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Hydroponic Screening at Early Seedling Stage Identified Sources of Salinity Tolerance in Wheat (*Triticum aestivum* L.) Crop

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Abstract: Wheat is a vital crop globally, essential for agriculture, economics, and food security. However, in arid and semi-arid conditions, wheat production faces significant challenges due to low water availability, uneven rainfall distribution, and high soil salinity. The germination and early seedling stages are particularly vulnerable to these stresses. Therefore, this study assessed 15 wheat genotypes for their tolerance to salinity stress during early growth stages, using a hydroponic system with four salt stress levels (0, 50, 100, and 150 mM NaCl). Significant differences were observed for genotype and salinity main effects and their interaction on all investigated traits, indicating considerable variability in the response to salt stress among the investigated wheat cultivars. High NaCl concentrations led to substantial reductions in measured parameters across genotypes, with some showing resilience while others exhibited heightened sensitivity. Stress tolerance indices, such as mean productivity (MP), geometric mean productivity (GMP), harmonic mean (HM), stress tolerance index (STI) and yield index (YI), were identified as reliable indicators for selecting salt-tolerant wheat cultivars. Consequently, Sidi Okba (G11), Ziad (G12), Tamezghida (G13) and Zidane (G14) emerged as the most promising, displaying acceptable performance under both non-stress and salt-stress conditions. These genotypes could serve as valuable genetic resources for breeding programs aimed at enhancing wheat's salinity tolerance, particularly in arid and semi-arid regions.

Keywords: abiotic stress; genetic variation; germination; stress tolerance indices; PCA analysis



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1. Introduction

Wheat (*Triticum* sp.) is a vital crop that occupies an important place in the human diet worldwide. This crop is an important component of farming systems integrating livestock and cereal production [1,2]. However, due to the increased demand for cereal products, especially wheat, cereals have to be grown even in stressful areas (arid and Saharan climate zones) where drought, often combined with salinity, represent the most limiting factor to crop production [3–5]. Most of these lands are located in arid and semi-arid areas, in North Africa, East Asia, Central Asia and South Asia [6]. Their proportion is notably high in the near East (Egypt, Algeria, Tunisia), Middle East (Iran, Pakistan, Bangladesh), Central Asia (Uzbekistan), Northern China and Argentina [7–14]. Sodic soils are particularly widespread

in Australia, but also in certain specific situations, such as in Hungary and Uzbekistan [15]. Hence, susceptibility to salinity stress presents a formidable challenge for wheat production in saline-affected regions.

With regard to salinity stress, various ameliorative strategies have been proposed to mitigate soil salinization, i.e., drainage, leaching, cultural practices, plant-microbial associations, omics and nanotechnologies, etc. [16–18]. While adopting better agronomic practices can contribute significantly to enhancing productivity, the choice of the right varieties also plays a crucial role [19,20].

Breeding salt-tolerant wheat varieties tailored to specific pedoclimatic conditions is, therefore, an alternate option to cope with salinity conditions and to sustain crop production in salty lands. However, progress in this field has been hindered by the complexity of the genetic system related to salt tolerance and the lack of a reliable and fast screening method. The slowness of breeding programs is attributed to the necessity of identifying salt-tolerant genetic resources, which involves screening wheat germplasm under salinity stress conditions. Tolerant cultivars are then selected and crossed to create improved breeding lines.

Screening techniques include both field experiments and controlled experiments in glasshouses and plant growth chambers. Hydroponic systems offer a controlled environment for studying plant responses to salt stress, allowing precise manipulation of salt levels in the nutrient solution and facilitating the observation of root and shoot growth dynamics [21]. By utilizing a hydroponic system, researchers can impose varying levels of salt stress on wheat genotypes while minimizing confounding factors associated with soil-based experiments, such as heterogeneity in soil properties and microbial interactions [22]. This approach enables the systematic evaluation of salt tolerance traits across multiple genotypes and provides valuable insights into the underlying physiological and molecular mechanisms involved in salt stress responses. When compared to soil screens, hydroponic systems have been reported to be more suitable for high throughput screening of large numbers of seedlings [23,24].

Moreover, salt stress can affect wheat plants at any growth stage but germination and early seedling growth are the most sensitive phases and can be used as criteria to screen germplasm and breeding material [25]. Various morphological, physiological, biochemical and molecular indicators for evaluation of salt tolerance at these phases of crop establishment have been developed, including germination rate, root and shoot structure and elongation, K^+/Na^+ discrimination and Na^+ exclusion from leaves, which is considered a key mechanism of salinity tolerance in wheat crop, preventing plants from reaching high toxic concentrations [26–35].

For this purpose, the present study aimed to dissect the differential responses of 15 bread wheat genotypes to four NaCl-induced salt stress levels during the germination and seedling growth stages in a hydroponic system. By subjecting these genotypes to controlled growth conditions, we sought to gain insights into the genetic diversity in salt tolerance among wheat genotypes and inform breeding efforts designed for developing resilient varieties capable of thriving in saline-affected environments.

2. Material and Methods

2.1. Plant Material

The experiment was performed at the regional laboratory of the National Seed and Plants Control and Certification Center (CNCC, Sétif, Algeria). A total of 15 bread wheat (*Triticum aestivum* L.) released varieties (Table 1) were screened for their salt tolerance at seedling stage, these varieties from diverse origins, range of genetic backgrounds and yield potential.

Table 1. Code, name, pedigree and origin of tested bread wheat varieties.

Code	Name	Pedigree	Origin
G1	Ain Abid	AS8189'A'	Spain
G2	Akhamokh	Irena/Badax//Pastor CMSS96 M05638T-040Y-010S-010M-010S-4M-0Y	Cimmyt-Mexico
G3	Andana	Cimmyt line \times eridiano	Italy
G4	Anforeta	Eg 83 \times Bel 118	Italy
G5	Guadalupe	1656-13 \times Recital	France
G6	Massine	Pastor CM85295-0101TOPY-2M-0Y-0M-3Y-0M-0SY	CIMMYT-Mexico
G7	Mawna	Acsad529/4/C182.24/C168.3/3/Cno*2/7C//CC/Tob-0s	ACSAD-Syria
G8	Mimouni	Inia/Napo//Tob/Hprew	CIMMYT-Mexico
G9	Nesser	W.3918.A/Jup	ICARDA-Syria
G10	Orion	Arche/Genial	Serasem-France
G11	Sidi Okba	Flk's/Hork's	CIMMYT-Mexico
G12	Siete Cerros	Front./Ken58/NThatcher/3/N10/Br/2/G55	CIMMYT-Mexico
G13	Tamezghida	(Geppeto \times Apache) 8248	Serasem-France
G14	Ziad	Alondra's/Era//Son64/Alondra's	CIMMYT-Mexico
G15	Zidane	Gv/Alondra's	CIMMYT-Mexico

CIMMYT: International Maize and Wheat Improvement Center, ACSAD: Arab Center for the Studies of Arid Lands and Dry Zones, ICARDA: International Center for Agricultural Research in Dry Areas.

2.2. Germination Assay

The germination tests conducted, as per Fellahi et al. (2019) [4], adhere to a standardized protocol encompassing procedures for seed preparation, salt concentration gradients, and germination conditions. This systematic approach aimed at ensuring consistency and reliability in the experimental setup.

2.2.1. Salt Solutions' Preparation

Solutions of sodium chloride (NaCl) at concentrations of 0 (control), 50, 100 and 150 mM were used. These concentrations were selected to achieve specific electrical conductivity (EC) values corresponding to 0, 4.56, 9.12 and 13.7 dS m⁻¹, respectively. Salt solutions were prepared dissolving varying concentrations of NaCl in deionized water and the EC was precisely verified using a conventional conductometer.

2.2.2. Wheat Growth Conditions

Each genotype in each treatment had three Petri dishes containing 100 seeds. The seeds were germinated in a growth chambers environment, maintained at 85% relative humidity, with a 16-h day and 8-h night photoperiod at a constant 22 °C temperature, ensuring optimal conditions for seed germination.

During the experimental period of 7 days, Petri dishes were monitored to ensure consistent conditions for seed germination. Germinated seeds were counted daily across all salt treatments to track the progression of germination over time. This daily monitoring provided valuable data on the germination kinetics and dynamics under varying salt stress conditions.

2.2.3. Germination Measurements

The following measurements were recorded using the 'germinationmetrics' package, version 0.1.4 in RStudio [36]: germination percentage (G%, %), mean germination time (MGT, day), mean germination rate (MGR, day⁻¹), coefficient of variation of germination time (CVt, seed day⁻¹), coefficient of velocity of germination (CVG, %), germination

index (GI, day), uncertainty of germination process (U, bit), synchronization index (Z, unit less), mean daily germination (MDG, %), peak value for germination (PV, day⁻¹) and germination value (GV, No). These measurements provide comprehensive insights into various aspects of the germination process, including its timing, rate, uniformity, and overall success.

2.3. Seedlings' Establishment Assay

The seedling establishment assay commenced after the germination assessment in non-stressed conditions. From each wheat variety, 40 germinated seeds were selected for further hydroponic growth experiments. These germinated seeds were individually transferred into test tubes, each containing 25 mL of salt solutions matching the same electrical conductivity (EC) levels applied during the germination assessments (0, 50, 100 and 150 mM NaCl). Each stress level was represented by 10 test tubes, with one germinated seedling transplanted into each tube. The seedlings were then cultivated under identical growth conditions to those of the germination assay, maintaining 85% relative humidity, a 16-h light period, and a constant temperature of 22 °C. These conditions were upheld to ensure the optimal environment for seedling development and to facilitate a comprehensive comparison of seedling responses to varying levels of salt stress.

Throughout the 12-day experiment, various parameters related to root and shoot growth were measured on each sample. These included: root number (RN, No), maximum root length (RL, cm) and coleoptile length (CL, cm). Additionally, the seedlings were separated into below-ground and aerial parts, and the following measurements were recorded: root fresh weight (RFW, mg) and shoot fresh weight (SFW, mg). Total fresh biomass was also determined as follows: TFB (mg plant⁻¹) = (RFW (mg plant⁻¹) + SFW (mg plant⁻¹)).

The results obtained from both germination and seed establishment assays serve as initial screening methods to assess the ability of bread wheat seeds to germinate and establish healthy seedlings under salt stress conditions.

2.4. Statistical Data Analysis

In this study, the variables measured were categorized into two sets: germination parameters (i.e., G%, MGT, MGR, CVt, CVG, GI, U, Z, MDG, PV and GV) and growth performance parameters (i.e., RL, RN, CL, RFW, SFW and TFB). The experiment was set up in a two-factor completely randomized design with 3 replicates for the first set of variables and 10 replicates for the second set. Data of the germination parameters and those of seedlings growth were analyzed by a two-way analysis of variance. The least significant difference test at $p < 0.05$ probability level ($LSD_{0.05}$) was used to separate the test treatment means.

The stress intensity across 15 wheat genotypes was calculated as the relative reduction in total fresh biomass (TFB) due to salinity stress, normalized by the total fresh biomass produced under saline conditions. This is expressed by the formula: $((Y_p - Y_s)/Y_p)$, where: Y_p represents the TFB under non-stress conditions (0 mM NaCl) and Y_s refers to the TFB under high stress conditions (150 mM NaCl). Additionally, to identify the salt-tolerant genotypes, nine stress tolerance indices were calculated based on total fresh biomass of control (0 mM NaCl) and the plants subjected to severe stress (150 mM NaCl). These indices were computed using mathematical equations specified in Table 2 with the aid of the iPASTIC toolkit [37].

Table 2. Stress tolerance indices calculated.

Indices	Equations	References
Tolerance	$TOL = Y_p - Y_s$	[38]
Mean Productivity	$MP = (Y_p + Y_s)/2$	[38]
Geometric Mean Productivity	$GMP = \sqrt{Y_s \times Y_p}$	[39]
Harmonic Mean	$HM = 2(Y_s \times Y_p)/(Y_s + Y_p)$	[40]
Stress Susceptibility Index	$SSI = [1 - (Y_s/Y_p)]/[1 - (\bar{Y}_s/\bar{Y}_p)]$	[41]
Stress Tolerance Index	$STI = (Y_s \times Y_p)/(\bar{Y}_p)^2$	[39]
Yield Index	$YI = Y_s/\bar{Y}_s$	[42]
Yield Stability Index	$YSI = Y_s/Y_p$	[43]
Relative Stress Index	$RSI = (Y_s/Y_p)/(\bar{Y}_s/\bar{Y}_p)$	[44]

Y_p : Performance under normal conditions, Y_s : Performance under stress conditions, \bar{Y}_p : Mean performance of the genotypes under normal conditions, \bar{Y}_s : Mean performance of the genotypes under stress conditions.

Pearson's correlation coefficients between Y_p (TFB under non-stress conditions), Y_s (TFB under stress conditions) and the stress tolerance indices were calculated to explore associations between these variables. A heat map was rendered using the 'corrplot' package in RStudio [45] which visually represents the correlation coefficients between pairs of variables. Additionally, Principal Component Analysis (PCA) based on the data matrix of the above cited indices was computed. The results of PCA were visualized using the 'FactoMineR' version 2.11, 'factoextra' version 1.0.7 and 'ggplot2' version 3.5.1 packages in RStudio [46–48]. PCA allows for the reduction of the dimensionality of the data while preserving most of the variation, enabling us to identify patterns and relationships among the stress tolerance indices.

3. Results

3.1. Effects of NaCl Stress on Germination Parameters

The analysis of variance (ANOVA) revealed highly significant differences among the studied wheat genotypes concerning various germination parameters across different growth conditions (Table 3).

The results also indicated that all germination-related traits were significantly influenced by salinity stress, as demonstrated by the significant stress effect observed in Table 3. Moreover, the interaction between genotypes and salinity stress was found to be significant for all parameters examined. This suggests that the response of wheat genotypes to salinity stress varied depending on the specific germination trait analyzed. The significant genotype * salinity stress interaction emphasizes the complexity of salt tolerance mechanisms in wheat, which underscores the crucial role of genotype-specific responses to environmental stress. This interaction significantly impacts various germination parameters, notably U and Z, as indicators of both germination capacity and seedling growth tendencies.

In Table 4, average values across different salinity levels are presented. The results demonstrate a decline in all germination parameters as the salt concentration increases. Consequently, with increasing salt levels, both the mean germination time (MGT) and the uncertainty in the germination process rise.

Under the treatment of 50 mM NaCl, germination percentage (G%) across all genotypes was significantly lower (90.34%) compared to the control treatment (92.24%). This suggests that wheat seeds are sensitive to this level of salinity stress. Among the genotypes, G3 exhibited the highest value of G% with estimates of 99.33% for both the control and 50 mM NaCl stress conditions. Conversely, the lowest G% was recorded for G9, with values of 70.33% in the absence of NaCl and 77.67% under 50 mM NaCl stress (Supplementary Materials).

Table 3. Morphological Two-way ANOVA test for germination parameters of bread wheat seedlings.

S.O.V	Genotype (G)		Stress (S)		G × S		Error
	$F_{(14, 117)}$	<i>p</i>	$F_{(3, 117)}$	<i>p</i>	$F_{(42, 117)}$	<i>p</i>	
G%	679.54	<0.001	1414.17	<0.001	173.08	<0.001	20.40
MGT	0.68	<0.001	1.43	<0.001	0.07	<0.001	0.02
MGR	0.10	<0.001	0.28	<0.001	0.01	<0.001	0.00
CVt	596.61	<0.001	246.71	<0.001	158.32	<0.001	39.65
CVG	1039.29	<0.001	2833.48	<0.001	136.55	<0.001	34.22
GI	1356.08	<0.001	4611.03	<0.001	190.44	<0.001	30.94
U	0.32	<0.001	1.36	<0.001	0.15	<0.001	0.03
Z	0.06	<0.001	0.23	<0.001	0.03	<0.001	0.01
MDG	3.47	<0.001	7.22	<0.001	0.88	<0.001	0.10
PV	1568.24	<0.001	5429.48	<0.001	263.22	<0.001	51.79
GV	92,306.06	<0.001	331,997.64	<0.001	17,384.05	<0.001	2760.64

S.O.V: Source of variation, *F*: Coefficient of Snedecor–Fisher with significance at $p < 0.05$, The numbers in brackets for the *F*-test represent the degrees of freedom of the treatment and residual sources or variation, respectively. G%: Germination percentage, MGT: Mean germination time, MGR: Mean germination rate, CVt: Coefficient of variation of germination time, CVG: Coefficient of velocity of germination, GI: Germination index, U: Uncertainty of germination process, Z: Synchronization index, MDG: Mean daily germination, PV: Peak value for germination, GV: Germination value.

Table 4. Mean germination parameters of bread wheat seeds growing at different salinity levels during the seven days of the experiment.

Stress Level	G%	MGT	MGR	CVt	CVG	GI	U	Z	MDG	PV	GV
0 mM	92.24 ^a	1.37 ^a	0.75 ^a	37.51 ^a	75.42 ^a	76.75 ^a	0.87 ^b	0.64 ^a	6.59 ^a	65.55 ^a	435.04 ^a
50 mM	90.34 ^b	1.45 ^b	0.72 ^b	35.70 ^{ab}	71.82 ^b	71.71 ^b	0.90 ^b	0.63 ^a	6.45 ^b	61.28 ^b	400.84 ^b
100 mM	89.29 ^b	1.66 ^c	0.62 ^c	41.33 ^{bc}	62.05 ^c	64.17 ^c	1.20 ^a	0.49 ^b	6.38 ^b	49.02 ^c	314.23 ^c
150 mM	79.62 ^c	1.76 ^d	0.58 ^d	38.90 ^c	58.39 ^d	53.27 ^d	1.17 ^a	0.53 ^b	5.69 ^c	41.41 ^d	242.88 ^d
Mean	87.88	1.56	0.67	38.36	66.92	66.48	1.03	0.57	6.28	54.32	348.25
LSD _{0.05}	1.89	1.89	0.05	0.03	3.13	2.64	2.31	0.08	0.04	0.14	3.03

G%: Germination percentage (%), MGT: Mean germination time (day), MGR: Mean germination rate (day^{−1}), CVt: Coefficient of variation of germination time (seed day^{−1}), CVG: Coefficient of velocity of germination (%), GI: Germination index (day), U: Uncertainty of germination process (bit), Z: Synchronization index (unit less), MDG: Mean daily germination (%), PV: Peak value for germination (day^{−1}), GV: Germination value. Means in each column followed by similar letter (s) are not significantly different at 5% probability level, using Fisher's Least Significant Difference Test (LSD_{0.05}).

The final germination percentage was not significantly affected by the presence of 100 mM NaCl (89.29%) compared to the 50 mM NaCl treatment (Table 4). However, it is worth noting that the extent of reduction in G% varied among the 15 studied genotypes (Supplementary Materials). This suggests that, while the overall germination performance was not significantly different between the 50 mM and 100 mM NaCl treatments, individual genotypes may exhibit differential responses to increased salinity levels.

At an elevated NaCl concentration of 100 mM, there was a decrease in germination percentage, with the value dropping to 89.29%. Among the cultivars, G1 performed the best with a germination percentage of 99.00%, while G15 showed the highest sensitivity with a germination percentage of 73.00%. Under a higher stress level of 150 mM NaCl, the germination percentage decreased further to 79.62%. G6 exhibited the highest germination percentage (95.33%) under this treatment, while G9 recorded the lowest (44.33%). The

most severe reduction in germination percentage was observed in G9, followed by G14, with reduction rates of 36.97% and 36.81%, respectively (Supplementary Materials). G9 appeared to be the most susceptible genotype across all salt treatment levels, while G13 seemed less affected by salinity, showing the lowest reduction in germination percentage with increasing stress up to 150 mM NaCl.

Figure 1 illustrates the accumulated germination in each treatment, calculated as the number of germinated seeds per 100 seeds subjected to different concentrations of NaCl in wheat genotypes. This graphical representation provides a visual depiction of how germination rates, averaged over wheat genotype, vary across different salt stress conditions.

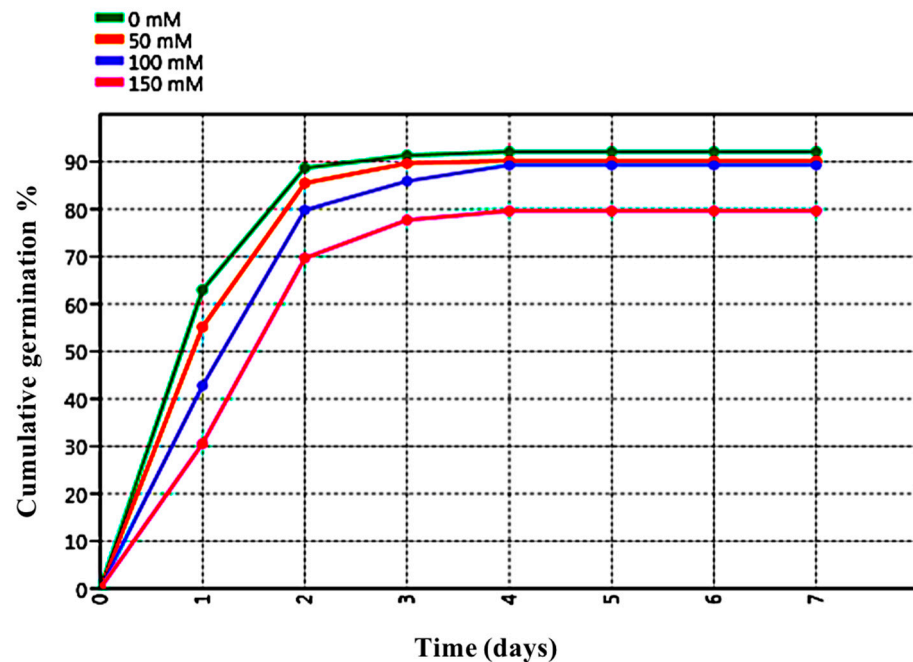


Figure 1. Germination time and cumulative mean number of bread wheat seeds germinated in each treatment during the experiment period.

3.2. Effects of NaCl Stress on Growth Parameters

The factorial analysis of variance (ANOVA), comparing the main effects of genotype and salinity stress levels, as well as the interaction effect genotype * salinity, on mean growth parameters of bread wheat seeds, revealed highly significant differences (Table 5).

Table 5. Two-way ANOVA test for growth parameters of bread wheat seedlings.

S.O.V	Genotype (G)		Stress (S)		G × S		Error
	$F_{(14, 540)}$	p	$F_{(3, 540)}$	p	$F_{(42, 540)}$	p	
RL	97.08	<0.001	2301.64	<0.001	34.29	<0.001	11.47
RN	11.33	<0.001	71.23	<0.001	4.57	<0.001	1.21
CL	6.24	<0.001	59.93	<0.001	2.32	<0.001	0.71
RFW	705.64	<0.001	3973.74	<0.001	242.56	<0.001	30.47
SFW	4529.13	<0.001	77,005.71	<0.001	1910.34	<0.001	404.00
TFB	7981.00	<0.001	112,712.00	<0.001	3053.00	<0.001	552.00

S.O.V: Source of variation, F : Coefficient of Snedecor-Fisher with significance at $p < 0.05$, The numbers in brackets for F -test represent the degrees of freedom of the treatment and residual sources or variation, respectively. RL: Root length, RN: Roots number, CL: Coleoptile length, RFW: Root fresh weight, SFW: Shoot fresh weight, TFB: Total fresh biomass.

Under low salinity stress (50 mM NaCl), certain growth parameters, such as RL, RFW, SFW, and TFB, decreased, while others, like RN, slightly increased compared to the control treatment. These findings suggest that low salinity stress negatively affects certain growth parameters but may have minor effects on others. However, when subjected to high salinity stress (150 mM NaCl), there was a significant reduction in all measured growth parameters across all wheat genotypes. The reductions were most severe for RL, RFW, SFW, and TFB, indicating the negative impact of high salinity stress on wheat seedling growth and development.

In the absence of stress, genotype G15 exhibited the highest RL, genotype G1 had the maximum RL, while genotype G7 displayed the longest CL. On the contrary, genotype G11 showed the highest RFW, SFW, and TFB, whereas genotype G9 had the lowest estimates for these traits (Supplementary Materials). Under high salt stress (150 mM NaCl), genotype G14 showed the longest RL and CL, while genotype G11 displayed the maximum RFW, SFW, and TFB. Conversely, genotype G9 exhibited the minimum values for all measured traits under high salt stress conditions (Supplementary Materials).

Table 6 illustrates the percentage change, averaged across all genotypes, of each parameter due to salinity stress. Under the low salinity stress level of 50 mM NaCl, the overall mean of the 15 genotypes decreased by 25.05%, 36.04%, 8.03% and 13.28% for RL, RFW, SFW, and TFB, respectively. However, RN of these genotypes was, on average, 10.40% higher, and their CL was 1.80% longer when grown in 50 mM NaCl compared to the control treatment. These findings suggest that, while low salinity stress negatively impacts certain growth parameters, such as RL, RFW, SFW, and TFB, it can lead to increased RN and slightly longer CL in wheat seedlings.

Table 6. Mean growth parameters of bread wheat seedlings growing at different salinity levels during the 10 days of the experiment.

Stress Level	Descriptor	RL	RN	CL	RFW	SFW	TFB
0 mM	Min	6.54	1.40	0.83	2.10	18.50	20.60
	Max	16.38	4.50	3.10	41.70	93.00	134.70
	Mean	12.07	3.46	2.17	14.78	64.11	78.89
	Std. Dev.	2.81	0.72	0.50	10.43	19.20	28.04
50 mM	Min	5.91	2.70	1.53	2.90	28.70	33.50
	Max	13.13	4.80	3.25	19.50	87.00	106.50
	Mean	9.04	3.82	2.21	9.45	58.96	68.41
	Std. Dev.	2.20	0.70	0.57	4.99	18.51	22.55
	Relative decrease (%)	−25.05	10.40	1.80	−36.04	−8.03	−13.28
100 mM	Min	3.99	2.10	0.99	3.00	20.80	24.00
	Max	10.80	4.70	3.10	8.50	61.50	70.00
	Mean	7.49	3.23	1.99	5.31	39.43	44.74
	Std. Dev.	2.09	0.63	0.60	1.69	12.76	14.12
	Relative decrease	−37.90	−6.74	−8.45	−64.05	−38.50	−43.29
150 mM	Min	0.37	0.10	0.00	0.10	0.00	0.10
	Max	6.03	3.80	2.04	6.70	34.40	41.10
	Mean	2.68	2.21	0.87	3.07	14.15	17.22
	Std. Dev.	1.69	1.05	0.62	2.60	12.33	14.83
	Relative decrease	−77.77	−36.03	−59.82	−79.21	−77.93	−78.17

RL: Root length (cm), RN: Roots number (No), CL: Coleoptile length (cm), RFW: Root fresh weight (mg plant^{−1}), SFW: Shoot fresh weight (mg plant^{−1}), TFB: Total fresh biomass (mg plant^{−1}), Min: Minimum, Max: Maximum, Std. Dev.: Standard deviation.

Increasing the concentration to 100 mM NaCl resulted in a significant reduction of 37.90%, 6.74%, 8.45%, 64.05%, 38.50% and 43.29% in the means of RL, RN, CL, RFW, SFW and TFB, respectively, over all wheat genotypes. When subjected to a much higher salinity stress level of 150 mM NaCl, the decreases in seedling parameters estimates were as follows: 77.77% for RL, 36.03% for RN, 59.82% for CL, 79.21% for RFW, 77.93% for SFW and 78.17% for TFB.

3.3. Stress Tolerance Indices

3.3.1. Stress Tolerance Indices Estimation

In this study, the stress intensity calculated as: $(Y_p - Y_s)/Y_p$ over the total fresh biomass of 15 wheat genotypes under 150 mM level of salinity was identified as 0.87 (Table 7), which suggests that the TFB under the 150 mM level of salinity was approximately 87% lower compared to the TFB under control conditions.

Table 7. Estimates of stress tolerance indices from fresh biomass yield data for bread wheat genotypes.

Genotypes	Y_p	Y_s	TOL	MP	GMP	HM	SSI	STI	YI	YSI	RSI
G1	108.20	8.30	99.90	58.25	29.97	15.42	1.18	0.14	0.48	0.08	0.35
G2	85.70	1.40	84.30	43.55	10.95	2.75	1.26	0.02	0.08	0.02	0.07
G3	72.60	9.20	63.40	40.90	25.84	16.33	1.12	0.11	0.53	0.13	0.58
G4	63.10	9.40	53.70	36.25	24.35	16.36	1.09	0.10	0.55	0.15	0.68
G5	55.30	0.50	54.80	27.90	5.26	0.99	1.27	0.00	0.03	0.01	0.04
G6	92.80	25.60	67.20	59.20	48.74	40.13	0.93	0.38	1.49	0.28	1.26
G7	76.20	8.00	68.20	42.10	24.69	14.48	1.14	0.10	0.46	0.10	0.48
G8	68.00	17.90	50.10	42.95	34.89	28.34	0.94	0.20	1.04	0.26	1.21
G9	20.60	0.10	20.50	10.35	1.44	0.20	1.27	0.00	0.01	0.00	0.02
G10	40.40	3.90	36.50	22.15	12.55	7.11	1.16	0.03	0.23	0.10	0.44
G11	134.70	41.10	93.60	87.90	74.41	62.98	0.89	0.89	2.39	0.31	1.40
G12	94.90	34.60	60.30	64.75	57.30	50.71	0.81	0.53	2.01	0.36	1.67
G13	83.60	32.90	50.70	58.25	52.44	47.22	0.78	0.44	1.91	0.39	1.80
G14	82.90	40.50	42.40	61.70	57.94	54.42	0.65	0.54	2.35	0.49	2.24
G15	104.30	24.90	79.40	64.60	50.96	40.20	0.97	0.42	1.45	0.24	1.09
Mean	78.89	17.22	61.67	48.05	34.12	26.51	1.03	0.26	1.00	0.19	0.89
Std. Dev.	28.04	14.83	21.46	19.69	21.93	21.14	0.19	0.26	0.86	0.15	0.69
SI	0.78										

Y_p : Total fresh biomass under normal conditions, Y_s : Total fresh biomass under stress conditions, TOL: Tolerance, MP: Mean Productivity, GMP: Geometric Mean Productivity, HM: Harmonic Mean, SSI: Stress Susceptibility Index, STI: Stress Tolerance Index, YI: Yield Index, YSI: Yield Stability Index, RSI: Relative Stress Index, Std. Dev.: Standard Deviation, SI: Stress Intensity.

Under non-stress conditions, the TFB of the wheat genotypes varied between 20.60 and 134.70 mg. However, in the presence of stress, TFB ranged from 0.10 to 41.10 mg. Among genotypes G11, G1 and G15 exhibited the greatest TFB under stress conditions, while the lowest values were found in genotypes G9, G10 and G5. Likewise, genotypes G11, G14 and G12 had the highest TFB under normal conditions, whereas the lowest TFB estimates were depicted in genotypes G9, G5 and G2 (Table 7). The stress intensity was identified as 0.87, which suggests that the TFB under the 150 mM level of salinity was approximately 87% lower compared to the TFB under control conditions.

3.3.2. Relationships among Indices and Produced Biomass

In order to get a clear picture of the relationships between the different stress indices of wheat genotypes grown under salt stress conditions, a heat map plot based on Pearson's correlation was generated (Figure 2). This heat map revealed a strong correlation between the TFB produced under normal conditions (Y_p) and the TFB under stress conditions (Y_s).

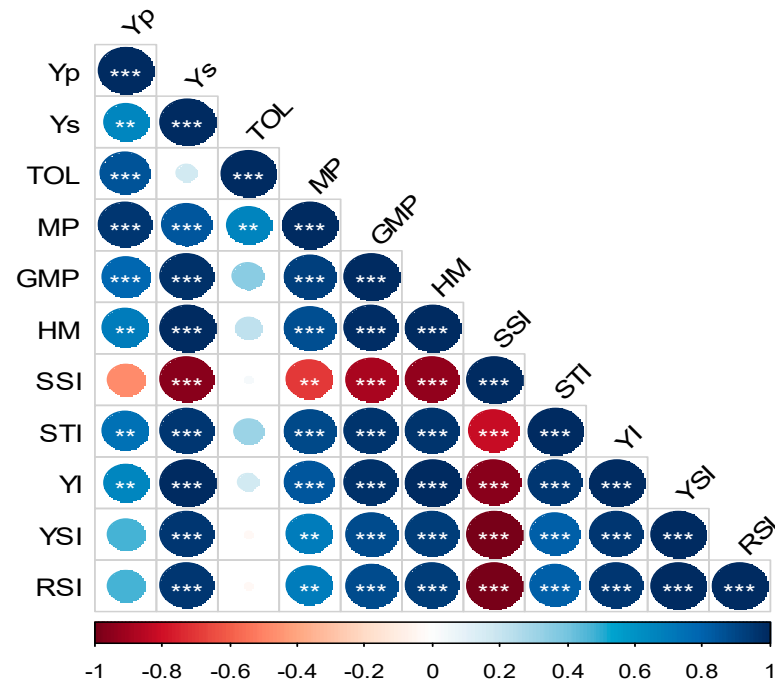


Figure 2. Heat map indicating the association among different total fresh biomass-based stress tolerance and susceptibility indices. ** Significant at $p < 0.01$, *** Significant at $p < 0.001$. Y_p : Total fresh biomass under normal conditions, Y_s : Total fresh biomass under stress conditions, TOL: Tolerance index, MP: Mean Productivity, GMP: Geometric Mean Productivity, HM: Harmonic Mean, SSI: Stress Susceptibility Index, STI: Stress Tolerance Index, YI: Yield Index, YSI: Yield Stability Index, RSI: Relative Stress Index.

The Y_p exhibited strong correlations with several stress tolerance indices, including TOL, MP, GMP, HM, STI) and YI. Notably, the GMP index also showed a high correlation with the TFB under stress conditions (Y_s) indicating its reliability in predicting genotype performance under salt stress. Alternatively, the Y_s showed a negative correlation with the SSI index, suggesting that genotypes with high SSI values tended to have lower values under stress conditions. Moreover, the Y_s demonstrated strong positive correlations with the other remaining indices, indicating consistent relationships between genotype performance under stress conditions and various stress tolerance parameters. However, the association with the TOL index was negative, suggesting an inverse relationship between genotype tolerance to stress and total fresh biomass production under stress conditions.

Additionally, a high positive relationship was observed between the TOL and MP indices, suggesting a strong association between genotype tolerance to stress and mean productivity. The heat map further revealed that five indices—MP, GMP, HM, STI and YI—were perfectly correlated with seedling performance in both non-stressed and stressed conditions.

Based on the average sum of ranks (ASR) method, genotypes G14, G11, G12, and G13 exhibited the greatest salt tolerance, as evidenced by their lowest ASR values. Conversely, genotypes G9, G5, and G2 were found to be the most susceptible to salinity stress, as indicated by their higher ASR values (Supplementary Materials).

3.3.3. Principal Component Analysis (PCA)

Principal component analysis (PCA) was conducted to explore the genetic relationships between genotypes and stress tolerance indices. Figure 3 illustrates these relationships, with red arrows representing each vector-variable, indicating the direction and strength of association between the original variables (stress tolerance indices) and the principal components. The genotypes evaluated in the study, depicted in blue on the PCA plot, are distributed across the first two axes based on their scores on these components. This positioning reflects their similarity or dissimilarity in terms of the included variables, aiding in understanding genetic relationships and associations with stress tolerance indices.

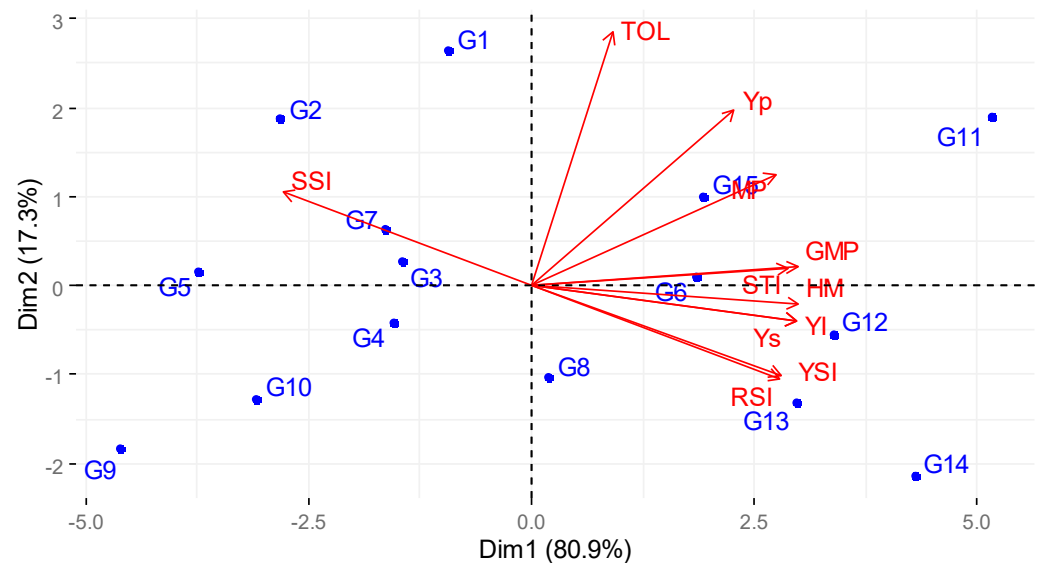


Figure 3. PCA-based biplot of stress-tolerance indices and wheat genotypes based on total fresh biomass under salt stress conditions. Y_p : Total fresh biomass under normal conditions, Y_s : Total fresh biomass under stress conditions, TOL: Tolerance index, MP: Mean Productivity, GMP: Geometric Mean Productivity, HM: Harmonic Mean, SSI: Stress Susceptibility Index, STI: Stress Tolerance Index, YI: Yield Index, YSI: Yield Stability Index, RSI: Relative Stress Index.

PC1 and PC2, with eigenvalues greater than one (8.89% and 1.91%, respectively), collectively explained 98.18% of the total variation in salt-tolerance indices among wheat genotypes (Table 8). PC1, explaining 80.9% of the variation, showed strong positive correlations with various indices, such as total fresh biomass under normal and stress conditions (Y_p and Y_s), MP, GMP, HM, STI, YI, YSI, and RSI, while it negatively correlated with SSI. Conversely, PC2 was positively influenced by the Tolerance (TOL) index. More so, genotypes G6, G11, G12, G13, G14, and G15 exhibited positive PC1 values and ranked strongly in MP, GMP, HM, STI, YI, YSI, and RSI, indicating their tolerance. Conversely, sensitive genotypes G2, G3, G4, G5, G7, G9, and G10 displayed negative PC1 values and ranked strongly in the SSI index, indicating susceptibility. The TOL index effectively separated genotypes G1 and G8 into susceptible and tolerant groups, with positive and negative PC2 values, respectively.

Table 8. Eigen value and vectors of principal component analysis for total fresh biomass of bread wheat genotypes under normal conditions (Y_p), stress conditions (Y_s) and stress tolerance indices.

Traits	PC1	PC2
Y_p	0.75	0.65
Y_s	0.99	−0.13
TOL	0.30	0.95

Table 8. Cont.

Traits	PC1	PC2
MP	0.91	0.42
GMP	0.99	0.07
HM	0.99	−0.07
SSI	−0.93	0.35
STI	0.96	0.07
YI	0.99	−0.13
YSI	0.93	−0.35
RSI	0.93	−0.35
Eigen value	8.89	1.91
Percentage of variation	80.84	17.34
Cumulative percentage	80.84	98.18

Y_p : Total fresh biomass under normal conditions, Y_s : Total fresh biomass under stress conditions, TOL: Tolerance, MP: Mean Productivity, GMP: Geometric Mean Productivity, HM: Harmonic Mean, SSI: Stress Susceptibility Index, STI: Stress Tolerance Index, YI: Yield Index, YSI: Yield Stability Index, RSI: Relative Stress Index.

4. Discussion

Results demonstrated that salt concentrations above 50 mM NaCl can delay and partially inhibit germination in wheat seeds, but they still have the ability to germinate even under high salinity levels [49,50]. This finding supports previous research indicating that germination percentage decreases as salinity levels increase. The genetic variation among different wheat genotypes had the most significant impact on various germination-related parameters, highlighting its importance in determining germination performance. Increasing NaCl concentration resulted in a longer mean germination time, indicating delayed germination initiation under higher salinity stress. However, some studies have shown that certain plant species, including wheat, may have faster germination rates under salt treatments [51–53], suggesting that quicker germination could be a strategy for seedling establishment under stressful conditions, aligning with broader plant resilience mechanisms [54].

The significant salinity effect reveals that the growing environment strongly influenced wheat seedling-associated traits. The significant genotype * salinity interaction indicates that genotypic performance under control conditions and under salt treatment exhibited different trends for all measured traits. This result suggests that the response of wheat genotypes to salt stress was influenced by their genetic makeup and that different genotypes may demonstrate distinct responses to salt stress.

The salinity stress exerted the strongest influence on the variance of all recorded traits, followed by genotype * stress interaction, while the genotype treatment ranked third in its influence on seedling growth. These findings suggest that salinity stress is the primary factor driving variability in seedling growth traits, highlighting the significant impact of environmental conditions on wheat seedling development. Moreover, the genotype * stress interaction underscores the importance of considering the complex interplay between genetic factors and environmental stressors in shaping seedling growth responses. Our results contribute to several recent studies that emphasize the contribution of different genotypes, environments, and their interactions to the expression of wheat plants at early growth stage [4,55–57].

Salinity stress negatively affects plants at the whole-plant level, leading to reduced productivity or plant death. A comparison of root and shoot fresh weight reductions across three salt treatments indicates that roots generally experience lower to moderate stress levels compared to shoots. This suggests that, under saline conditions, plants prioritize root growth to enhance water and nutrient uptake and maintain physiological functions.

However, severe stress (150 mM NaCl) results in similar decreases in both root and shoot fresh weights, indicating significant overall plant development impact. This reduction in root growth may disrupt biomass allocation balance between roots and shoots as plants prioritize stress tolerance mechanisms, like osmolyte accumulation and ion exclusion, to cope with salinity stress.

Cirillo et al. (2016) [58] observed that the root/shoot ratio did not increase under salinity stress, attributing this to a simultaneous reduction in both root and shoot biomass. Our findings corroborate this, as treating wheat seedlings with 50 mM NaCl led to shorter roots with a more branched root system compared to control conditions. Similar alterations in root architecture have been reported in earlier studies [31,55,59,60].

Previous research studies conducted on various cultivated crop species, such as wheat [61], barley [57], maize [62], rice [63], and sorghum [53], have consistently provided evidence of the detrimental effects of salinity stress on key aspects of plant growth and development, including germination, root and shoot growth, biomass accumulation, and overall productivity. According to Guttieri et al. (2001) [64], genotypes with Stress Susceptibility Index (SSI) less than or equal to one ($SSI \leq 1$) are considered stress-tolerant; those with Stress Susceptibility Index (SSI) values greater than one ($SSI > 1$) are deemed more susceptible to stress. Based on this criterion, genotypes G6, G8, G11, G12, G13, G14 and G15 were identified as stress-tolerant, as they demonstrated the lowest SSI values.

The YI, YSI, and RSI indices offer valuable insights into the performance and stability of wheat genotypes under various growth conditions. Genotypes demonstrating high values for these indices are considered tolerant [37]. Genotypes G11, G14, and G12 displayed superior vigor, stability, and salt tolerance, with high YI values. Additionally, G14, G13, and G12 performed consistently well across varying conditions, showing high values for YSI and RSI. Conversely, G9, G5, and G2 exhibited lower stability in performance, consistently displaying low values across YI, YSI, and RSI. The consistent rankings of YSI and RSI emphasize their effectiveness in identifying wheat genotypes with enhanced salt tolerance.

In the present study, a high positive relationship was observed between the TOL and MP indices, suggesting a strong association between genotype tolerance to stress and mean productivity. Furthermore, the heat map further revealed that five indices—MP, GMP, HM, STI and YI—were perfectly correlated with seedling performance in both non-stressed and stressed conditions. Such a finding elucidates their ability to identify genotypes with high performance and tolerance to saline conditions. In addition, the strong association between these indicators shows that they can be used interchangeably in the selection of salt-tolerant genotypes. These results are consistent with the findings reported by Pour-Aboughadareh et al. (2019) [37], who assessed the effect of water stress on the shoot dry weight of cultivated and wild wheat species, indicating the robustness of these indices in evaluating genotype performance under stress conditions.

In a recent study by Ivic et al. (2021) [65], MP, GMP, HM, STI, and YI were found to be strongly correlated with genotype performance and grain quality under low and sufficient amount of nitrogen conditions. YSI and RSI were positively and significantly related to total fresh biomass under stress conditions (Y_s), indicating their association with seedling performance under stress. Conversely, the TOL index showed a strong correlation with total fresh biomass under normal conditions (Y_p), suggesting its suitability for selecting genotypes with robust performance under optimal environments. This suggests that, while YSI and RSI are linked to performance under stress, TOL is more appropriate for selecting genotypes for optimal conditions.

The SSI exhibited negative correlations with MP, GMP, HM, STI, YI, YSI, and RSI. However, its correlation with the total fresh biomass under normal conditions (Y_p) was weak. While YSI, RSI, TOL, and SSI may not be suitable for simultaneous selection of genotypes with high performance and stress tolerance, they provide valuable insights into genotype responses to stress conditions. Ivic et al. (2021) [65] revealed weak or no correlations of TOL, YSI, and RSI with performance and grain protein content under stress and optimal conditions. Bahrami et al. (2014) [66] observed that SSI, TOL, and YSI

were more effective in identifying genotypes with higher yields under stress rather than under control conditions. Similarly, Ekbic et al. (2017) [67] reported that the TOL index was not distinctive in identifying salt tolerance in watermelon genotypes. These findings underscore the complexity and variability of stress tolerance indices across different plant species and environmental conditions.

In Fernandez's study (1992) [39], wheat genotypes were categorized into four groups based on their performance under control and stress conditions. Group A included genotypes with consistent performance under both conditions, while Group B comprised genotypes excelling only under control conditions. Group C consisted of genotypes showing high performance only under stress, and Group D contained genotypes performing poorly under both conditions. Our results confirm that MP, GMP, HM, STI, and YI were effective indices for identifying salt stress-tolerant wheat genotypes (Group A). The TOL index identified genotypes from Group B, while YSI and RSI distinguished genotypes from Group C, and SSI differentiated genotypes from Group D. This classification approach has also been used by Ivic et al. (2021) [65] and Bahrami et al. (2014) [66] in their studies.

Indeed, as highlighted by Pour-Aboughadareh et al. (2019) [37], relying solely on a single index to identify salt-tolerant genotypes may pose challenges. To address this, Zhao et al. (2019) [68] found no clear advantage when targeting selection based only on MP and GMP indices, which could lead to errors, since selected genotypes demonstrate mean yield performance under different nitrogen levels. These authors recommended combining these indices along with the STI index to improve the selection of wheat cultivars. Ivic et al. (2021) [65] proposed proceeding for selection based on a combination of several stress tolerance indices, such as MP, GMP, HM, STI and YI combination, to improve the accuracy of genotype selection for stress tolerance.

The average sum of ranks (ASR) (Supplementary Materials) offers another complementary approach to select potentially superior genotypes with acceptable performance under both non-stress and stress conditions [65]. Based on ASR criterion, G14, G11, G12 and G13 were qualified as the most salt-tolerant genotypes, whereas, G9, G5 and G2 were identified as the most susceptible to salinity stress. Pour-Aboughadareh et al. (2020) [69] also employed this ranking method to determine the most tolerant genotypes in a set of a durum wheat collection subjected to polyethylene glycol-induced water stress at seedling stage.

This study demonstrated that MP, GMP, HM, STI, and YI effectively identified genotypes satisfactorily under both stress and non-stress conditions, which aligns with previous experiments on various crops. Bahrami et al. (2014) [66] evaluated drought tolerance indices for safflower genotypes and demonstrated the discriminative ability of GMP and STI between drought-sensitive and -tolerant genotypes. Krishnamurthy et al. (2016) [70] highlighted the effectiveness of GMP and STI indices in identifying salt-tolerant genotypes, while TOL and SSI were effective in identifying sensitive ones [71]. These indices were also successful in screening watermelon genotypes for salt stress [67]. Studies focusing on bread wheat, maize, and beans showed that MP, GMP, and STI were highly correlated with grain yield under both control and salinity stress conditions [72,73]. Additionally, HM, along with MP, GMP, and STI, was effective for drought tolerance selection in bread and durum wheat [74,75]. Tahmasbali et al. (2020) [76] noted a positive and significant relationship between yield values under non-stress and stress conditions with MP, GMP, HM, STI, and YI in tobacco cultivars. These reports collectively indicate that tolerance indices can effectively identify stress-sensitive and tolerant genotypes with stable performance under variable environmental conditions.

5. Conclusions

Based on the obtained results, it can be concluded that both genotype and salinity factors, along with their interaction, were highly significant sources of variance for seed germination-related parameters and seedling growth-associated characteristics. As expected, increasing the severity of salinity stress resulted in a reduction of all measured traits, except MGT and U values, which increased, indicating lower germination rates,

lower homogeneity of seeds germinability, lower synchrony of germination and a slower germination pattern under stress conditions. Nevertheless, the decreases in assessed seedling features depended on the genetic background and the level of salinity stress, wherein some genotypes showed a relatively good ability to cope with stress effects. Finally, Sidi Okba (G11), Ziad (G12), Tamezghida (G13) and Zidane (G14) were qualified as the most promising salt-tolerant genotypes that could be recommended as interesting genetic resources in wheat breeding programs targeting improvement of salinity tolerance during the seedling growth stage.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy14050984/s1>.

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