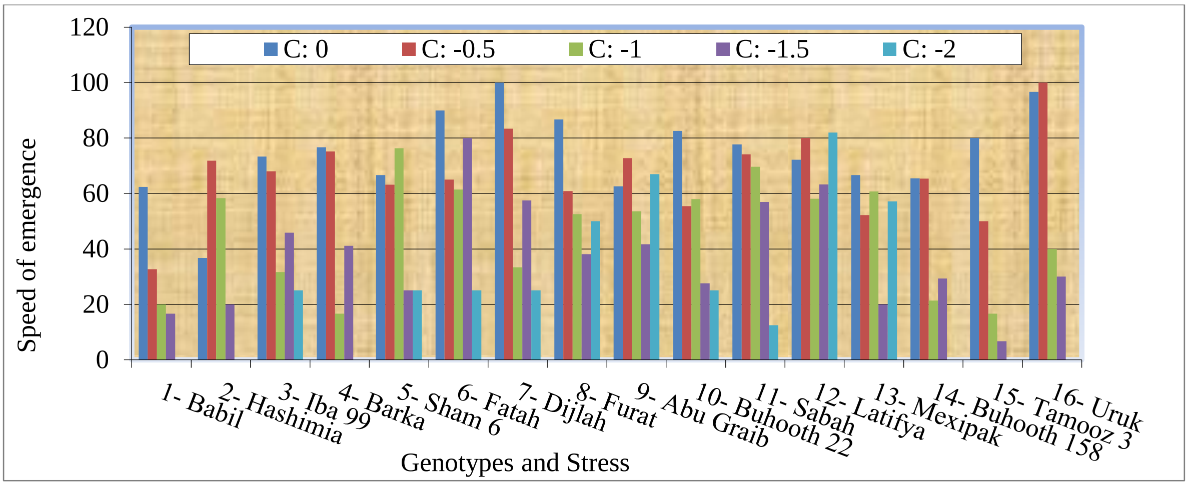

Figure 2. Effect combined drought and salinity stresses on speed of emergence, LSD=24.24

The vigor index of seedlings significantly decreased as the osmotic potential decreased to -1.0 and significantly decreased at -1.5 and -2.0 MPa in most genotypes, except for the osmotic potential of -0.5 MPa. This osmotic potential did not significantly differ from the control, except for genotype Tamooz3 (2.19), which showed a significant decrease from the control (7.08) (**Figure 3**). The Latyfia genotype showed a high vigor index of 8.5, while Tamooz3 showed the lowest at -0.5 MPa. Interestingly, -2.0 *Mpa* exhibited a seedling vigor index of 5.70 and did not differ significantly from the control (6.03). At -2.0 MPa, out of 16 genotypes, 6 showed no seedling vigor index; as for the rest of the genotypes, they showed little vigor index ranging from 0.10 to 1.76 (**Figure 3**).

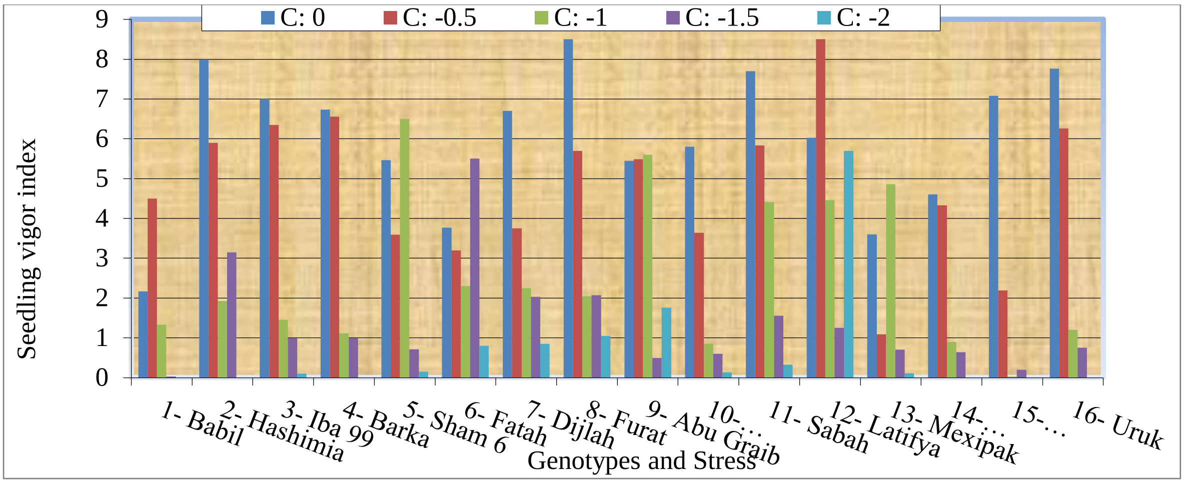

Figure 3. Effect combined drought and salinity stresses on seedling vigor index of 16 wheat genotypes, LSD=4.41

3.2. Seedling Growth Traits

Water and salt stress play a vital role in seedling growth. The seedling length of the genotypes ranged from 1.67 to 6.62 cm. When applying combined drought and salt, significant differences *($P \leq 0.01$) were shown* between control and stress treatments, which decreased gradually by 18.58, 42.75, 67.82, and 74.77% with the decrease of the osmotic potential to -0.5, -1.0, -1.5, and -2.0 *MPa*, respectively (**Table 4**). Other parameters are the weights of seedlings, which can evaluate seed vigor and seedling stress tolerance. There were significant differences among stress treatments (**Table 4**). It was observed that shoot, root, and whole plant fresh weight were affected considerably due to stress treatments (combined PEG and salt solutions) increment, by 45.45, 71.12, 83.33, and 93.33% for shoot fresh weight; 36.72, 63.99, 67.56, and 82.00% for root fresh weight; and 39.12, 66.67, 72.62, and 86.93% for whole plant fresh weight compared to control (**Table 4).**

Table 4. Growth traits recorded after 15days of exposed to combined stress

Stress (*Mpa*)	Growth traits						
	Seedling Length (cm)	**Shoot fresh weight (g)**	**Shoot dry weight (g)**	**Root fresh weight (g)**	**Root dry weight (g)**	**Seedling fresh weight (g)**	**Seedling dry weight (g)**
0	6.620	0.066	0.0362	0.0561	0.0344	0.1209	0.0697
-0. 5	5.390	0.036	0.0186	0.0355	0.0202	0.0736	0.0389
-1.0	3.790	0.019	0.0085	0.0202	0.0132	0.0403	0.0213
-1.5	2.130	0.011	0.0039	0.0182	0.0080	0.0331	0.0116
-2.0	1.670	0.0044	0.0022	0.0101	0.0048	0.0158	0.0070
LSD	0.254**	0.0064 **	0.0045**	0.0038**	0.0049**	0.0068**	0.0069**

** ($P \leq 0.01$)

The seedling length of the genotypes ranged from 2.68 to 6.16 cm, with significant differences *($P \leq 0.01$)*. The Latyfia genotype had the longest seedlings (6.16 cm), followed by the Furat genotype (6.06 cm) and the AbuGraib genotype (5.08 cm). The Buhooth22 and Tamooz3 genotypes recorded the lowest seedling lengths, measuring 2.68 and 2.75 cm, respectively. The lengths of other genotypes ranged between them (**Table 5**). There were significant differences between control and stress treatments for dry weights of seedlings, which showed the same decreasing trends for fresh weights of seedlings. The shoot fresh weight of the given data ranged from 0.0165 to 0.0536 g. The shoot fresh weight showed that the genotypes Latyfia (0.0490 g) and Sabah (0.0536 g) were the highest, and the genotypes

Tamooz3 and Babil showed the lowest values of 0.0165 and 0.0172 g (**Table 5**). As described in Table 4, dry weights of seedlings varied significantly ($P \leq 0.01$) between studied genotypes. The values ranged from 0.0061 to 0.0311 g for the shoots, 0.0077 to 0.0268 g for the roots, and from 0.0158 to 0.0579 g for the entire plant. Also, the genotypes Latyfia and Sabah had maximum mean values of dry weight (0.0200 and 0.0311 g) for shoots, (0.0268 and 0.0246 g) for roots, and (0.0446 and 0.0579 g) for the whole plant. Among all the genotypes, Babil and Ipa99 showed the lowest dry weight (0.0061 and 0.0081 g) for shoots, and Ipa 99 and Hashimia (0.0077 and 0.0085 g) for roots, and Ipa99 and Tamooz3 (0.0158 and 0.0187 g) for the whole plant (**Table 5**).

Table 5. seedling growth traits of studied wheat genotypes.

Genotypes	Growth traits						
	Shoot Length (cm)	**Shoot fresh weight (g)**	**Shoot dry weight (g)**	**Root fresh weight (g)**	**Root dry weight (g)**	**Seedling fresh weight (g)**	**Seedling dry weight (g)**
Babil	3.07	0.0172	0.0061	0.0102	0.0150	0.0274	0.0211
Hashimia	4.39	0.0269	0.0107	0.0232	0.0085	0.0501	0.0192
Iba 99	3.88	0.0211	0.0081	0.0190	0.0077	0.0401	0.0158
Baraka	3.36	0.0282	0.0144	0.0263	0.0142	0.0545	0.0286
Sham 6	4.08	0.0198	0.0099	0.0350	0.0163	0.0548	0.0253
Fatah	3.74	0.0221	0.0108	0.0259	0.0120	0.0480	0.0228
Dijlah	4.04	0.0288	0.0175	0.0359	0.0228	0.0647	0.0403
Furat	6.06	0.0293	0.0146	0.0333	0.0174	0.0626	0.0320
Abu Graib	5.08	0.0349	0.0168	0.0347	0.0173	0.0696	0.0341
Buhooth 22	2.68	0.0232	0.0135	0.0280	0.0157	0.0512	0.0292
Sabah	4.33	0.0536	0.0311	0.0520	0.0268	0.1056	0.0579
Latifya	6.16	0.0490	0.0200	0.0423	0.0246	0.0913	0.0446
Mexipak	2.82	0.0215	0.0103	0.0258	0.0168	0.0473	0.0271
Buhooth 158	2.88	0.0298	0.0152	0.0247	0.0162	0.0545	0.0314
Tamooz 3	2.75	0.0165	0.0097	0.0157	0.0090	0.0322	0.0187
Uruk	3.42	0.0238	0.0143	0.0266	0.0174	0.0504	0.0317
LSD	0.937 **	0.0064 **	0.0081**	0.0068**	0.0087**	0.012**	0.0124**

** ($P \leq 0.01$)

Interestingly, the lengths of Babil, Sham6, Abu Graib, and Mexipak at -1.0 MPa were longer than at control. There was a sharp and significant decrease in the length of all genotypes when the osmotic potential was decreased to -1.5 and -2.0 MPa, except for the Fatah genotype at -1.5 MPa, which increased significantly to 6.5 cm compared to the control (3.90 cm), and of Latifya (7.50 cm) at -2.0, which was at par with the control (7.30 cm), whereas the genotypes Baraka, Buhooth158, Tamooz3, and Uruk died at -2.0*Mpa* (**Figure 4**). The genotype Latyfia experienced a higher reduction in shoot fresh weight at -1.5 and -2.0 MPa, compared to 0.0070 g at -1.5 MPa. Of 16 genotypes, 5 died at -2.0 MPa (**Figure 5**). The genotypes under study exhibit significant genetic variability. Shoot dry weight dropped sharply when the stress level dropped below -0.5 MPa, especially when it dropped below -2.0 MPa. However, it is interesting that the genotypes of Dijlah, Furat, and Latyfia gave higher weights (0.0020, 0.0051, and 0.0099 g) than when the stress level was -1.5 MPa (0.0009, 0.0033, and 0.0018 g), in that order. Six genotypes were unable to endure and ultimately perished (**Figure 6**).

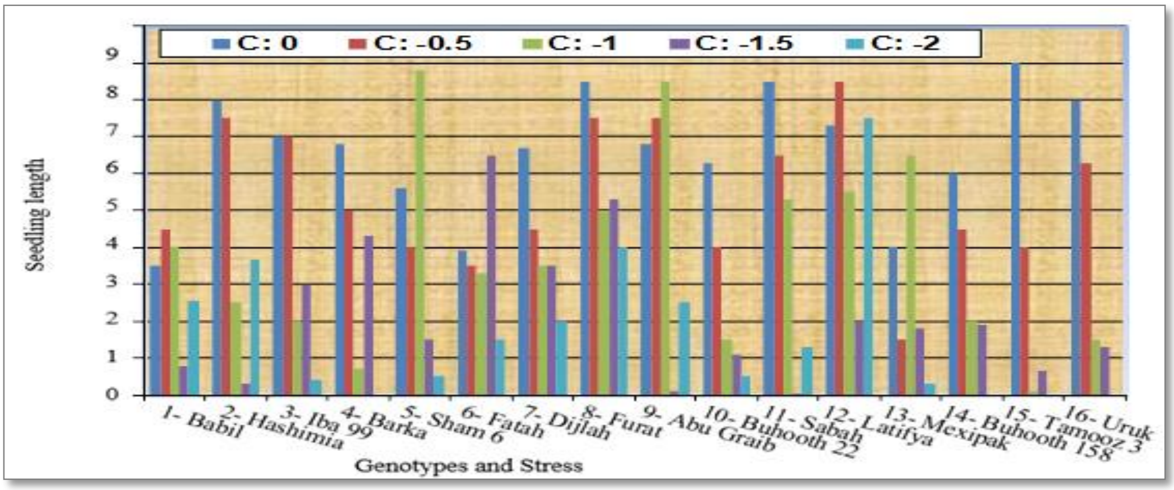

Figure 4. Effect of combined drought and salinity stresses on seedling length of 16 wheat genotypes, LSD=2.09

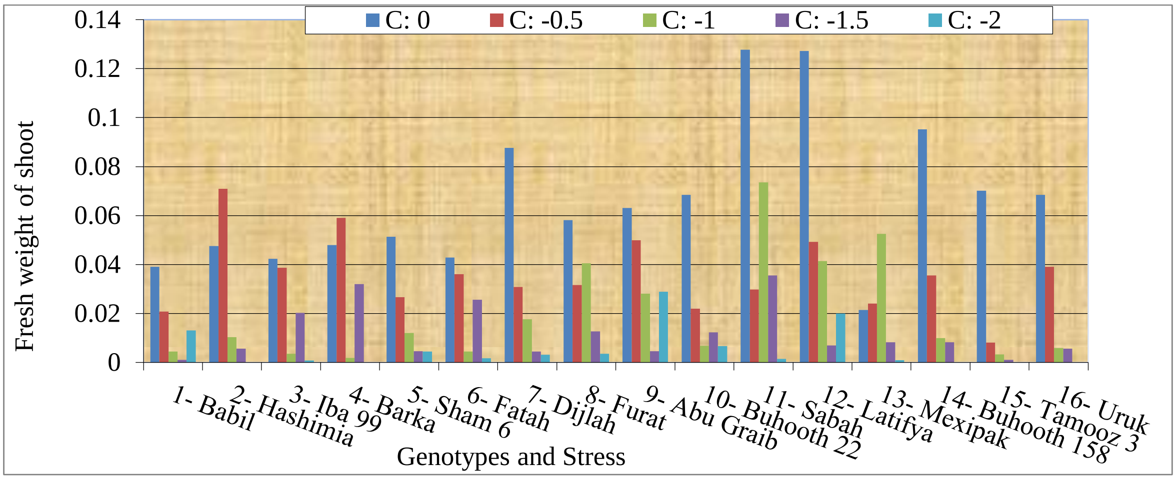

Figure 5. Effect of combined drought and salinity stresses on shoot fresh weight of 16 wheat genotypes, LSD=0.014.

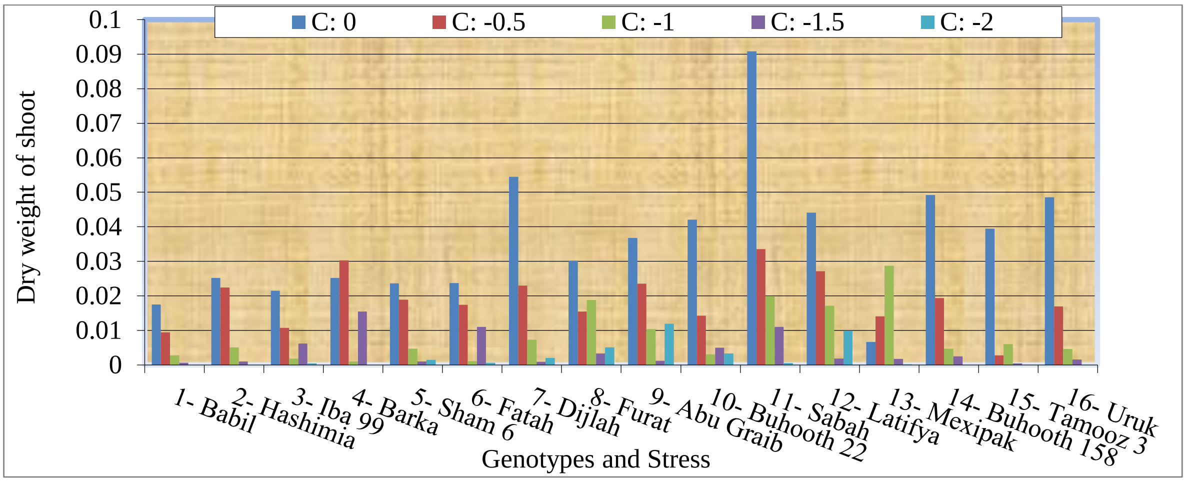

Figure 6. Effect of combined drought and salinity stresses on shoot dry weight of 16 wheat genotypes, LSD=0.018

All genotypes saw a sharp decrease in root fresh weight at -1.5 and -2.0 MPa, with the exception of the genotype Fatah (0.0426 g) at -1.5 MPa. However, it's noteworthy that the genotype Latyfia experienced an increase to 0.0450 g, surpassing all other stress levels. Simultaneously, six genotypes perished (**Figure** 7). The Latvian genotypes were more tolerant. A strong drop in root growth is seen when the osmotic potential makes roots less able to absorb water, especially when the pressure is -2.0 MPa. However, it is interesting to note that the genotype Latyfia produced root dry mass (0.0312 g) that was similar to control (0.0385 g). While some genotypes experienced a significant decline in the dry weight of their roots, six genotypes struggled to grow and ultimately perished without producing any dry weight (**Figure 8**).

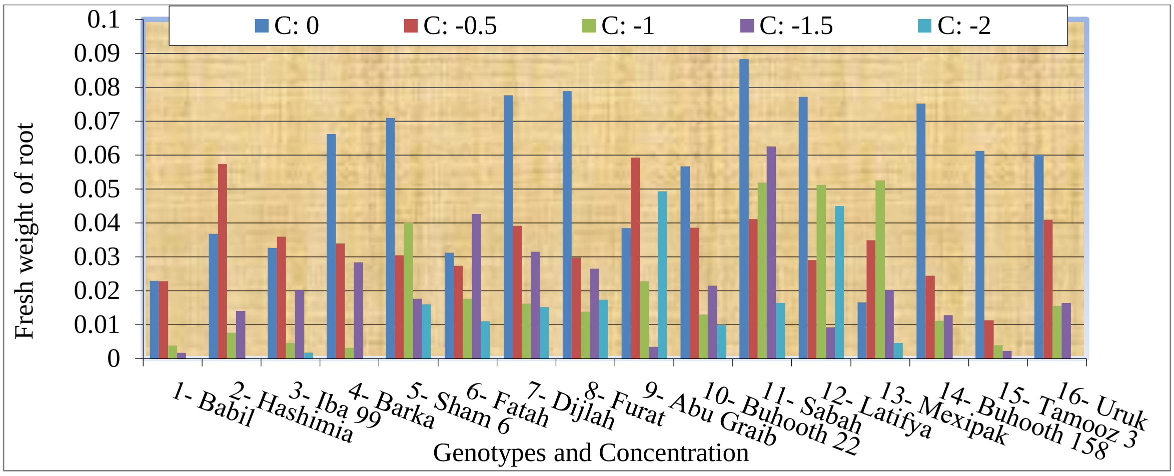

Figure 7. Effect of combined drought and salinity stresses on root fresh weight of 16 wheat genotypes, LSD=0.015.

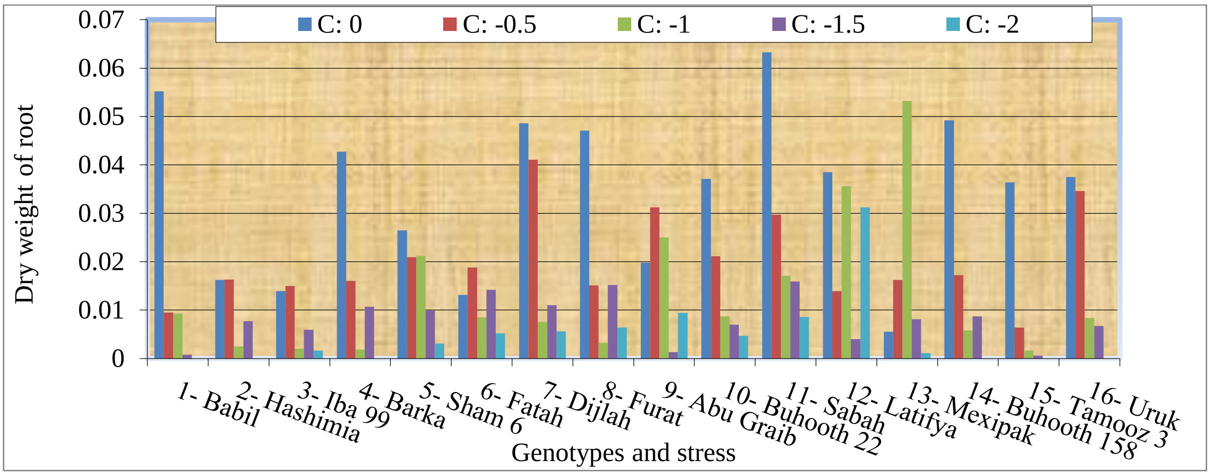

Figure 8. Effect of combined drought and salinity stresses on root dry weight of 16 wheat genotypes, LSD=0.019.

The total seedling weight is the product of dry matter accumulation in the root and shoot, and thus it is affected by the pattern of their response to stress. It is noted that the fresh seedling weight decreases with increasing stress to -0.5 and -1.0 MPa, except for the genotypes Hashimia, Abu Graib, and Mexipak at -0.5 MPa, and Sabah and Mexipak at -1.0 MPa; then there was an increase in the weights of most genotypes at -1.5 MPa compared to -1.0 MPa, followed by a strong decrease in the weights of most genotypes at -2.0 *MPa*, with the exception of the two genotypes Latyfia (0.0650 g) and Abu Graib (0.0782 g), which regained some of their weight compared to their weights (0.0162 and 0.0081 g) at -1.5 MPa, while 6 genotypes died (**Figure 9**), which appear to be the most sensitive to stress. Increased stress caused a substantial decrease in dry weight. The highest reduction was observed in genotypes Babil, Hashimia, Baraka, Buhooth158, Tamooz3, and Uruk, which eventually died at the high level of 2.0 MPa with no production of dry matter. The outcomes of the current study exhibited that Latyfia genotype was tolerant ,since it shows the enhancement of dry matter (0.0411g) that very close to the dry matter (0.0410g) at -0.5*Mpa* (**Figure 10**), indicating the stress adaptation mechanism of this genotype to increase seedling dry weight.

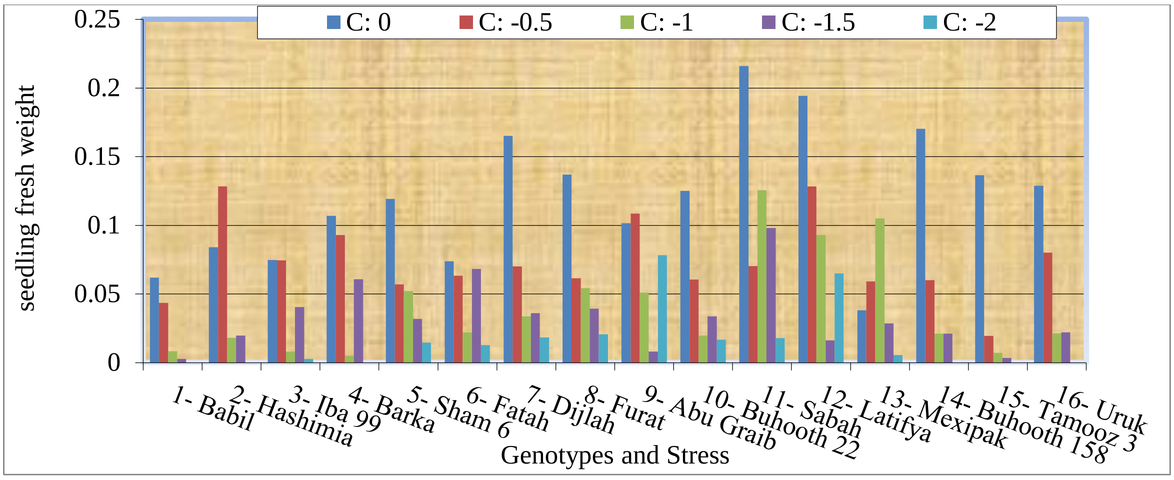

Figure 9. Effect of combined drought and salinity stresses on seedling fresh weight of 16 wheat genotypes, LSD=0.027.

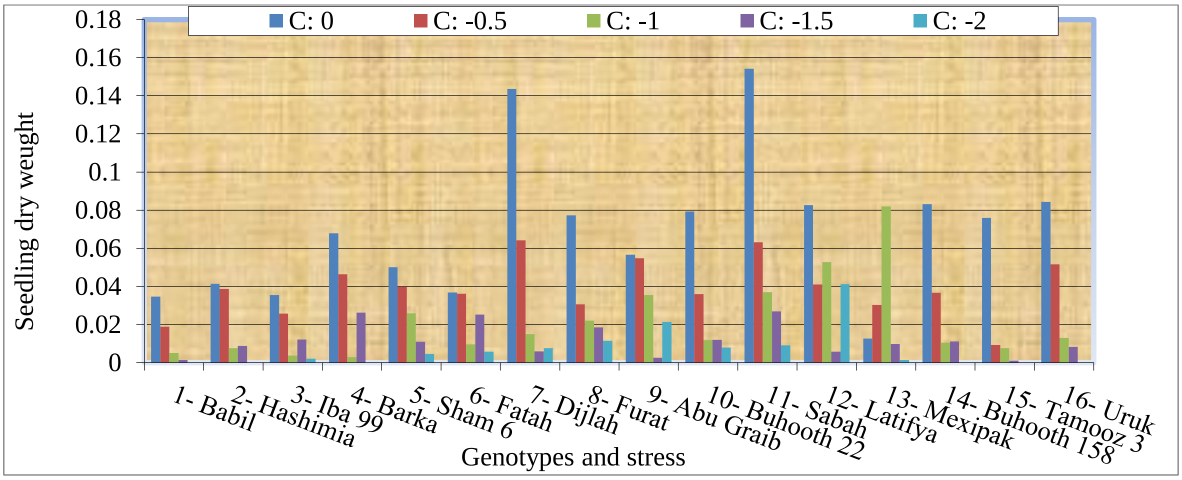

Figure 10. Effect of combined drought and salinity stresses on seedling dry weight of 16 wheat genotypes, LSD=0.0277.

4. Discussion

4.1. Germination Traits

Crop plant seed germination is a crucial transitional stage from seeds to seedlings, and high seed germination under demanding and favorable environmental conditions aids plant growth and increases yield (28). One of the most obvious seed quality and performance tests is the germination test, which is always connected to vigor tests, such as the seedling growth test (29). With regard to germination traits, there were significant variations *(P0.01)* between stress levels (**Table 2**), genotypes (**Table 3**), and their interactions (**Figures 1** to **3**). The tolerance to stress (combined PEG-induced drought stress and NaCl salt) varied significantly between genotypes. According to previous studies (9, 30, 31), the highest reduction in seed germination and growth features was observed under the -2.0 MPa osmotic potential level compared to the control treatment. The seedbed environment and genetic potential of the crop plants govern seed germination. As the level of osmotic stress increased, it was discovered that the genotypes under study experienced decreased germination. Under the maximum level of osmotic stress (-2.0*Mpa*), germination was entirely prevented in some genotypes. Wheat genotypes responded differently to treatments for osmotic stress, as evidenced by significant interactions (**Figures 1** to **3**). As a result, there is a better possibility to choose cultivars that are tolerant of drought at the germination stage (32). In the current investigation, the highest germination % was found at treatments of 0.0 and -0.5 *MPa*, while the lowest was found at a stress level of -2.0 *MPa* (**Table 2**). High viscosity, where oxygen solubility and diffusion were decreased in comparison to the control (free water), may be the cause of this significant decline in germination rate (33). The reduced seed germination at higher negative osmotic potential (-2.0*Mpa*) in the current study may be related to lower water imbibitions and subsequently reduced enzyme activities necessary for seed germination. Lower imbibitions of water were cited by (9) as another significant factor in poor seed germination and the failure to meet the seeds' moisture requirements. The genotypes with the highest germination percentage at zero potential, -0.5, -1.0, -1.5, and -2.0 *MPa* were Latifya and AbuGraib. Therefore, these genotypes could be grown under decreased osmotic conditions.

According to the study's findings (**Table 2**), negative osmotic potential (NaCl and PEG) indicated a detrimental impact on germination traits. Generally, the presence of the lowest osmotic potential (-2.0*MPa*) resulted in the lowest germination percentage, speed of emergence, and seedling vigour index. Furthermore, under various stress levels, there were discernible differences between several wheat genotypes (**Figures 1** to **3**). The most susceptible genotypes were Babil, Hashimia, Buhooth158, Tamooz3, and Uruk at the lowest osmotic potential (-2.0 *MPa*). Vigor decreased gradually as osmotic potential decreased from 0 to -1.0 *MPa* before reaching an extreme level at -2.0 *MPa*. It was found that an osmotic potential of -2.0 MPa could affect most types of wheat, especially the Babil, Hashimia, Baraka, Buhooth128, Tamooz3, and Uruk types. Surprisingly, Latifya showed the highest vigor at -2.0 *MPa*, which did not differ from 0*Mpa*. In their investigations, (34) and (35) discovered varying levels of sensitivity in wheat genotypes based on seedling growth, and they concluded that this trait is crucial for determining a plant's susceptibility to stress in the early stages of development, where the seedling vigour index regards the ability to produce dry matter and promise for better seedling growth and completion of their life cycle.

In general, most wheat genotypes were sensitive to the impact of osmotic potential of -2.0 *MPa*, and some genotypes their values drastically fall to zero, except for Latifya genotype which not affected compared to 0*Mpa* (fresh water). (35) also discovered different susceptibilities in wheat genotypes based on seedling growth, and they hypothesized that seedling growth is one of the most crucial traits for screening stress tolerance during early growth during osmoconditioning that allows seeds to develop a higher germination potential (radical thrusting) or the capacity to remove the seed coat obstruction (36). This could potentially explain the enhancement of the Latifya genotype's germination and emergence rate in the current study. It's possible that the moisture deficit in the seeds below the critical level for germination is the cause of the reduced germination observed in other genotypes tested at -1.0 MPa osmotic potential and below.

4.2. Seedling Growth Traits

NaCl and PEG-induced water deficit had an impact on the outcomes of shoot length. Shoot length values were highest at zero potential. The seedling growth was gradually slowed down by all other treatments (**Table 4**). These results are corroborated by (37), who found that the length of the seedlings reduced as the stress level increased. By varying the intensity of stress conditions, wheat genotypes considerably varied in shoot length (**Figure 4**). Stress severely shortens seedlings, and at the beginning of the ideal range (at 0*Mpa*), it begins to increase dramatically. With increased stress, genotypes' shoot lengths fell in varying degrees. Mexipak genotype demonstrated the maximum sensitivity, while Latifya genotype demonstrated the best tolerance capacity, and the extent of decline was modest in comparison to the other 15 genotypes. At -2.0 MPa, some genotypes perished (**Figure 4**). These results supported what (38) found: when plants were exposed to low osmotic stress, the shoot length of Sarsabz and Anmol cultivars decreased very little, which showed that they could handle more stress. As a result, seedling traits may be useful as excellent indicators for drought tolerance. The majority of genotypes are additionally sensitive to the lowest osmotic potential (-2.0 *MPa*), and several genotypes (Hashimia, Baraka, Buhooth158, Tamooz3, and Uruk) have values that sharply decrease to zero. The current study's findings (**Figure 5**) indicate that stress affected the shoot fresh weight, which aligns with the findings of other authors (37, 39) who also reported a decrease in shoot fresh weight due to stress. In terms of root fresh weight, there was no discernible difference between the control and -0.5 MPa conditions (**Figure 7**). According to (15), the decreased root and shoot development may be linked to the unbalanced seedlings' nutrient uptake as well as the harmful effects of the high level of NaCl concentration.

More biomass was produced by the Latifya genotype growing under combined stress compared to the others, proving that it is the most tolerant. Additionally, previous research indicates that plants can adapt their growth allocation to their roots and shoots in response to adverse circumstances such as salinity and water stress (40). The current findings corroborate previous research, as the Latifya genotype exhibited higher shoot and root biomass under combined stress (**Table 4**), potentially due to the enhanced water absorption capacity of the roots. However, in Babil and Tamooz3, combined stress treatment resulted in a decrease in root biomass. Furthermore, the current investigation showed that, under combined stress, in particular, the Hashimia.Iba99 and Tamooz.3 genotypes had more severe inhibitory effects and showed lower tolerance than the Latifya genotype. The current findings often indicate that the Latifya genotype is more resilient to stress. According to the findings of (26), the PI31 soybean exhibits greater resilience to stress, regardless of whether it encounters salt stress, drought stress, or both.

Stress significantly reduced the shoot and root dry weight at the greater stress level (-2.0 MPa), and **Table (4)** identified significant differences between wheat genotypes. At a stress of -2.0 MPa, the genotypes Latifya and AbuGraib had the highest average dry weights for the shoots and roots. Between control and low stress, there were variations in shoot and root dry weight that were statistically significant $(P \leq 0.01)$. The lowest root dry weight was displayed by genotype Iba99 at -2.0 *MPa* (**Figure 8**). An excellent seedling development and germination rate were achieved in all genotypes under study at a water potential of -0.5 MPa. However, all genotypes displayed a decrease in all examined attributes when the stress was increased to -2.0 *MPa*. When subjected to stress treatments, the genotype Latifya had the highest dry weights for the shoots and roots. Due to the compatibility between the accumulation of dry matter and the physical measurement of seedling growth, dry weight might indicate the development of a seedling (11). Stress-related situations have an impact on a significant attribute of wheat seedlings, which results in a decrease in seedling dry weight (41).

After 15 days at the highest stress level (-2.0 MPa), most of the 16 genotypes are unable to sustain and continue their growth, including shoot length, shoot fresh and dry weight, root fresh and dry weight, and seedling fresh and dry weight (**Figures 4** to **10**). (42) and (43) reported similar findings, indicating that stress significantly reduced the majority of growth parameters, including shoot length, root length, shoot fresh weight, and root fresh weight. Stress treatment significantly reduces the examined seedling characteristics. The majority of the genotypes under investigation were unable to sustain stress levels more than -0.5 MPa and continue to develop. In this study, using the same stress treatment, the Latyfia genotype exhibited higher means for all the traits compared to the other genotypes. The Iba99, Babil, and Tamooz3 genotypes had the lowest means (**Table 5**). As a result, the Latyfia genotype has a high level of stress tolerance, whereas Iba99, Babul, and Tamooz3 have a low level. Similar results were reported by (43), who discovered that the Mahmoudi variety was more resistant than the other studied varieties.

At higher stress levels (-2.0 MPa) and without developed roots, Babul, Hashimia, Buhoot128, Tamooz3, and Uruk all perished. Due to this lack of root development, seedling establishment would be impossible (44). The water potential in which all genotypes under study had successfully germinated seeds and experienced healthy seedling growth was typically -0.5 MPa. All genotypes decreased under the severe stress of -2.0 *MPa*, with the exception of Latifya, which had somewhat positive growth in comparison to some other stress levels. According to the results, selecting any of these seedling features would produce stress-tolerant wheat genotypes. In this context, (38) asserts that selecting for any of these seedling traits will result in seedlings that are tolerant to drought. According to (45), knowledge of seedling growth, including average germination time, coleoptiles length, root length, seedling height, and relative water content (RWC), allows for the selection of genotypes that are tolerant to salinity and drought.

5. Conclusions

As expected, there were significant variations in genotypes' ability to withstand the stress brought on by PEG and NaCl and each genotype behaved differently. Similar to how, in compared to the study's control treatment, seed germination and growth characteristics showed the highest reduction under -2.0MPa osmotic potential level. The genotype Latifya performed better in the study compared to the other genotypes because it was better able to endure the effects of these stresses. Consequently, it can be utilized as breeding stock to increase the wheat crop's tolerance to salinity and drought. The highest amount of osmotic stress, however, completely prevented the germination of the majority of genotypes.

Acknowledgment

Many thanks to the staff of Ministry of Agriculture, Directorate of Seed Examination and Certification– Iraq.

Conflict of Interest

The authors declare that they have no conflicts of interest

Funding

No funding**.**

References

1. Qamer Z, Chaudhary MT, Du X, Hinze L, Azhar MT. Review of oxidative stress and antioxidative defense mechanisms in Gossypium hirsutum L. in response to extreme abiotic conditions. J Cotton Res. 2021;4:9. https://doi.org/10.1186/s42397-021-00086-4
2. Ma Y, Dias MC, Freitas H. Drought and salinity stress responses and microbe-induced tolerance in plants. Front Plant Sci. 2020;11:591911. https://doi.org/10.3389/fpls.2020.591911
3. Wen W, Timmermans J, Chen Q, van Bodegom PM. Monitoring the combined effects of drought and salinity stress on crops using remote sensing. Hydrol Earth Syst Sci. 2022;26:4537–4552. https://doi.org/10.5194/hess-26-4537-2022
4. Poudel R, Finnie S, Rose DJ. Effects of wheat kernel germination time and drying temperature on compositional and end-use properties of the resulting whole wheat flour. J Cereal Sci. 2019;86:33–40.

5. Koornneef M, Bentsink L, Hilhorst H. Seed dormancy and germination. Curr Opin Plant Biol. 2002;5(1):33–36. https://doi.org/10.1016/S1369-5266(01)00219-9
6. Liu Y, Han C, Deng X, Liu D, Liu N, Yan Y. Integrated physiology and proteome analysis of embryo and endosperm highlights complex metabolic networks involved in seed germination in wheat (*Triticum aestivum* L.). J Plant Physiol. 2018;229:63–76. https://doi.org/10.1016/j.jplph.2018.06.011.
7. Shah T, Latif S, Khan H, Munsif F, Nie L. Ascorbic acid priming enhances seed germination and seedling growth of winter wheat under low temperature due to late sowing in Pakistan. Agronomy. 2019;9:757. https://doi.org/10.3390/agronomy9120757.
8. Abido WAE, Zsombik L. Effect of water stress on germination of some Hungarian wheat landraces. Shengtai Xuebao Acta Ecol Sin. 2020;38:422–428. https://doi.org/10.1016/j.chnaes.2018.03.004.
9. Mahpara S, Zainab A, Ullah R, Kausar S, Bilal M, Latif MI. The impact of PEG-induced drought stress on seed germination and seedling growth of different bread wheat (Triticum aestivum L.) genotypes. PLoS One. 2022;17(2):e0262937. https://doi.org/10.1371/journal.pone.0262937
10. Yan M, Xue C, Xiong Y, Meng X, Li B, Shen R. Proteomic dissection of the similar and different responses of wheat to drought, salinity and submergence during seed germination. J Proteomics. 2020;220:103756. https://doi.org/10.1016/j.jprot.2020.103756
11. Khaeim H, Kende Z, Balla I, Gyuricza C, Eser A, Tarnawa Á. The effect of temperature and water stresses on seed germination and seedling growth of wheat (*Triticum aestivum* L.). Sustainability. 2022;14:3887. https://doi.org/10.3390/su14073887.
12. Farooq S, Onen H, Ozaslan C, Baskin CC, Gunal H. Seed germination niche for common ragweed (*Ambrosia artemisiifolia* L.) populations naturalized in Turkey. S Afr J Bot. 2019;123:361–371. https://doi.org/10.1016/j.sajb.2019.03.031.
13. Muhu-Din Ahmed HG, Zeng Y, Shah AN, Majid Yar M, Ullah Z, Ali M. Conferring of drought tolerance in wheat (*Triticum aestivum* L.) genotypes using seedling indices. Front Plant Sci. 2022;13:961049. https://doi.org/10.3389/fpls.2022.961049
14. Alam H, Khattak JZK, Ksiksi TS. Comparative effects of NaCl and polyethylene glycol on seed germination of four native species for landscaping under arid environment. Sarhad J Agric. 2020;36(2):655–657. http://dx.doi.org/10.17582/journal.sja/2020/36.2.655.667.
15. Fellahi ZEA, Zaghdoudi H, Bensaadi H, Boutalbi W, Hannachi A. Assessment of salt stress effect on wheat (*Triticum aestivum* L.) cultivars at seedling stage. Agric Conspect Sci. 2019;84(4):347–355.
16. Al-Temimi AH, Abed ZA. Evaluation the performance and stability of cowpea selected generations under drought tolerance. Iraqi J Agric Sci. 2016;47(3). https://doi.org/10.36103/ijas.v47i3.569.
17. Al-Jobori KM, Al-Waiely MM. Screening of Iraqi wheat (*Triticum aestivum* L.) genotypes against salinity at early growth stage: studies in hydroponic culture. Int J Agric Biol Res. 2017;7(3):524–534.
18. Berger B, de Regt B, Tester M. Trait dissection of salinity tolerance with plant phenomics. In: Shabala S, editor. *Plant Salt Tolerance: Methods and Protocols*. Totowa (NJ): Humana Press; 2012. p. 399–413.
19. Asfaw KG, Danno FI. Effects of salinity on days to heading, days from heading to maturity and days to maturity of tef (*Eragrostis tef* (Zucc.) Trotter) accessions and varieties in Ethiopia. Asian J Agric Sci. 2011;3(4):250–256.
20. Michel BE, Kaufmann MR. The osmotic potential of polyethylene glycol 6000. Plant Physiol. 1973;51(5):914–916. https://doi.org/10.1104/pp.51.5.914.
21. El Harfi M, Hanine H, Rizki H, Latrache H, Nabloussi A. Effect of drought and salt stresses on germination and early seedling growth of different color-seeds sesame (*Sesamum indicum*). Int J Agric Biol. 2016;18:1088–1094.
22. International Seed Testing Association (ISTA). The Germination Test. In: *International Rules for Seed Testing*. Bassersdorf (Switzerland): ISTA; 2015. p. 5-1–5-6.

23. Zewdie T, Welka K. Effect of micropyle orientation on germination of Millettia ferruginea and Delonix regia. Ecol Process. 2015;4(12):1–7. https://doi.org/10.1186/s13717-015-0036-7
24. Gowda B, Naik K, Rakesh A, Mathad C, Raghu BN, Lokesh GY. Seed germination and vigour index as influenced by method of testing in Kabuli chickpea. Indian J Pure Appl Biosci. 2019;7(6):356–361.
25. Nazeer H, Rauf M, Gul H, Yaseen T, Shah AA, Rehman KU. Salt stress affects germination and seedling establishment in different wheat (*Triticum aestivum* L.) varieties. J Pure Appl Agric. 2020;5:42–51.
26. Begum N, Hasanuzzaman M, Li Y, Akhtar K, Zhang C, Zhao T. Seed germination behavior, growth, physiology and antioxidant metabolism of four contrasting cultivars under combined drought and salinity in soybean. Antioxidants. 2022;11:498. https://doi.org/10.3390/antiox11030498
27. SAS Institute. *SAS/STAT User's Guide*. Version 9.1. Cary (NC): SAS Institute Inc.; 2012.
28. Gašparovič K, Živčák M, Brestič M, Hauptvogel P. Diversity of leaf cuticular transpiration and growth traits in field-grown wheat and Aegilops genetic resources. Agronomy. 2021;11:522. https://doi.org/10.3390/agronomy11030522
29. da Silva LJ, de Medeiros AD, Oliveira AMS. SeedCalc, a new automated R software tool for germination and seedling length data processing. J Seed Sci. 2019;41:250–257. https://doi.org/10.1590/2317-1545v42n2217267.
30. Yassin M, El Sabagh A, Mekawy AMM, Islam MS, Hossain M, Barutcular C. Comparative performance of two bread wheat (Triticum aestivum L.) genotypes under salinity stress. Appl Ecol Environ Res. 2019;17:5029–5041. https://doi.org/10.15666/aeer/1703_50295041
31. Awan SA, Khan I, Rizwan M, Zhang X, Brestic M, Khan A. Exogenous abscisic acid and jasmonic acid restrain polyethylene glycol–induced drought by improving the growth and antioxidative enzyme activities in pearl millet. Physiol Plant. 2021;172:809–819. https://doi.org/10.1111/ppl.13391
32. Hefny YAM, Mohamed NE, Abdelraoof AO, Sleem HA. Germination parameters of some bread wheat genotypes under drought stress conditions. SVU Int J Agric Sci. 2020;2(2):226–241. https://doi.org/10.21608/svuijas.2020.40227.1029.
33. Delachiave MEA, de Pinho SZ. Germination of Senna occidentalis Link seeds at different osmotic potential levels. Braz Arch Biol Technol. 2023;46(2):163–166. https://doi.org/10.1590/S1516-89132003000200004.
34. Khatun M, Hafiz MHR, Hasan MA, Hakim MA, Siddiqiui MN. Response of wheat genotypes to salt stress in relation to germination and seedling growth. Int J Bioresour Stress Manag. 2013;4(4):635–640.
35. Jovović M, Tunguz V, Mirosavljević M, Pržulj N. Effect of salinity and drought stress on germination and early seedling growth of bread wheat (*Triticum aestivum* L.). Genetika. 2018;50(1):285–298. https://vaseljena.ues.rs.ba/handle/123456789/544.
36. Khan AA. Preplanting physiological seed conditioning. Hortic Rev. 1993;20:131–181.
37. Bhutto TA, Gola AQ, Bhutto MH, Wahocho SA, Buriro M, Wahocho NA. Evaluation of wheat varieties to salt stress (NaCl) for seed germination and early seedling growth under laboratory conditions. Pure Appl Biol. 2019;8(1):609–618.
38. Baloch MJ, Dunwell J, Khakwani AZ, Dennett M, Jatoi WA, Channa SA. Assessment of wheat cultivars for drought tolerance via osmotic stress imposed at early seedling growth stage. J Agric Res. 2012;50(3):299–310.
39. Pour-Aboghadareh A, Mehrvar MR, Sanjani S, Amini A, Chaman-Abad HN, Asadi A. Effects of salinity stress on seedling biomass, physiochemical properties and grain yield in different breeding wheat genotypes. Res Sq. 2021;1–17. https://doi.org/10.21203/rs.3.rs-258193/v1
40. Yu L, Dong H, Li Z, Han Z, Korpelainen H, Li C. Species-specific responses to drought, salinity and their interactions in Populus euphratica and P. pruinosa seedlings. J Plant Ecol. 2020;13:563–573. https://doi.org/10.1093/jpe/rtz030

41. Ahmed HGM D, Zeng Y, Shah AN, Yar MM, Ullah A, Ali M. Conferring of drought tolerance in wheat (*Triticum aestivum* L.) genotypes using seedling indices. Front Plant Sci. 2022;13:961049. https://doi.org/10.3389/fpls.2022.961049
42. Soni P, Sharma R, Rizwan M, Singh G. Optimization of screening protocol for evaluation of drought tolerance in Vigna aconitifolia using hydroponic system. Indian Res J Genet Biotechnol. 2014;6(4):617–628.
43. Othmani O, Ayed S, Slama-Ayed O, Slim-Amara H, Younes MB. Durum wheat response (*Triticum durum* Desf.) to drought stress under laboratory conditions. IOSR J Agric Vet Sci. 2019;12(2):1–4. https://doi.org/10.9790/2380-1202010104.
44. Coons MJ, Kuehl RO, Simons NR. Tolerance of ten lettuce cultivars to high temperature combined with NaCl during germination. J Am Soc Hortic Sci. 1990;115:1004–1007.
45. Kizilgeci F, Mokhtari NEP, Hossain A. Growth and physiological traits of five bread wheat (*Triticum aestivum* L.) genotypes are influenced by different levels of salinity and drought stress. Fresen Environ Bull. 2020;29(9):8592–8599.