International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 24s, 2025
https://www.theaspd.com/ijes.php

Selection Of Salinity-Tolerant Wheat (*Triticum Aestivum L.*) Lines To Improve Production Yields Under Climate Change

Malek H Wall [1,2*], Fatima Duksi [2], Yassir Dakheel Kremsh Alasadiy [3], Meisam Zargar [1], Zina Al-jubouri [4]

[1]Department of Agrobiotechnology, Institute of Agriculture, RUDN University, 117198 Moscow, Russia.
zargar_m@pfur.ru , https://orcid.org/0000-0002-5208-0861 ,
f.duksi@gmail.com , https://orcid.org/0000-0002-7353-7816,
[2]Department of Biology, College of Nursing, Al-Muthanna University, Samawah 66001, Iraq
malek_h88@yahoo.com * https://orcid.org/0000-0002-4884-6481.
[3]Department of Biology, College of education for pure Sciences, Al-Muthanna University, Samawah 66001, Iraq.
dr.yassiralasadiy@mu.edu.iq, https://orcid.org/0000-0003-2128-7533
[4]Department of Accounts, College of Education for Management and Economics, Al-Muthanna University, Samawah 66001, Iraq. Zinah480@gmail.com , https://orcid.org/0009-0005-6908-5385

Abstract

This study aimed to identify salinity-tolerant wheat genotypes under conditions of salt stress in irrigation water, particularly in regions like Iraq affected by soil and irrigation water salinization. Twenty-three bread wheat (Triticum aestivum L.) genotypes were evaluated under three salinity levels (5, 10, and 15 dS·m⁻¹) in a split-plot design during the two seasons 2022-2024. Morphological (yield per m², biomass, flag leaf area, 1000 grain weight) and physiological traits (chlorophyll content, proline accumulation, Na^+/K^+ ratio) were measured. Molecular analysis involved SSR markers (Xcfd-18 and Xgwm-493) to identify genetic loci associated with salinity tolerance, followed by Sanger sequencing of selected genotypes. Results revealed significant genotypic variation in salinity tolerance. Tolerant genotypes (NF, SA, TB, UD) exhibited minimal yield reduction (12–19%) at high salinity, while sensitive genotypes ones (VN, PB) showed severe declines (up to 77%). Physiological markers, including higher proline content and balanced Na^+/K^+ ratios, correlated with tolerance. SSR markers distinguished tolerant genotypes, and sequencing confirmed homology with stress-resilient (Triticum turgidum durum). Four genomic sequences were deposited in NCBI (PP873642–PP873645). The study demonstrates the potential of integrating phenotypic and genotypic screening for developing salt-tolerant wheat varieties, supporting sustainable agriculture in salinity-affected regions.

INTRODUCTION

Wheat (*Triticum aestivum*) is one of the most important food crops worldwide, providing the world's population with more than 20% of energy (Braun et al. 2010). Soil and water salinization, is taking a huge toll on global agricultural productivity and food security; salinization's economic impact on developing countries by 2050 includes US$7-8 billion in adaptation costs and 10-25% reduced crop yields (Cline 2007; Adger et al. 2003). It may have economic impacts of up to 45% of crop production(Walli and AL-Jubouri 2022) The wheat grain production begins to decline at a salinity level of 6–8 ds/m (Royo and Abió 2003). Iraq is one of the important breeding centres for wheat, and its production rate reached 3,684 tons, yield rate 2,544 kg/ha from 2016 to 2022 (FAO 2022; Walli et al. 2025). Iraq's share of global wheat production is approximately 0.48%, while its contribution to production within the Asian continent stands at around 1.10%, attributable to Iraq's geographical position in Western Asia (FAO 2022). In Iraq, where the productivity of irrigated areas is extremely low with wheat yield estimated at 2100 kg. ha^{-1}(Ray et al. 2013). Salinity rates in central and southern Iraq rise from 10-20 gm/l south of Baghdad, Kut, and Samawah, reaching over 50 gm/l in some areas (Chabuk et al. 2020) 70% of irrigated land in central and southern Iraq is affected by salinity, with 30% lost to production. The diminishing water supply of the Tigris and Euphrates rivers has exacerbated soil and water salinity, necessitating vertical agricultural growth and the development of resistant cultivars (Jha 2019). Wheat genotypes vary significantly in their field and physiological responses to salinity, with salinity-tolerant genotypes generally showing superior performance (Ehtaiwesh and Rashed, n.d.). Genetic engineering and breeding programs for wheat that can withstand high soil salinity produce quantifiable agronomic and financial gains. According to field reports, one study's yield improvements ranged from 1-13% (with an average gain of roughly 4%), while another study's yield increases ranged from 22.5-52.9%. According to one study, the benefit-cost ratio for specific salt-tolerant lines ranges from 2.0 to 3.0 (mean 2.4), while the local interests

rate has a ratio of 1.8 (Meena et al. 2020). Simple sequence repeat (SSR) markers play a crucial role in identifying genetic loci associated with salt and drought tolerance in wheat. SSR markers enable marker-assisted selection (MAS) for salt and drought tolerance (Khalid et al. 2023). SSR markers are crucial in assessing drought and salinity tolerance, enabling breeders to efficiently select for these traits under stress conditions (Shawai et al. 2023). DNA sequencing technologies have revolutionised wheat research and agricultural enhancement (Amiteye 2021; Bidyananda et al. 2024; I. Udoh et al. 2021), enabling the identification of genetic variations associated with important traits (Shaheenuzzamn et al. 2020).
The research aimed to Evaluate Morphological, Physiological and Molecular markers Responses to 23 wheat genotypes under varying salinity levels (5, 10, and 15 dS.m-1) in field experiments. To identify Genetic Loci for Salinity Tolerance, providing insights for marker-assisted selection in breeding programs. To classify Genotypes Based on Tolerance. To contribute to the development of salt-tolerant wheat cultivars to mitigate the adverse effects of soil and water salinization on agricultural productivity, particularly in regions like Iraq where salinity threatens food security. This research aligns with global efforts to address climate change impacts on agriculture by offering practical solutions to sustain wheat production in saline-affected regions.

MATERIALS AND METHODS

The study utilized 23 genotypes of bread wheat (*Triticum aestivum L.*), as listed in **Table 1**. These included 18 genotypes developed through hybridization and irradiation at agricultural research institutions in Iraq, along with additional genotypes sourced from the Gene Bank in Abu Ghraib and other accredited research centers under the Ministry of Agriculture and the College of Agriculture at Al-Muthanna University. The remaining genotypes were obtained from the Russian Federation, France, Italy, Spain, and Turkey. All genotypes belong to the winter wheat type, typically sown from early October to late November and harvested by the end of April.

Table 1. Genetic sources of genotypes involved in the experiment.

	Genotypes	**Sample**	**Pedigree**		**Genotypes**	**Sample**	**Pedigree**
1	Baraka	AB	IARI × STD	13	Baghdad	MB	MX105-6MVLT40 / BNSN
2	Wafia	BW	-	14	Faris	NF	STAR/TR77/773/SLMS
3	Latifiya	CL	Australian breed × Aras	15	Tammuz	OT	Exposing the resulting hybrid (Maxipac x Saber Beek) to radiation
4	Binakal	DB	BISU/3/YAV79/ALOI/ALTARS4/CD93683.7Y.040M-03OY-LPAP.B	16	Buhuth	PB	118//S2/57-S2-CR7-S2
5	Uruk	EU	Inia 66 (Rad) Irradiation of seeds of Enya 66	17	Abaa 95	QA	Veery eer
6	Sham	FS	W-3018-A/JUPATECO-73	18	Abaa 99	RA	Ures/Boww/oowwJup/ Biyiy
7	Fateh	GF	MixPac × Aras	19	Abo ghurayb	SA	Ajeeba × Lian 12 × Mexico24
8	Buhuth 10	HB	Abaa 95 × Abaa 99	20	Buhuth 22	TB	CMSS96Y03236M-050M-040M-020M-050Sy-020sy-IM-0Y
9	Buhuth 158	IB	119-S2/57-S2. Cr7.S2	21	Dujela	UD	8409644HS2-6H
10	Babul 113	JB	MEXIPAK/R23	22	Nemchinovka	VN	
11	Al Iraq	KA	Irradiation of Mexipac seeds with full cobalt 60 doses and 10 kilos rad, Max. (Rad)	23	Abo Raghif	WA	
12	Bwru	LB	H31/Trapf21 / Enesco				

METHODS

Twenty-three wheat genotypes were evaluated under three salinity levels (5, 10, 15 dS·m^{-1}) in a split-plot field design. Morphological, physiological, and yield traits were measured. Molecular analysis used SSR markers (Xcfd-18, Xgwm-493) and Sanger sequencing of selected tolerant genotypes.

Field Study:

Experiment Site: The experiment was conducted on a farm in southern Iraq, located at latitude 45.28 and longitude 31.34, during the 2022–2023 and 2023–2024 growing seasons. The climate of Al-Muthanna is arid, consistent with desert conditions, and is classified as BWh according to the Köppen-Geiger climate classification system (*Climate Data of IRQ* 2023).

Soil analysis and crop service

Samples were taken from field soil at different depths were analyzed, revealing a pH of 8, EC of 3.5 dS.m^{-1}, 0.55 g/kg organic matter and the soil texture (Clay - mixture). Fertilization and weed control were carried out according to scientific recommendations, using DAP fertilizer and urea fertilizer (Ibraheem 2018). Seed rates were 10 g/m^2 and 250-300 seeds/m^2 (Shifa et al. 2021).

Experiment Design

Split-plot design with Randomized Complete Block Design (RCBD), the design used in the experiment. The land was divided into three main blocks (Replicates). Each block contains three main plots (Treatments), and is randomly distributed in the experiment. Three treatments of salt were (T1=5, T2=10, T3=15) ds.m^{-1}, the treatment T1=5 (control) because the salinity of irrigation water from the river ranged from 4-5 ds.m^{-1}. Each main plot was classified to sup-plots representing the genotype (23 genotypes), where the experimental units were distributed randomly in the experiment. The space between blocks was 1.5m and between plots was also 1.5m. The space between one experimental unit and another is 35c. The sup-plot (genotypes) was divided into 69 experimental units for each treatment, 207 experimental units in field experiment. The area of the experimental unit was 1m^2, with dimensions of (1x1m). The seeds were planted on November in 3 lines in the experimental unit, parallel to the irrigation pipes, and the space between one line and another was 20-30c (Rao et al. 2016). The experiment utilized drip irrigation to manage water distribution and prevent leaking into other treatments or experimental units. Three tanks, each with a 2000 liters capacity, were allocated for each treatment, ensuring an organized and controlled water supply. The salinity percentage was measured before the irrigation process using a portable device (HANNA, HI98304 DiST4).

Assessment of Growth and Yield Parameters in Wheat Plants:
Chlorophyll Content, Flag Leaf Area, and Agronomic Traits"

After 100% of the plants in the experimental unit reached the flowering stage, 10 plants were randomly selected from each experimental unit. Chlorophyll content, an indication of the growth status of the crop, was measured after the flag leaf appeared by SPAD meter (Kamarianakis and Panagiotakis 2023). Measure the area of a flag leaf. The following equation calculated the flag leaf area:

Flag Leaf Area (F.L.A) = maximum length of the leaf × maximum width of the middle of the leaf × correction factor (0.95) (Thomas 1975).

After the plants reached full maturity, an area of 0.5 m2 was determined in each experimental unit. The plants were harvested on the April. The following parameters were considered for data collection: Biomass per plant trait, Number of tillers per plant trait, The Number of Spikes per plant trait, Grains Production per square meter trait, Weight of Grains per Spike trait, Weight of 1000 Grains trait, Plant Height Trait was measured from the base of the plant to the spike of the main stem.

Estimation of proline content in flag leaf (µg. g-1 D. Wt.)

The proline content of flag leaves was evaluated according to Bates (1973). 0.5 gram from the dried leaves was homogenized in 10 ml of 3% aqueous sulfosalicylic acid and then filtered through Whathman's No.2 filter paper. 3 ml of filtrate was reacted with 3 ml of ninhydrin acid and 3 ml of glacial acetic acid (1:1:1) in test tubes that were placed in a water bath at 100°C for one hour. The reaction was terminated in an ice bath, and the reaction mixture was extracted with 5 ml of toluene. Proline content in toluene was measured using spectrophotometer at a wavelength of 520 nm. Proline content was determined from a standard curve and calculated according to (Bates et al. 1973) as follows:

Proline = {[(proline in extract x 2)/ 115.5] / (g sample / 5)}

Estimation of Na^+ / K^+ in leaves

For the measurement of Na^+ and K^+ contents, 0.1 g of oven-dried leaf samples was pulverised and subsequently washed with 25 mL of 1 N HCl, as previously outlined by Yoshida et al (1976). The Na^+ / K^+ contents were measured using an atomic absorption spectrophotometer (Model: 170e30, Hitachi, Japan) in accordance with the methodology established by (Hasan et al. 2015). The Na^+ / K^+ ratio at varying salinity levels, based on dry weight, was represented as a percentage for all assessed genotypes.

Statistical Analyses

Analysis of variance (ANOVA) was performed for the split-plot design in three replicates across the two growing seasons since yearly differences were insignificant. Combined ANOVA was performed to analyses the salinity and genotypic differences across the two growing seasons using the Shapiro-Wilk test and Bartlett's test for the normality distribution of the residuals and homogeneity of variances, respectively. The combined analysis indicated homogenous variances across the two growing seasons for different parametric measurements, and therefore, the data of the two growing seasons were combined. Salinity level, genotype group, and their interaction were considered fixed effects. The growing season, replicate, and their interaction were considered random effects. The mean differences among salinity level, genotype group, and their interaction were compared using Fisher's protected Least Significant Difference test (LSD) at a $p \leq 0.05$ significance level according to (Gomez and Gomez 1984). Programs used in data tabulation and statistical analysis: Excel 2019, Statistix 8, and OPSTAT website. In this study, a dendrogram was created based on morphological and physiological traits by using SPSS. Euclidean distances between genotypes were measured based on the standardised data by Ward's method.

Molecular analysis

DNA Extraction

DNA was extracted from fresh, 10-day-old leaves of selected genotypes for testing by the CTAB method with minor modifications(Dellaporta et al. 1983; Porebski et al. 1997). The extraction kit (DNA Extraction Maxi Kit, Plant Genomic) was used, and the extraction method was followed according to Doyle & Doyle (1990) the instructions attached to the extraction kit by FAVORGEN BIOTECH CORP, were employed to isolate genomic DNA from 23 genotypes of bread wheat.

PCR amplification by using SSR (microsatellite) markers

Following the acquisition of field results, group (A) of the genotypes (AB, CL, NF, SA, TB, UD), shown in **Figure 6**, was chosen due to its demonstrated tolerance to salt treatments, alongside the inclusion of one salinity-sensitive genotype (PB) from group (D) in **Figure 6**. DNA was extracted from fresh leaves of selected genotypes (Dellaporta et al. 1983; Porebski et al. 1997). Genomic DNA of wheat genotypes was subjected to SSR analysis using two primers Xcfd-18 (Hannan et al. 2021) and Xgwm-493(Vaja et al. 2016) in the **Table 2** as genetic markers associated with salt tolerance and approved in several sources in selective breeding program (Vaja et al. 2016). The primers are made in Alpha ADN, S.E.N.C. company, www.alphaadn.com . The total of samples in the SSR (microsatellite) test was14 samples.

Table 2. Primer sequence, temperature, Sources, and length for twenty ISSR markers in this study

Primer to Salinity

	Primer	Sequences	Length (meres)	Annealing. °C	Sources
3	Xcfd-18	F CATCCAACAGCACCAAGAGA R GCTACTACTATTTCATTGCGACCA	20 -24	55-60	(Hannan et al. 2021)
4	Xgwm-493	F ATCGCATGATGCACGTAGAG R ACATGCATGCCTACCTAATGG	20-21	55-59	(Vaja et al. 2016)

To prepare the template, there are several materials added according to the manufacturer of the Master Max PCR. Samples were numbered before DNA was added. The PCR reaction mixture (25 µl) contained 10X Taq Master Mix with Standard Buffer (12.5 µl), template DNA (2 µl), nuclease-free water (9.5 µl), 10 µM forward primer (0.5 µl), and 10 µM reverse primer (0.5 µl).

The reaction mixtures were heated to 94°C for 3 min by 1 cycle, followed by 32 cycles at 94°C for 30s., 40,55°C for 30s according to used primer and 72°C for 1 min. A final extension for 5 min at 72°C. SSR-PCR products were separated by using the agarose gel electrophoresis method as the following steps: 3% agarose gel was prepared using 3 g of agarose with 100 ml of 1X TBE. Then 2 µL of ethidium bromide stain were added into the agarose gel solution. DNA marker ladder (100-1500 bp) provided by (TRANS-

China) in one well. Then electric current was performed at 125 V for 25 min, then 75 V for 1 h. SSR-PCR products were visualized by using Ultraviolet (UV) light (365 nm) using a photo imaging system.

Identification of target genetic loci

Four DNA samples of the genotypes (NF, UD, SA, TB) that showed tolerance to salinity in PCR-SSR test in **Figure 6**, were sent to Alpha ADN Canadian, www.alphaadn.com , by comparing local samples' nucleic acid sequences with retrieved sequences, using Sanger dideoxy sequencing technology. Madison's BioEdit Sequence Alignment Editor Software Version 7.1 was utilized to analyse PCR products, comparing observed sequences with retrieved ones, identifying virtual positions and fragment details. PCR amplicons were used to accurately analyse samples, identifying variations in sequences. These were translated to amino acid sequences using the Expasy translate suite. The NCBI Bankit portal was utilized to submit sequences for investigation, which were then analysed and provided as nucleic acid sequences to GenBank for unique accession numbers.

Results and discussion

The results of statistical analysis and averages in the tables indicated that there was a significant effect of irrigation water salinity on the study traits. The genetic factor of the genotypes had a clear effect on the study traits and the genotypes varied in tolerance and sensitivity to salt levels.

Morphological and physiological characterization of wheat genotypes

Production (yield) per m^2 (Pro.m^2) g

The results of **Table 4** showed that there were significant differences in the Pro.m^2 at a significance level of (0.05), as the T_3 level gave the lowest average for this trait, which amounted to (451.9) g/m^2, compared to the T_1 level, which gave the highest average of (700.1) g/m^2. Yield reductions caused by salinity and drought are primarily due to reduced spike counts per plant and reduced grain counts per spike (Maas and Grieve 1990). Overall, these stresses significantly reduce the potential of wheat plants by affecting stem growth and development, and above-ground dry weight (Hu and Geesing 2008). In **Table 3** for the varieties, the TB variety gave the highest average of (872.4) g/m^2, while the lowest average was (345) g/m^2 for the VN variety. In **Table 3** as for the SD values for the average genotype under the influence of salinity, the highest value (265) was for the genotype PB, and the lowest value (53.4) was for the genotype NF, while the effect of genotypes and overlap was significant (48.8) at the significance level of (0.05). Tolerant wheat varieties show less reduction in tiller number and yield compared to sensitive varieties under stress conditions (Kumar et al. 2018). The percentage of yield loss(YL) at T3 of salinity is represented, as tolerant varieties showed the least yield loss, thus demonstrating the importance of selecting tolerant genotypes in maintaining the level of agricultural economy under salinity conditions. YL in the NF genotype was the lowest at 12% compared to the highest decrease of 69% for the VN variety. It is clear from the **Table 3** shows that the NF genotype is the most tolerant to salinity, with a tolerance of cultivar (TOL) value of value of 105.8. It can be asserted that salinity affects wheat grain yield at different rates depending on the controlling genetic factor.

Table 3. Varieties and Salinity treatments irrigation water on the production (yield) trait per square meter (g/m2)

V	*T1(Control)*	*T2*	*T3*	*Mean*	*SD*	*TOL*	*YL%*
AB	457.5	400.8	338.3	398.9	59.6	119.2	26
BW	777.5	667.5	595.0	680.0	91.9	182.5	23
CL	642.5	586.7	506.7	578.6	68.3	135.8	21
DB	780.0	562.5	530.0	624.2	135.9	250.0	32
EU	592.5	404.8	325.0	440.8	137.3	267.5	45
FS	627.5	462.5	315.0	468.3	156.3	312.5	50
GF	667.5	597.5	477.5	580.8	96.1	190.0	28
HB	567.5	460.0	302.5	443.3	133.3	265.0	47
IB	517.5	365.0	267.5	383.3	126.0	250.0	48
JB	767.5	555.0	445.0	589.2	163.9	322.5	42
KA	728.3	557.5	393.3	559.7	167.5	335.0	46
LB	686.7	600.0	525.0	603.9	80.9	161.7	24

MB	860.0	684.2	581.7	708.6	140.8	278.3	32
NF	900.8	835.8	795.0	843.9	53.4	105.8	12
OT	692.5	595.0	322.5	536.7	191.8	370.0	53
PB	680.0	480.0	155.0	438.3	265.0	525.0	77
QA	532.5	407.5	297.5	412.5	117.6	235.0	44
RA	850.0	690.0	610.0	716.7	122.2	240.0	28
SA	927.5	820.0	790.0	845.8	72.3	137.5	15
TB	966.7	863.3	787.3	872.4	90.0	179.3	19
UD	712.5	675.0	553.3	646.9	83.2	159.2	22
VN	535.0	332.5	167.5	345.0	184.1	367.5	69
WA	631.7	460.0	312.5	468.1	159.7	319.2	51
Mean	**700.1**	568.0	**451.9**	573.3			

Biomass per plant (Bio.P) g/plant

The results of **Table 4** showed that there were significant differences in the Bio.P at a significance level of (0.05), as the T_3 salinity level gave the lowest average for this trait, which amounted to (60)g per plant, compared to the T_1, and T_2 salinity concentration, which T_1 gave the highest average of (103.9) g per plant, The relationship between yield and salinity concentration is usually a negative linear relationship, as confirmed by linear (Richards et al. 1987), as salinity affects several agricultural traits, including plant height, number of tillers, and flag leaf area, and these traits determine the plant's biological yield index (Sima Taheri 2011). In **Figure 1** for the varieties, the UD variety gave the highest average of (129.7) g per plant, while the lowest average was (49) g per plant for the FS variety. In **Figure 1**, the SD values for the genotype under the influence of salinity show that the genotype (LB) had the highest value (40.8) and the genotype (CL) had the lowest value (2.5). The effect of interaction between genotypes and salinity concentration treatments of irrigation water was significant (6.9) at a significance level of 0.05. Studies have shown that wheat genotypes respond differently to salinity, although there are large differences in biomass and grain yield under salt stress (Yumurtaci and Uncuoğlu 2012). In another study, the degree of yield loss varies by genotype and plant growth stage (Rawtiya and Kasal 2021).

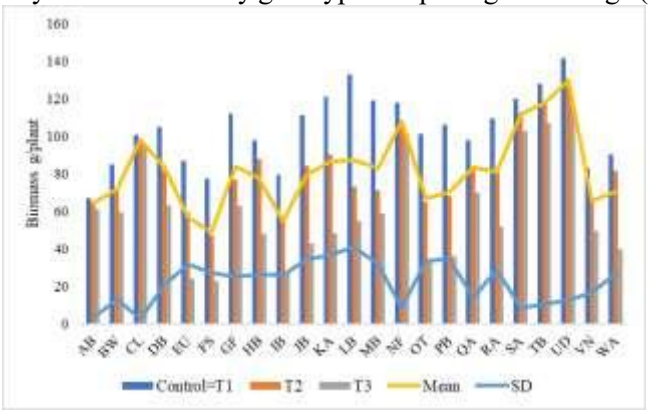


Figure 1. Varieties and Salinity treatments irrigation water on Biomass g /plant.

The Flag Leaf Area (F.L.A) cm^2

The results of **Table 4**, it was found that the effect of Salinity concentration treatments of irrigation water was significant on F.L.A, as T_3 salinity level gave the lowest average for this trait (32.5), the reason for this discrepancy may be attributed to the fact that the flag leaf growth rate is one of the important adaptive activities associated with avoiding salinity [18], Salt stress in the vegetative stage limits leaf expansion and photosynthesis (Taiz et al. 2022). In **Figure 2** for the varieties, the AB variety gave the highest average of (68.2) c^2, while the lowest average was (11.8) c^2 for the UD variety, while the effect of the interaction between the genotypes and salt concentration was significant, as the value of the LSD was (7.8). As for the F.L.A, the standard deviation values (SD) of the genotypes under the influence of salinity were indicated. The lowest value (1.13) was for the genotypes (UD) and the highest value (14.62) was for the genetic composition (JB).

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 24s, 2025
https://www.theaspd.com/ijes.php

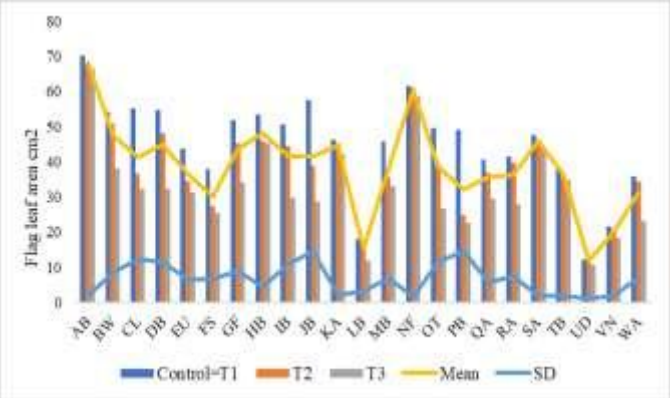


Figure 2. Effect of varieties and salinity treatments irrigation water on the flag leaf area.

Grains weight per spike (G.W.S) g

The results of **Table 4** showed that there were significant differences in G.W.S, as the T_3 salinity level gave the lowest average for this trait amounting to (2) g, compared to the T_1 salinity level, which gave the highest average of (2.8) g. In **Table 4** the effect of interaction between varieties and salinity treatments (VXT) was significant, as the value of LSD was (0.5) at a significance level of (0.05). The varieties (V) and the salinity treatments (T) had a significant effect at a significance level (0.05) on the G.W.S.T by an amount of (0.3, and 0.1) respectively. In **Figure 3** for the varieties, the AB variety gave the highest average of (4.5) g, while the lowest average was (0.7) for the VN variety. The lowest SD value was (0.09) in the variety SA, and it was the SD highest value (0.92) for the variety (KA). The osmotic pressure resulting from salinity affects the availability of water and nutrients, especially during the reproductive and grain-filling stages, which causes a decrease in grain weight and thus affects grain yield (Pradhan et al. 2012).

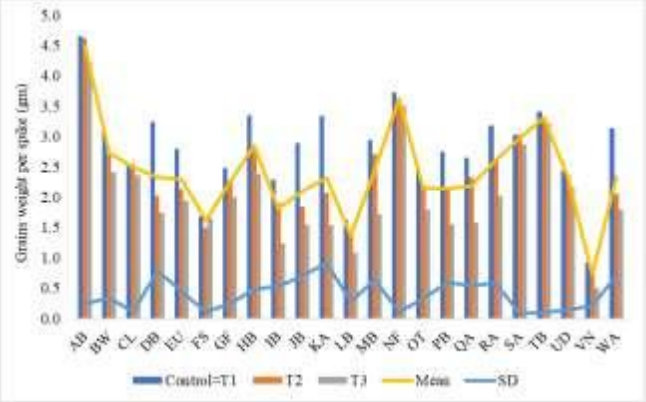


Figure 3. Varieties and salinity treatments irrigation water on grains weight per spike.

Weight 1000 grains Trait (W.1000grs) g

In **Table 4**, the analysis of variance (ANOVA) at LSD between the means and at a significance level of (0.05) showed that the interaction between the varieties and salinity treatments was not significant on W.1000grs. The varieties (V) and salinity treatment (T) had a significant effect at a significance level of (0.05) on W.1000grs by (9.7 and 5.8) respectively. In **Figure 4** for the varieties, the NF variety gave the highest average of (60.9) g, while the lowest average was (14.8) for the VN variety, while the effect of interaction between varieties and salinity treatments of irrigation water was not significant, at a significance level of (0.05). The lowest SD value was (0.2) in the genotype UD, and it was the highest value (16.4) for the variety (KA). The results of **Table 4** showed that the T_3 salinity level gave the lowest average for this trait amounting to (28.7)g, compared to the T_1, and T_2 salt levels, in which the T_1 gave the highest average of (41.7)g, these stressors limit the availability of water and nutrients required for grain filling, resulting in smaller and lighter grains (Mostafazadeh-Fard et al. 2009).

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 24s, 2025
https://www.theaspd.com/ijes.php

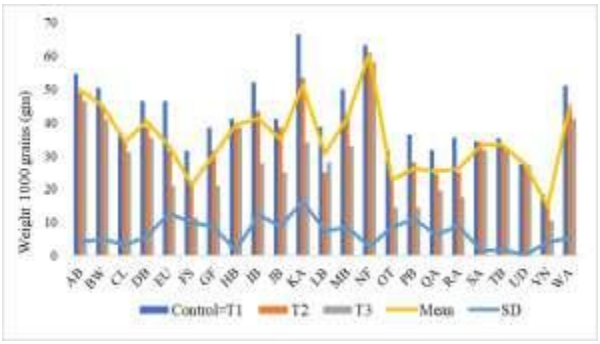


Figure 4. Varieties and salinity treatments of irrigation water on weight 1000 grains

Chlorophyll (SPAD)

The results of **Table 4** showed that there were significant differences in the chlorophyll content trait, as the T_3 salinity level gave the lowest average for this trait amounted to (46.7), compared to the T_1 salt concentration, which gave the highest average of (51.4). Salinity reduces chlorophyll a but increases chlorophyll b, and carotenoids (Dehnavi et al. 2017). This reduction in chlorophyll levels resulted in a decline in photosynthetic efficiency, which is crucial for the optimal growth and productivity of the wheat crop (Shah et al. 2017). In **Figure 5** for the varieties, the UD variety gave the highest average of (55.4), while the lowest average was (44.1) for the RA variety, while the effect of interaction between Varieties and Salinity treatments irrigation water was not significant, at a significance level of (0.05). The lowest SD value was (0.5) in the genotype TB, and it was the highest value (4.6) for the variety (OT).

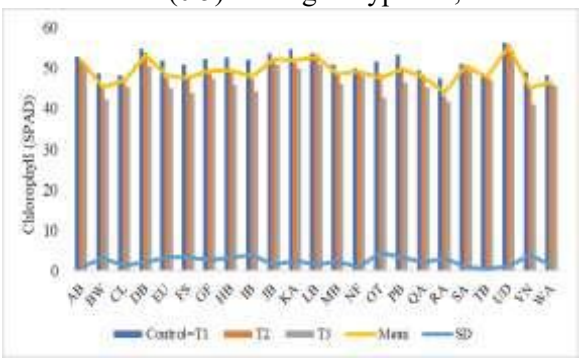


Figure 5. Varieties and Salinity treatments irrigation water on Chlorophyll (SPAD).

Table 4. Analysis of variance (ANOVA) table for study traits and salinity treatments (T), varieties (V) by using LSD between data means at significance level 0.05

Trans		***(Control)T1***	***T2***	***T3***	***Mean***	***LSD.05***
Biomass (g/plant)	*Mean*	103.9	81.3	60.0	81.7	*T X V* 6.9
	LSD.05	*T*= 2.9			*V*= 4.0	
Production (yield) per m^2 (g)	*Mean*	700.1	571.4	459.5	577	*T X V* 48.8
	LSD.05	*T*= 19.3			*V*= 28.2	
Flag leaf area (cm^2)	*Mean*	45.1	38.6	32.5	38.7	*T X V* 7.8
	LSD.05	*T*= 1.3			*V*= 4.5	
Grain weight per spike /g	*Mean*	2.8	2.4	2.0	2.4	*T X V* 0.5
	LSD.05	*T*= 0.1			*V*= 0.3	
Weight 1000 grains (gm)	*Mean*	41.7	35.1	28.7	35.1	*T X V* N/A
	LSD.05	*T*= 5.8			*V*= 9.7	
Chlorophyll (SPAD)	*Mean*	51.4	49.1	46.7	49.0	*T X V* N/A
	LSD.05	*T*= 1.2			*V*= 1.8	

Proline acid content (µg. g^{-1} dw)

In **Table 5** Salinity and drought stresses affected the proline accumulation of plants with increasing stress intensity. Under salinity conditions at T3, the average proline concentration increased by 0.82 mg. g^{-1} and was significantly higher than T2 level and control level. By comparing the means of genotypes at the

significance level in the statistical analysis, it was found that the genotypes (SA, UD, NF, TB, CL) were significantly higher than all genotypes, with averages of 0.59, 0.57, 0.57, 0.56 and 0.5 µg. g^{-1}dw, respectively. These genotypes may be classified as salinity-tolerant, as noted by [25,44] in his research, which indicates that proline concentration rises in salinity-tolerant genotypes, but it falls in sensitive genotypes relative to the control group.

Table 5. Effect of salinity and genotypes on proline (µg. g^{-1}dw) in the leaves.

	(Control)T1	***T2***	***T3***	***Means ± SD***
AB	0.15±0.01	0.44±0.01	0.83±0.11	0.47±0.34
BW	0.1±0	0.27±0.02	0.82±0.02	0.4±0.37
CL	0.24±0.01	0.38±0.07	1.02±0.09	0.55±0.42
DB	0.09±0	0.24±0.03	0.67±0.04	0.34±0.3
EU	0.14±0.02	0.24±0.02	0.68±0.01	0.36±0.29
FS	0.06±0.01	0.22±0.06	0.58±0.01	0.29±0.26
GF	0.09±0.04	0.31±0	0.85±0.01	0.42±0.39
HB	0.06±0.01	0.32±0.03	0.92±0.04	0.43±0.44
IB	0.1±0.03	0.3±0.03	0.81±0.07	0.4±0.37
JB	0.09±0.02	0.24±0.02	0.58±0.1	0.3±0.25
KA	0.04±0.02	0.24±0.02	0.74±0.03	0.34±0.36
LB	0.09±0.05	0.26±0.05	0.75±0.06	0.37±0.35
MB	0.03±0.01	0.28±0.05	0.76±0.05	0.36±0.37
NF	0.21±0.01	0.44±0.01	1.04±0.2	0.57±0.43
OT	0.09±0	0.22±0.02	0.54±0.04	0.29±0.23
PB	0.07±0	0.22±0.08	0.58±0.01	0.29±0.26
QA	0.09±0.02	0.25±0.04	0.88±0	0.41±0.42
RA	0.09±0	0.29±0.05	0.77±0.05	0.38±0.35
SA	0.15±0	0.49±0.01	1.13±0.09	0.59±0.5
TB	0.18±0.03	0.43±0.04	1.07±0.12	0.56±0.46
UD	0.19±0.01	0.45±0	1.08±0	0.57±0.46
VN	0.1±0	0.27±0.04	0.85±0.08	0.41±0.4
WA	0.05±0.02	0.29±0.05	0.83±0.07	0.39±0.4
Means	0.11±0.06	0.31±0.09	0.82±0.17	0.41±0.1
LSD.05	T=0.028	V=0.045	TXV	0.078
LSD.01	T=0.046	V=0.060	TXV	0.103

Sodium and potassium ion ratios in leaves.

The results demonstrated that exposure of wheat plants to high salinity caused an over-accumulation of Na+ and a decrease in K+ uptake, consequently an increase in Na+ / K+ ratio, in all genotypes as compared to the control level in **Table 6**, Because high Na concentrations in soil and irrigation water inhibit the flow of K into cells, this leads to decreased cellular K requirements(Nieves-Cordones et al. 2016). through statistical analysis of sodium absorption data in wheat leaves, it was found that the highest sodium content was in the third treatment T3 (0.22%) compared to the control treatment (0.14%), while the potassium content in wheat leaves at the control level of 0.33% and at the levels of T2 and T3 decreased to 0.28% and 0.2%, respectively. As a result, Na+ / K+ content increased significantly at all levels of NaCl treatment. The treatment level had a significant effect at $p < 0.05$, with the control level being 0.46%, the T2 level being 0.71%, and the T3 level being 1.35%. However, SA, UD, TB, NF, and CL exhibited lower accumulation of Na+ and lower Na+/K+ ratio relative to other cultivars, indicating that these cultivars maintained better Na+ and K+ homeostasis, which minimized the salt stress-induced injuries of cellular constituents in **Table 6**, Salinity-tolerant genotypes contribute to plant adaptations that contribute to maintaining ionic balance by reducing sodium uptake, increasing intracellular sodium sequestration, and regulating osmotic balance(Mostofa et al. 2015).

Table 6. Effect of salinity and genotypes on *Na% and K%* in the leaves.

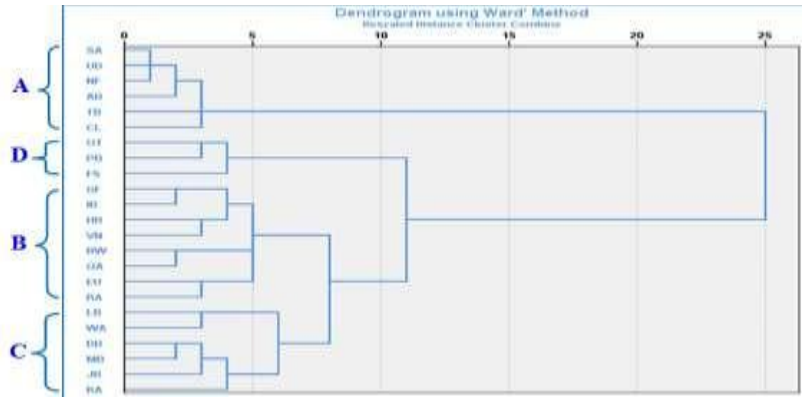
Na%					***K%***				***Na/K***
	(Control)T1	*T2*	*T3*	Means	*(Control)T1*	*T2*	*T3*	Means	Means

AB	0.13	0.19	0.19	0.17	0.4	0.3	0.28	0.33	0.57
BW	0.15	0.18	0.22	0.18	0.37	0.29	0.16	0.27	0.81
CL	0.13	0.18	0.18	0.16	0.35	0.35	0.29	0.33	0.51
DB	0.15	0.19	0.23	0.19	0.3	0.21	0.12	0.21	1.14
EU	0.15	0.18	0.23	0.19	0.34	0.25	0.19	0.26	0.82
FS	0.17	0.21	0.28	0.22	0.22	0.18	0.15	0.18	1.29
GF	0.14	0.17	0.24	0.18	0.36	0.36	0.21	0.31	0.67
HB	0.14	0.16	0.24	0.18	0.41	0.33	0.15	0.29	0.86
IB	0.15	0.17	0.22	0.18	0.38	0.3	0.23	0.3	0.65
JB	0.14	0.19	0.24	0.19	0.28	0.24	0.1	0.21	1.24
KA	0.15	0.2	0.26	0.2	0.27	0.22	0.13	0.21	1.17
LB	0.15	0.17	0.24	0.19	0.32	0.23	0.16	0.24	0.92
MB	0.15	0.17	0.24	0.19	0.3	0.19	0.14	0.21	1.09
NF	0.12	0.18	0.17	0.16	0.37	0.37	0.35	0.37	0.43
OT	0.17	0.21	0.26	0.21	0.25	0.2	0.15	0.2	1.14
PB	0.15	0.23	0.27	0.22	0.24	0.2	0.16	0.2	1.15
QA	0.14	0.18	0.23	0.19	0.33	0.29	0.16	0.26	0.85
RA	0.14	0.18	0.23	0.19	0.36	0.24	0.13	0.24	0.99
SA	0.11	0.15	0.15	0.14	0.42	0.43	0.39	0.41	0.33
TB	0.13	0.18	0.18	0.16	0.37	0.34	0.29	0.34	0.5
UD	0.13	0.17	0.17	0.16	0.4	0.38	0.33	0.37	0.43
VN	0.14	0.17	0.23	0.18	0.38	0.3	0.17	0.28	0.81
WA	0.15	0.18	0.23	0.19	0.26	0.21	0.17	0.21	0.94
Means	0.14	0.18	0.22	0.18	0.33	0.28	0.2	0.27	0.84
LSD.05	T=0.008	V=0.021	TXV	0.037	T=0.008	V=0.043	TXV	0.074	0.285

Dendrogram analysis for 23 genotypes based on morphological and physiological traits

In this study, Dendrogram was created based on morphological and physiological traits that showed great diversity. In the Euclidean distances between 23 genotypes were measured based on the standardised data from the data mean and standard deviation values by Ward's method. In **Figure 6**, the 23 genotypes were classified into four groups according to their ability to tolerate salinity. Salt-tolerant genotypes (A), moderately salt-tolerant genotypes (B), moderately salt-sensitive genotypes (C), and salt-sensitive genotypes (D). Cluster (A) included genotypes (AB, CL, NF, BW, UD, SA, and TB), which showed salt tolerance through the results of morphological and physiological traits, while cluster (B), which included (EU, VN, BW, GF, HB, IB, QA, and RA), genotypes showed moderate tolerance through some morphological traits in biomass and chlorophyll percentage, but grain productivity was less than cluster (A), cluster (C) which included (DB, KA, MB, WA, JB, and LB) These genotypes showed sensitivity to moderate salt levels at a degree of 12 ds-1, Cluster (D) included genotypes (FS, OT, and PB), which showed sensitivity to salt levels through grain productivity and yield-related traits, which showed deterioration with increasing salt concentration. Mention (AL-Salim 2018; AL.GHANMI 2021) in research papers of some genotypes in the principle of genetic variation and similarity in morphological traits, which supports the results.

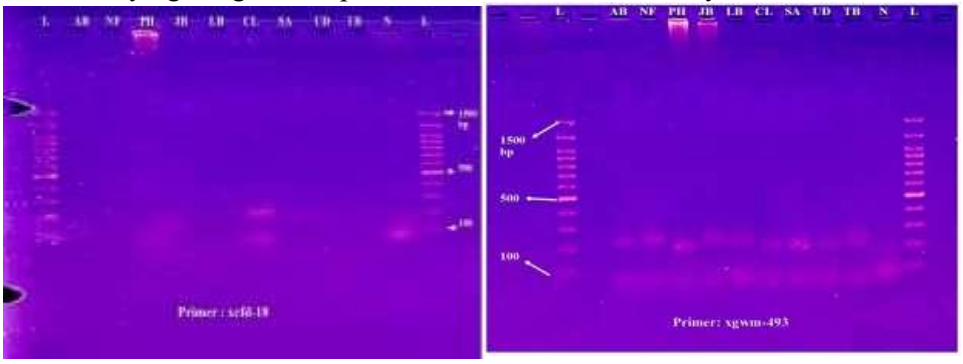
Figure 6. Dendrogram for 23 genotypes based on morphological and physiological traits by using ward's method.



Molecular characterization of wheat genotypes by Molecular markers

PCR- SSR (microsatellite) markers analysis

The results of the gel electrophoresis analysis of the samples in the primer (xcfd-18) revealed the presence of a radioactive band indicating the sample (CL) in the **Figure 7.a**, while the primer (xgwm-493) revealed the presence of the samples (TB, UD, SA, NF) in the **Figure 7.b**. These radioactive bands indicate the interaction of the primers with the DNA genome of the candidate samples, which confirms the validity of the morphological and physiological results in the field, although the sample PB did not show any bands in all the selected primers. These results pave the way for moving to the stage of genetic sequencing and identifying the genes responsible for tolerance to salinity.



(a) **(b)**

Figure 7. Gel electrophoresis analysis: (**a**) Gel electrophoresis analysis of primer (xcfd-18)**;** (**b**) Gel electrophoresis analysis of primer (xgwm-493).

Genetic sequencing results

The sequencing reactions indicated the exact identity after performing NCBI BLASTn for PCR amplicons. Where the NCBI BLASTn engine showed the presence of entire sequence similarity between the sequenced samples (Q1, Q2, Q3, and Q4) and the microsatellite sequences of *Triticum trugidum* (GenBank acc. AF275898.1), The results indicated a sequence match with the genotype. The genotype (*T. turgidum durum*) is one of the parents of bread wheat and durum wheat, it is a tetraploid wheat (*2n = 4x = 28, AABB*)(Peng et al., n.d.). The geographical distribution of wild wheat is in the Fertile Crescent region in southwest Asia, Palestine, Jordan, Lebanon, Syria, southern Turkey, northern Iraq, and southwest Iran (Breseghello and Sorrells 2006; Balter 2007). It can serve as one of the most important genetic resources to improve durum (*Triticum turgidum L. ssp.* Durum (Desf.) and bread wheat (*Triticum aestivum L.*) and it has been used for allele mining for many needs of wheat breeding, including, but not limited to, drought (Börner et al. 1998; Bolot et al. 2009) and salinity tolerance (Buckler et al. 2001), and for biotic stress factors.

All the investigated genetic sequences were deposited in the NCBI web server, and unique accession numbers were obtained for all analysed sequences. Four GenBank accession numbers of the microsatellite amplicons (PP873642, PP873643, PP873644, and PP873645) were deposited in NBCI to respectively represent the (Q1, Q2, Q3, and Q4) samples, it is clear in **Table 7**.

Table 7. GenBank accession numbers for nucleotide sequences https://www.ncbi.nlm.nih.gov/genbank/.

Seq. №	**Accession.** №	**GenBank.** №	**Amplicon**	**Primers**	**strain**	**Samples**	**Genotypes**
Seq1	PP873642	BankIt2835923	microsatellite	xgwm-493	Malek-Q1	Q1	NF
Seq2	PP873643				Malek-Q2	Q2	UD
Seq3	PP873644				Malek-Q3	Q3	SA
Seq4	PP873645				Malek-Q4	Q4	TB

DISCUSSION

Results of study provide a comprehensive evaluation of salinity tolerance in 23 bread wheat genotypes, integrating agronomic, physiological, and molecular analyses. The primary objective was to identify robust, salt-tolerant wheat lines that can sustain productivity in regions severely affected by soil and irrigation water salinization, such as Iraq. Our findings demonstrate significant genotypic variation in response to salinity stress, identifying specific genotypes with superior tolerance mechanisms and linking these traits to genetic markers.

The severe yield reduction observed in sensitive genotypes (VN and PB with up to 77% loss at 15 $dS \cdot m^{-1}$) underscores the devastating impact of salinity on wheat production. In contrast, the minimal yield loss in tolerant lines like NF, SA, TB, and UD (12–19%) highlights their potential for cultivation in saline-affected areas. This differential response aligns with established literature confirming that genetic variation is a key determinant of salinity tolerance in wheat, primarily mediated through traits like maintained tillering, spike fertility, and 1000 grain weight under stress (Kumar et al. 2018; Maas and Grieve 1990). The correlation between high biomass production, stable flag leaf area, and sustained yield in tolerant genotypes suggests that the ability to maintain photosynthetic capacity and assimilate partitioning under stress is a critical component of their resilience (Taiz et al. 2022; Sima Taheri 2011).

At the physiological level, the tolerant genotypes exhibited classic adaptive mechanisms to osmotic and ionic stress. The significant accumulation of proline in genotypes like SA, NF, TB, and UD under high salinity is a well-documented osmoprotectant response. Proline helps maintain cellular turgor, stabilizes proteins and membranes, and scavenges reactive oxygen species (ROS), thereby mitigating the detrimental effects of water deficit caused by high osmotic pressure in the soil solution [25,44]. More importantly, the ability of these genotypes to maintain a lower Na^+/K^+ ratio compared to sensitive lines is a cornerstone of ionic homeostasis. Sodium toxicity and potassium deficiency are major causes of cellular damage under salinity stress (Nieves-Cordones et al. 2016). The tolerant genotypes' capacity to limit Na^+ uptake and/or compartmentalize it in vacuoles, while sustaining K^+ acquisition—a nutrient vital for enzymatic function and stomatal regulation—is a key indicator of their internal ion regulation efficiency (Mostofa et al. 2015). This balanced Na^+/K^+ ratio likely contributed to their superior physiological performance and yield stability.

The integration of molecular markers provided a genetic validation of the phenotypic observations. The SSR markers Xcfd-18 and Xgwm-493, previously associated with salt tolerance loci [30, 31], successfully amplified specific alleles in the tolerant genotypes (NF, SA, TB, UD, CL) but not in the sensitive control (PB). This not only confirms the genetic distinction between the groups but also demonstrates the utility of these markers for marker-assisted selection (MAS) in breeding programs. The subsequent Sanger sequencing of amplicons from the most tolerant genotypes and their high homology with *Triticum turgidum durum* (GenBank acc. AF275898.1) is a particularly significant finding. Wild relatives and ancient cultivars like *T. turgidum* are renowned reservoirs of stress-resilience alleles that have often been diluted in modern bread wheat through intensive breeding for yield under optimal conditions (Buckler et al. 2001; Peng et al., n.d.). The identification of homologous sequences suggests that the salinity tolerance in these bread wheat genotypes may be derived from or shared with these hardy ancestral species. Depositing these sequences in NCBI (PP873642–PP873645) provides a valuable genetic resource for the global research community to probe the specific genes and mechanisms conferring this tolerance. The cluster analysis (dendrogram) based on Euclidean distances effectively grouped the 23 genotypes into four distinct categories (tolerant, moderately tolerant, moderately sensitive, and sensitive), which strongly correlated with their performance across all measured traits. This multivariate approach reinforces the

conclusion that salinity tolerance is a complex, polygenic trait manifested through a suite of coordinated morphological and physiological responses, rather than a single characteristic.

CONCLUSION

Based on the comprehensive evaluation of 23 wheat genotypes under saline conditions, this study successfully identifies several promising salt-tolerant lines, including NF, SA, TB, and UD. These genotypes exhibited minimal yield reduction (12–19%) at high salinity (15 $dS \cdot m^{-1}$), maintained superior physiological performance through higher proline accumulation and balanced Na^+/K^+ ratios, and showed greater morphological stability. Molecular analysis using SSR markers (Xcfd-18 and Xgwm-493) confirmed the genetic basis of this tolerance, with sequencing revealing homology to resilient *Triticum turgidum durum*. The integration of phenotypic and genotypic screening provides a robust framework for selecting parental material in breeding programs. The identified genotypes and deposited genomic sequences (NCBI accessions PP873642, PP873643, PP873644, PP873645) are valuable resources for developing high-yielding, salt-tolerant wheat varieties. This approach is essential for enhancing productivity in salinity-affected regions like Iraq, contributing to food security and sustainable agricultural resilience under climate change. Future work should focus on multi-environment trials and advanced genomic strategies to further exploit these genetic resources.

REFERENCES

- Adger, W. Neil, Saleemul Huq, Katrina Brown, Declan Conway, and Mike Hulme. 2003. 'Adaptation to Climate Change in the Developing World'. *Progress in Development Studies* 3 (3): 179–95. https://doi.org/10.1191/1464993403ps060oa.
- AL.GHANMI, MARWA. 2021. 'Response of Wheat Cultivars Triticum Aestivum L. to Bio -Organic and Mineral Fertilization on Growth and Yield'. https://agr.mu.edu.iq.
- AL-Salim, Salih Hadi. 2018. 'Evaluation the Bread Wheat Triticum Aestivum L. Genetics Variations Using Biochemical and Molecular Techniques to Morphological Characterization'. Agriculture College – AL-Muthanna university.
- Amiteye, Samuel. 2021. 'Basic Concepts and Methodologies of DNA Marker Systems in Plant Molecular Breeding'. *Heliyon* 7 (10): e08093. https://doi.org/10.1016/j.heliyon.2021.e08093.
- Balter, Michael. 2007. 'Seeking Agriculture's Ancient Roots'. *Science*, ahead of print, June 29. world. https://doi.org/10.1126/science.316.5833.1830.
- Bates, L. S., R. P. Waldren, and I. D. Teare. 1973. 'Rapid Determination of Free Proline for Water-Stress Studies'. *Plant and Soil* 39 (1): 205–7. https://doi.org/10.1007/BF00018060.
- Bidyananda, Nongthombam, Imlitoshi Jamir, Karolina Nowakowska, et al. 2024. 'Plant Genetic Diversity Studies: Insights from DNA Marker Analyses'. *International Journal of Plant Biology* 15 (3): 3. https://doi.org/10.3390/ijpb15030046.
- Bolot, Stéphanie, Michael Abrouk, Umar Masood-Quraishi, et al. 2009. 'The "Inner Circle" of the Cereal Genomes'. *Current Opinion in Plant Biology*, Genome Studies and Molecular Genetics, vol. 12 (2): 119–25. https://doi.org/10.1016/j.pbi.2008.10.011.
- Börner, A., V. Korzun, and A.J. Worland. 1998. 'Comparative Genetic Mapping of Loci Affecting Plant Height and Development in Cereals'. *Euphytica* 100 (1): 245–48. https://doi.org/10.1023/A:1018364425150.
- Braun, Hans-Joachim, Gary Atlin, and Thomas Payne. 2010. 'Multi-Location Testing as a Tool to Identify Plant Response to Global Climate Change'. *Climate Change and Crop Production* 1: 115–38. https://doi.org/10.1079/9781845936334.0115.
- Breseghello, Flavio, and Mark E. Sorrells. 2006. 'Association Mapping of Kernel Size and Milling Quality in Wheat (Triticum Aestivum L.) Cultivars'. *Genetics* 172 (2): 1165–77. https://doi.org/10.1534/genetics.105.044586.
- Buckler, Edward S., Jeffry M. Thornsberry, and Stephen Kresovich. 2001. 'Molecular Diversity, Structure and Domestication of Grasses'. *Genetics Research* 77 (3): 213–18. https://doi.org/10.1017/S0016672301005158.
- Chabuk, Ali, Qais Al-Madhlom, Ali Al-Maliki, Nadhir Al-Ansari, Hussain Musa Hussain, and Jan Laue. 2020. 'Water Quality Assessment along Tigris River (Iraq) Using Water Quality Index (WQI) and GIS Software'. *Arabian Journal of Geosciences* 13 (14): 654. https://doi.org/10.1007/s12517-020-05575-5.
- *Climate Data of IRQ*. 2023. https://en.climate-data.org/asia/iraq/muthanna/as-samawah-2921/.
- Cline, William R. 2007. 'Global Warming and Agriculture: Impact Estimates by Country'. *Peterson Institute Press: All Books*. https://ideas.repec.org//b/iie/ppress/4037.html.
- Dehnavi, M., Tayebeh Zarei, Rahil Khajeeyan, and Mitra Merajipoor. 2017. 'Drought and Salinity Impacts on Bread Wheat in a Hydroponic Culture: A Physiological Comparison'. June 1. https://www.semanticscholar.org/paper/Drought-and-Salinity-Impacts-on-Bread-Wheat-in-a-A-Dehnavi-Zarei/4fb6e42d4070305a7333ec431216e4ac8c01b7e7#citing-papers.
- Dellaporta, Stephen L., Jonathan Wood, and James B. Hicks. 1983. 'A Plant DNA Minipreparation: Version II'. *Plant Molecular Biology Reporter* 1 (4): 19–21. https://doi.org/10.1007/BF02712670.
- Ehtaiwesh, Amal F, and Fatma H Rashed. n.d. *Growth and Yield Responses of Libyan Hard Wheat (Triticum Durum Desf) Genotypes to Salinity Stress*.
- FAO. 2022. *Food and Agriculture Organization of the United Nations. FAOSTAT*. https://www.fao.org/faostat/en/#data/HS.
- Gomez, Kwanchai A., and Arturo A. Gomez. 1984. *Statistical Procedures for Agricultural Research*. John Wiley & Sons.
- Hannan, Muhammad Abdul, Nihar Ranjan Saha, Swapan Kumar Roy, Sun-Hee Woo, and Muhammad Shahidul Haque. 2021. 'Genetic Diversity Analysis and Molecular Screening for Salinity Tolerance in Wheat Germplasm'. *Plant Breeding and Biotechnology* 9 (3): 185–98. https://doi.org/10.9787/PBB.2021.9.3.185.

- Hasan, Abu, Hafizur Rahman Hafiz, Nurealam Siddiqui, M. Khatun, Rabiul Islam, and Abdullah -Al Mamun. 2015. 'Evaluation of Wheat Genotypes for Salt Tolerance Based on Some Physiological Traits'. *Journal of Crop Science and Biotechnology* 18 (5): 333–40. https://doi.org/10.1007/s12892-015-0064-2.
- Hu, Y., and D. Geesing. 2008. 'Interactive Effects of Nutrients and Salinity and Drought on Wheat Growth'. https://www.semanticscholar.org/paper/Interactive-effects-of-Nutrients-and-Salinity-and-Hu-Geesing/e590c5281a9a4cd6530f06d0e54ae7486a7aec37.
- I. Udoh, Lovina, Willie Peggy Obaseojei, and Chiebuka Uzoebo. 2021. 'Single Nucleotide Polymorphisms: A Modern Tool to Screen Plants for Desirable Traits'. In *Plant Breeding - Current and Future Views*, edited by Ibrokhim Y. Abdurakhmonov. IntechOpen. https://doi.org/10.5772/intechopen.94935.
- Ibraheem, Saleh. 2018. 'Influence of Different Levels of Biofertilizer Em1 and Nitrogen Fertilizer on Growth Traits and Yield in Wheat'. *Mesopotamia Journal of Agriculture* 46 (1): 151–68. https://doi.org/10.33899/magrj.2018.161427.
- Jha, Shweta. 2019. 'Transgenic Approaches for Enhancement of Salinity Stress Tolerance in Plants'. In *Molecular Approaches in Plant Biology and Environmental Challenges*, edited by Sudhir P. Singh, Santosh Kumar Upadhyay, Ashutosh Pandey, and Sunil Kumar. Energy, Environment, and Sustainability. Springer Singapore. https://doi.org/10.1007/978-981-15-0690-1_14.
- Kamarianakis, Zacharias, and Spyros Panagiotakis. 2023. 'Design and Implementation of a Low-Cost Chlorophyll Content Meter'. *Sensors* 23 (5): 2699. https://doi.org/10.3390/s23052699.
- Khalid, Mishal, Rehana Kausar, Armghan Shahzad, Ghulam Muhammad Ali, and Sania Begum. 2023. 'Screening and Validation of Salt-Stress Responsive Cg-SSR Markers in Wheat (Triticum Aestivum L.) Germplasm of Pakistan'. *Molecular Biology Reports* 50 (7): 5931–40. https://doi.org/10.1007/s11033-023-08519-w.
- Kumar, Ashwani, Surinder Kumar Sharma, Charu Lata, et al. 2018. 'Impact of Water Deficit (Salt and Drought) Stress on Physiological, Biochemical and Yield Attributes on Wheat (Triticum Aestivum) Varieties'. *The Indian Journal of Agricultural Sciences* 88 (10): 1624–32. https://doi.org/10.56093/ijas.v88i10.84255.
- Maas, E. V., and C. M. Grieve. 1990. 'Spike and Leaf Development of Sal-Stressed Wheat'. *Crop Science* 30 (6): 1309–13. https://doi.org/10.2135/cropsci1990.0011183X003000060031x.
- Meena, Moti Lal, Dheeraj Singh, and Dheeraj Singh. 2020. 'Dissemination of Salt Tolerant Wheat Varieties through Frontline Demonstrations Approach for Sustainable Wheat Production in Pali District of Rajasthan'. *Journal of Cereal Research* 12 (1). https://doi.org/10.25174/2582-2675/2020/87064.
- Mostafazadeh-Fard, Behrouz, Hamed Mansouri, Sayed-Farhad Mousavi, and Mohammad Feizi. 2009. 'Effects of Different Levels of Irrigation Water Salinity and Leaching on Yield and Yield Components of Wheat in an Arid Region'. *Journal of Irrigation and Drainage Engineering* 135 (1): 32–38. https://doi.org/10.1061/(ASCE)0733-9437(2009)135:1(32).
- Mostofa, Mohammad G., Daisuke Saegusa, Masayuki Fujita, and Lam-Son Phan Tran. 2015. 'Hydrogen Sulfide Regulates Salt Tolerance in Rice by Maintaining Na+/K+ Balance, Mineral Homeostasis and Oxidative Metabolism Under Excessive Salt Stress'. *Frontiers in Plant Science* 6 (December). https://doi.org/10.3389/fpls.2015.01055.
- Nieves-Cordones, Manuel, Fouad Razzaq Al Shiblawi, and Hervé Sentenac. 2016. 'Roles and Transport of Sodium and Potassium in Plants'. *Metal Ions in Life Sciences* 16: 291–324. https://doi.org/10.1007/978-3-319-21756-7_9.
- Peng, Junhua, Dongfa Sun, and Eviatar Nevo. n.d. *Wild Emmer Wheat, Triticum Dicoccoides, Occupies a Pivotal Position in Wheat Domestication Process.*
- Porebski, Sue, L. Grant Bailey, and Bernard R. Baum. 1997. 'Modification of a CTAB DNA Extraction Protocol for Plants Containing High Polysaccharide and Polyphenol Components'. *Plant Molecular Biology Reporter* 15 (1): 8–15. https://doi.org/10.1007/BF02772108.
- Pradhan, Gautam P., P. V. Vara Prasad, Allan K. Fritz, Mary B. Kirkham, and Bikram S. Gill. 2012. 'Effects of Drought and High Temperature Stress on Synthetic Hexaploid Wheat'. *Functional Plant Biology* 39 (3): 190. https://doi.org/10.1071/FP11245.
- Rao, K. V. R., A. Bajpai, S. Gangwar, L. Chourasia, and K. Soni. 2016. 'Maximising Water Productivity of Wheat Crop by Adopting Drip Irrigation'. *Research on Crops* 17 (1): 163. https://doi.org/10.5958/2348-7542.2016.00029.2.
- Rawtiya, Akshay Kumar, and Y. Kasal. 2021. 'Drought Stress and Wheat (Triticum Aestivum L.) Yield: A Review'. https://www.semanticscholar.org/paper/Drought-stress-and-wheat-(Triticum-aestivum-L.)-A-Rawtiya-Kasal/5f837f4e4b765f26db6cc4ca46ddfa614fc94b8b.
- Ray, Deepak K., Nathaniel D. Mueller, Paul C. West, and Jonathan A. Foley. 2013. 'Yield Trends Are Insufficient to Double Global Crop Production by 2050'. *PloS One* 8 (6): e66428. https://doi.org/10.1371/journal.pone.0066428.
- Richards, R.A., C.W. Dennett, C.O. Qualset, E. Epstein, J.D. Norlyn, and M.D. Winslow. 1987. 'Variation in Yield of Grain and Biomass in Wheat, Barley, and Triticale in a Salt-Affected Field'. *Field Crops Research* 15 (3–4): 277–87. https://doi.org/10.1016/0378-4290(87)90017-7.
- Royo, A., and D. Abió. 2003. 'Salt Tolerance in Durum Wheat Cultivars'. *Spanish Journal of Agricultural Research* 1 (3): 27–35. https://doi.org/10.5424/sjar/2003013-32.
- Shah, Syed, Rasmus Houborg, and Matthew McCabe. 2017. 'Response of Chlorophyll, Carotenoid and SPAD-502 Measurement to Salinity and Nutrient Stress in Wheat (Triticum Aestivum L.)'. *Agronomy* 7 (3): 61. https://doi.org/10.3390/agronomy7030061.
- Shaheenuzzamn, Md, Tianxiang Liu, Shandang Shi, Peipei An, Hongqi Wu, and Zhonghua Wang. 2020. 'Development of Sequencing Technology and Role of next Generation Sequencing Technologies in Wheat Research: A Review'. *Pakistan Journal of Botany* 52 (5). https://doi.org/10.30848/PJB2020-5(33).
- Shawai, R. S., A. I. Magashi, I. M. Bello, U. H. Gaya, and K. D. Dawaki. 2023. 'Molecular and Morphological Characterization of Wheat Genotypes Under Drought Condition in Nigerian Sudan Savannah'. *European Modern Studies Journal* 7 (5): 120–31. https://doi.org/10.59573/emsj.7(5).2023.11.

- Shifa, Q. I., Zhang Jiafu, G. a. O. Huihui, R. a. O. Xiaojuan, Zhang Zhanmei, and J. I. N. Gailong. 2021. 'Effect Analysis of Cultivation Mode of Drip Irrigation Winter Wheat Precise Hole Sowing'. *Xinjiang Agricultural Sciences* 58 (6): 1021. https://doi.org/10.6048/j.issn.1001-4330.2021.06.006.
- Siddiqui, Md. Nurealam, Mohammad Golam Mostofa, Mst. Mahmuda Akter, et al. 2017. 'Impact of Salt-Induced Toxicity on Growth and Yield-Potential of Local Wheat Cultivars: Oxidative Stress and Ion Toxicity Are among the Major Determinants of Salt-Tolerant Capacity'. *Chemosphere* 187 (November): 385–94. https://doi.org/10.1016/j.chemosphere.2017.08.078.
- Sima Taheri. 2011. 'Effects of Drought Stress Condition on the Yield of Spring Wheat (Triticum Aestivum) Lines'. *AFRICAN JOURNAL OF BIOTECHNOLOGY* 10 (80). https://doi.org/10.5897/AJB11.352.
- Taiz, Lincoln, Ian Max Møller, Angus Murphy, and and Eduardo Zeiger. 2022. *Plant Physiology and Development*. Seventh Edition, New to this Edition:, Seventh Edition, New to this Edition: Oxford University Press.
- Thomas, Henry. 1975. 'The Growth Responses to Weather of Simulated Vegetative Swards of a Single Genotype of Lolium Perenne'. *The Journal of Agricultural Science* 84 (2): 333–43. https://doi.org/10.1017/S0021859600052485.
- Vaja, Komal N, Hp Gajera, Zinkal A Katakpara, Sv Patel, and Ba Golakiya. 2016. 'Microsatellite Markers Based Genetic Diversity Analysis for Salt Tolerance in Wheat Genotypes'. *Indian Journal of Agricultural Biochemistry* 29 (2): 140. https://doi.org/10.5958/0974-4479.2016.00023.X.
- Walli, Malek, and Zina AL-Jubouri. 2022. 'Genetic and Environment Diversity to Improve Wheat (Triticum Spp.) Productivity: A Review'. *Research on Crops* 23 (2): 295–306. https://doi.org/10.31830/2348-7542.2022.041.
- Walli, Malek Hubaish, Meisam Zargar, Arshad Naji Al-Hasnawi, and Zina Al-Jubouri. 2025. 'Impact of Global Climate Change on Soil Properties and Water Resources: Review Article'. *IOP Conference Series: Earth and Environmental Science* 1487 (1): 012203. https://doi.org/10.1088/1755-1315/1487/1/012203.
- Yumurtaci, Aysen, and A. A. Uncuoğlu. 2012. 'Tissue Specific Responses Alter the Biomass Accumulation in Wheat under Gradual and Sudden Salt Stress'. *Journal of Stress Physiology & Biochemistry*. https://www.semanticscholar.org/paper/Tissue-specific-responses-alter-the-biomass-in-and-Yumurtaci-Uncuo%C4%9Flu/f1a5ad15dd13cd2b7b4f5a4ff65d5c07dc7920b9.