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Comprehensive Evaluation of Morpho-Physiological and Ionic Traits in Wheat (*Triticum aestivum* L.) Genotypes under Salinity Stress

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Abstract: Salinity is the foremost abiotic stress that severely affects plant growth and constrains its productivity worldwide. In the present investigation, genetic variation in wheat genotypes was evaluated to identify novel salt-tolerant genetic resources, which could be used in the bread wheat improvement program. A diverse panel of 44 different wheat genotypes was evaluated at seedling stage to characterize morphological and ionic traits under salt stress (150 mM NaCl). Salt treatment caused 33.33, 45.31, 55.17, and 72.53% reduction in root dry weight (RDW), root fresh weight (RFW), shoot dry weight (SDW), and shoot fresh weight (SFW), respectively. Under salt stress, maximum inhibition of Na^+ ion uptake was observed in tolerant genotypes, and this was accompanied by a high Ca^{2+} uptake. Wheat genotypes showed a wide spectrum of responses under salt stress; however, four genotypes, EC576356, IC533596, IC279230, and IC290188, exhibited consistent performance, which was strongly linked to proper Na^+ and K^+ discrimination in leaves. The tolerant genotypes acquired a better ability to maintain stable relative water content (RWC), chlorophyll (CHL), and photosynthesis rate (PS), resulting in significantly higher dry matter production under salt stress. Further, biomass, shoot K^+, root Ca^{2+}, and shoot K^+/Na^+ were identified as the most effective parameters for screening wheat germplasm for salinity tolerance. The identified germplasm could be used as donors for transferring salt tolerance to improved cultivars as well as in further genetic studies to uncover the genetic mechanisms governing salt stress response in wheat.

Keywords: bread wheat; salt tolerance index (STI); plant biomass; shoot K^+/Na^+; root Na^+/Ca^{2+}

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1. Introduction

Wheat is one of the major cereal crops, supplying about ~20% of calorie intake and proteins in the human diet worldwide [1,2]. Globally, the annual production of wheat is 768 million metric tons, and India represents the second-largest production of 103 million tons [3]. Ensuring food supply to the ever-growing world population, which is expected to reach 9.5 billion by 2050, is a challenging task and would require a substantial jump in the world total food production [4]. Among the abiotic stresses, soil salinity is considered a major challenge in many parts of the world, and this problem is likely to increase further due to climate change as rise in sea levels could lead to the increased salinization of soils in the coastal region. Salinity is often referred to as a "white death", and almost 32 million ha of dry land and 60 million ha of irrigated land have been salinized worldwide by the dint of improper anthropogenic activities [5]. Unfortunately, recent scientific evidence indicates that ~12 million hectares (MHA) of productive lands are salinized every year by natural and anthropogenic factors, and more than half of the total cultivated area will be saline by 2050. Salinity is the major factor limiting wheat productivity globally by affecting plant growth and development. In Australia, about 69% of wheat grows in salt-affected lands. Globally, an annual economic loss of USD 27.3 billion has been estimated because of salinity [6]. In India, ~4.1 MT of production loss covering USD 0.76 billion monetary return

has been projected in the wheat crop grown under salt-affected soils. As a consequence, to ameliorate saline lands, USD ~27.3 billion are invested annually [6]. Enhancement in wheat productivity under salt-affected areas was predominantly achieved through the application of management practices such as leaching and drainage. However, the costs of these practices have increased prohibitively. Therefore, to increase the wheat production in salt-affected areas through phyto-melioration, i.e., cultivation of salt-tolerant wheat cultivars, seems to be the most effective strategy [7]. The higher intensity of salt stress severely affects seedling growth, membrane stability, and chlorophyll and biomass in early vegetative stages, and reduced tillering and grain yield in the reproductive stages [8]. Conversely, to achieve global food security and sustainability, there is a necessity to identify salt-resistant genotypes to be utilized for developing salt-tolerant wheat cultivars [9].

Salt tolerance is a complex mechanism that involves cellular and tissue level responses to minimize Na^+ and Cl^- toxicity [8]. Salinity impairs the plant developmental processes by inhibiting ionic exchanges, which lead the low photosynthetic activity in plants. Salt tolerance is articulated via three important mechanisms in plants: osmotic tolerance, shoot ion exclusion, and tissue tolerance [10–14]. Among these, shoot ion exclusion played a vital role in attributing tolerance against salt stress, as it linked with one or more salt tolerance mechanisms governing different biological pathways in plants. However, plant growth/biomass-related traits contributing to salt tolerance might be affected by ion-independent phenomena of salt accumulations in the shoot. Several morpho-physiological traits have been identified and exploited as indexes for screening of salt tolerance in plants, such as chlorophyll reduction, relative water content, plant height, fresh and dry weight of shoot and roots, and photosynthesis rate, as well as Na^+, K^+, and Ca^{2+} content [15,16]. Among them, shoot Na^+, shoot K^+, root Ca^{2+}, shoot K^+/Na^+, and root Na^+/Ca^{2+} ratios have been recognized as the most decisive and crucial morphological markers to identify salt tolerance in bread wheat. In the past, several researchers have reported that Ca^{2+} is involved as a second messenger in the structural and functional integrity of the cell membrane, enzymatic activities of the cell wall, and regulation of the ion transport selectivity. Eventually, it could be the ideal trait for further investigating the ionic interactions under salt stress in wheat genotypes [17–20]. Moreover, [21] mentioned that Ca^{2+} content in durum wheat decreased during salinity stress and declined the role of calcium signaling required for salt tolerance. Several studies have also been carried out to discriminate the important traits for screening of salt-tolerant wheat genotypes and dissecting the tolerance mechanism through genetic, physiological, and molecular mapping approaches [22–31]. Previous studies claimed relation between Na^+ and Ca^{2+} during cytosolic Ca^{2+} accumulation under drought and salinity stress and K^+/Na^+ selectivity in the saline condition in various crops [32–38]. Generally, bread wheat (*Triticum aestivum* L.) is considered moderately tolerant to salinity stress in comparison to durum wheat (*Triticum turgidum* L.), since *Kna1* locus on chromosome 4D regulates lower Na^+ and higher K^+ accumulation in the young leaves of bread wheat [39]. Similar findings were also reported by [40], through capturing the trait variation for Xylem Na^+ and K^+ content in 49 wheat cultivars (25 bread/24 durum wheat genotypes), which revealed that the Na^+ sequestration capability of bread wheat was better than durum wheat. Similarly, [41] reported that salinity-tolerant genotypes showed a higher PSII activity, photochemical efficiency, osmotic potential, K^+ content, biomass production, and lower Na^+ content under salinity stress, in comparison to the control. Collectively, most of the studies were focused on a limited number of traits, whereas multiple trait evaluation would be more efficient for a better understanding of variation and genetic dissection of salt tolerance among the genotypes rather than using a limited number of parameters [15,25].

For a better understanding of salt tolerance mechanisms, mapping of genes/QTLs, and developing the salt-tolerant wheat cultivars, the identification of salt-tolerant germplasm is a prerequisite. Several salt-tolerant bread wheat genotypes, such as KRL99, KRL3-4, KRL1-4, KRL19, KRL210, KRL 283, and KRL213, have been developed in India through conventional breeding approaches. These genotypes have shown differential response to

several abiotic stresses as well as microelement toxicities. The salinity tolerant varieties such as KRL283, KRL213, and KRL210 have become very popular in different salt-affected agro-ecosystems of Indian sub-continents, and the latest projection indicated they have covered approximately 2.4 lakh ha of wheat growing area in India [42]. Globally, the availability of donors for salt tolerance is very limited in bread wheat; for instance, "Kharchia local" is a well-known landrace of bread wheat that evolved in saline-sodic ecosystems of (Kharchia village) Rajasthan, India, and is one of the high salt tolerant donors. Therefore, identifying the new donors for salt tolerance to broaden the genetic base in bread wheat through a multivariate approach is an urgent need. With these perspectives, the present investigation was designed to identify the salt tolerant germplasm and the most important traits contributing to salt tolerance at germination and seedling stages through a multivariate approach under hydroponics. The identified germplasm will be a valuable genetic resource for breeding and further genetic studies, such as gene mapping, gene pyramiding, and a better understanding of the physiological and biochemical mechanisms of salt tolerance in wheat.

2. Materials and Methods

2.1. Plant Materials

A total of 44 bread wheat genotypes, including indigenous and exotic germplasm lines, were used in this study. These genotypes were procured from the Indian National Gene Bank, located at ICAR-NBPGR, New Delhi, India. The passport data information of the wheat genotypes is provided in Table S1.

2.2. Experimental Design

The sand culture technique was applied to evaluate the wheat genotypes for salt tolerance at the seedling stage. The experiment was performed using a complete randomized design (CRD) with 3 replications along with 2 standard checks (KRL-210 as salt-tolerant and HD2009 as salt-sensitive) with two treatments: control (Hoagland solution) and salinity stress (Hoagland solution + 150 mM NaCl) in glass-house condition with hydroponic setup. Experiments were set up in plastic plug trays containing deep pots (5 inches) with holes in the bottom for the uptake of the nutrient solution by roots. The plastic plug trays were filled with small size gravel to support wheat seedlings. Glass-house conditions were set at 15/26 °C as day/night temperature cycle and ~65% average humidity throughout the experiments. Eight seeds were sown in each pot. At the initial phase of up to 15 days, all plants were grown under normal conditions without any stress. On the 16th day, salt treatment was applied when the plants reached up to second leaf stage, whereas under control conditions plants remained in Hoagland's solution without salt stress. The solutions of both control and stress conditions were regularly monitored for pH and electrical conductivity (EC). The EC of the solution was maintained by adding water or salt. Further, solutions for both conditions were changed every 8th day. The tray containing the nutrient solution was marked, and only 80% of the solution (by volume) was changed every time so that the plants did not experience any sudden shock or microclimate change. All parameters were recorded on 32 day-old seedlings.

2.3. Plant Morphological Data

After 32 days of the experiment, plants were harvested and separated into the shoot and root parts; further, shoot fresh weight (SFW), root fresh weight (RFW), shoot length (SL), and root length (RL) were measured through weighing and metric scales. Further, the samples were kept in a dried air oven at 50–60 °C for 72 h, and shoot dry weight (SDW) and root dry weight (RDW) were recorded. Total fresh weight (TFW), total dry weight (TDW), and root shoot ratio (R/S) were estimated using the variables SFW, RFW, SDW, and RDW. The relative water content of shoot and root was also calculated using the formula: RWC = (FW − DW)/FW × 100 (fresh weight: FW, dry weight: DW). The salt tolerance index (STI) for morpho-physiological traits was estimated using the following formula.

STI: mean value under salt stress/mean value under control [43].

2.4. Chlorophyll Content and Photochemical Efficiency of PSII

Chlorophyll content (CHL) was measured in the topmost leaves of plants, using SPAD-502 plus (Konica-Minolta, Osaka, Japan) on 32 day-old seedlings. Photochemical efficiency of the plant leaves was recorded at 9:30–11:00 am by using an chlorophyll fluorometer (OPTI-SCIENCES-30p) instrument. For measurements, four plants per replication of both control and salt-treated were considered.

2.5. Membrane Stability Index

Freshly harvested 0.1 g of leaves were cut and kept in 50 mL glass test tubes and filled with double distilled water (10 mL) in three replicates. The samples were kept at 40 °C for 40 min in a water bath, and their electrical conductivity (EC1) was recorded using a digital conductivity meter. Again, the same samples were kept at 100 °C for 10 min in the water bath, and electrical conductivity (EC2) was recorded. Membrane stability index (MSI) was calculated using the equation (MSI = 1 − EC1/EC2) × 100) [44].

2.6. Na^+, K^+, Ca^{2+} Content in Shoot and Root

Approximately 1.0 g of the dried shoot and root tissue samples was kept in 75 mL digestion tubes. Then, 5 mL nitric acid (69% HNO_3) was added to each tube and heated in a digestion block till the separation of the extract in transparent phases. After that, it was cooled and diluted with distilled water to a final volume of 25 mL and filtered through ash-free quantitative filter papers placed in a glass funnel, and filtrate was collected. Na^+, K^+, and Ca^{2+} were measured using flame photometry [45] and, subsequently, the K^+/Na^+ and Na^+/Ca^{2+} ratios were estimated.

2.7. Statistical Analysis

The analysis of variance (ANOVA) was performed to estimate the effects due to genotype (G), treatment (T), and genotype × treatment (G × T) interaction using the general linear model (GLM). Descriptive statistics and frequency distribution were analyzed to check the range of variability among the traits. The Pearson correlation coefficients between salt stress traits were estimated. Principle component analysis (PCA) and predictive screening were performed to find out the important contributor's response to salt stress using SAS 9.3 (JMP) program. Heritability (H_2) was estimated using the formula $H_2 = 1 - [MS\ (genotype \times year)]/MS\ (genotype)$ according to [46].

3. Results

3.1. Effects of Salt Stress on Morpho-Physiological and Ionic Traits

Salt stress, like any other stress, has a detrimental effect on morphological and physiological traits. In this study, observations on 25 parameters, including 15 morpho-physiological and 10 ionic traits, were recorded. After 17 days of salt treatment, adverse effects were observed on plant growth and biomass-related parameters in comparison to the control condition. Analysis of variance revealed a significant difference ($p \leq 0.05$) among the genotypes (G), treatment (T), and genotypes x treatment (G × T) interaction. Mean squares (MS) for all studied traits were highly significant ($p \leq 0.05$) (Table 1). The treatment effect (salinity stress) was also highly significant and obtained in all the measured traits. All the monitored traits exhibited a higher level of variability under control and salt stress conditions except five traits, including SDW, PS, RWC_S, RWC_T, and TDW, which showed a low coefficient of variation (CV) under control conditions. The highest CV was observed for RFW (49.28%), RDW (45%), and MSI (31.17%) under salt stress conditions. Further, highest variability was observed for Na^+/Ca^{2+}_R (86.04%), root Na^+ (62.35%), K^+/Na^+_R (61.31%), and shoot Ca^{2+} (57.44%) under control conditions and Na^+/Ca^{2+}_S (57.89%) under salt stress conditions. However, the lowest variability was observed for SDW (2.75%), PS (2%), RWC_S (3.07%), TDW (3.42%), and SFW (6.72%) under salt-stressed

conditions (Table S2). The estimates of heritability (H2) ranged from 0.02 (K^+/Na^+_R) to 0.79 (root RWC) with an average of 0.51. The highest heritability was observed in physiological traits, such as root RWC (0.79), CHL (0.73), MSI (0.69), and RDW (0.66), indicating the major role of a genotypic constitution for the expression of these traits. Frequency distribution histograms of all traits (fifteen morpho-physiological traits and ten shoot ionic traits) are represented in Figure S1.

Table 1. Mean squares (MS) of the analysis of variance of all studied traits under salinity stress condition; CHL: chlorophyll, MSI: membrane stability index, RFW: root fresh weight, SFW: shoot fresh weight, TFW: total fresh weight, RDW: root dry weight, SDW: shoot dry weight, TDW: total dry weight, R/S: root/shoot ratio, RL: root length, SL: shoot length, PS: photosynthetic rate, RWC_S: relative water content of shoot, RWC_R: relative water content of root, RWC_T: total relative water content, K^+/Na^+_S: shoot K^+/Na^+, K^+/Na^+_R: root K^+/Na^+, Na^+/Ca^{2+}_S: shoot Na^+/Ca^+, Na^+/Ca^{2+}_R: root Na^+/Ca^{2+}.

S. No	Traits	Genotypes (G)	Salinity Treatments (T)	Genotype × Treatment
	df	43	1	43
1	CHL	41.028 **	649.89 **	10.90 **
2	MSI	685.03 **	45900.15 **	209.11 **
3	RFW	0.48 **	5.53 **	0.13 **
4	SFW	5.62 **	279.36 **	3.71 **
5	TFW	8.92 **	363.52 **	4.94 **
6	RDW	0.006 **	0.074 **	0.002 **
7	SDW	0.07 **	1.73 **	0.04 **
8	TDW	0.11 **	2.52 **	0.05 **
9	R/S	0.13 **	1.27 **	0.04 **
10	RL	69.90 **	1392.74 **	44.68 **
11	SL	119.00 **	8684.74 **	46.58 **
12	PS	5234.92 **	227274.68 **	3654.37 **
13	RWC_S	78.90 **	2484.55 **	27.71 **
14	RWC_R	314.79 **	459.89 **	65.31 **
15	RWC_T	86.78 **	2293.51 **	22.53 **
16	Shoot Na^+	2344.95 **	692762.80 **	2064.73 **
17	Root Na^+	1593.13 **	215913.41 **	751.39 **
18	Shoot K^+	5014.66 **	233.04 *	1568.55 **
19	Root K^+	1354.47 **	25986.23 **	952.10 **
20	Shoot Ca^+	1865.33 **	4383.89 **	556.15 **
21	Root Ca^+	166824.19 **	36555.03 **	43997.20 **
22	K^+/Na^+_S	7.75 **	1642.41 **	6.86 **
23	K^+/Na^+_R	4.39 **	529.30 **	4.30 **
24	Na^+/Ca^+_S	5.17 **	634.66 **	4.23 **
25	Na^+/Ca^+_R	0.05 **	2.08 **	0.02 **

* and ** represents significance at $p \leq 0.05$ and $p \leq 0.01$ respectively.

A significant reduction was observed in plant growth and biomass-related parameters under salt stress as compared to the control condition (Table S2). The traits, including, RDW, RFW, SDW, and SFW, decreased to the tune of 33.33, 45.31, 55.17, and 72.53%, respectively, under the salt stress condition. Similarly, salt stress significantly decreased RL, SL, and MSI by 23.15, 31.5, and 37.44%, respectively. On the contrary, salt stress has little impact on root RWC (3.19%), shoot RWC (6.83%), PS (7.95%), and CHL (10.1%). The most significant change was observed in K^+/Na^+_S, K^+/Na^+_R, Na^+/Ca^{2+}_S, and shoot Na^+ concentration. The average values of Na^+ concentration under control conditions were 15.01 mg g^{-1} in shoot and 17.93 mg g^{-1} in root, while salt treatment remarkably increased the Na^+ content in wheat accessions such as shoot (117.5 mg g^{-1}) and root (75.2 mg g^{-1}) (Table S2). Under salinity stress, Na^+ content was significantly higher; conversely, K^+ content remarkably declined in contrast to the control condition. Further, salt treatment increased Ca^{2+} content eight times in root (344.7 mg g^{-1}) in comparison to shoot (39.63 mg g^{-1}), which in turn

affects Na^+/Ca^{2+} ratio in plants. Shoot K^+, root Ca^{2+}, shoot K^+/Na^+, and root Na^+/Ca^{2+} were considered imperative traits for the salt tolerance phenomenon in bread wheat.

3.2. Categorization of Wheat Genotypes Based on Salt Tolerance Index (STI)

The salt tolerance indices (STI) of all the 44 wheat accessions considered in the experiment were estimated based on the reduction of plant biomass under salt stress in comparison to the control condition. Wheat accessions showed a broad range of the STI values having an average of 51.8%. Interestingly, all wheat genotypes could be further divided into five cluster groups based on their salt tolerance index: Cluster 1 = highly susceptible genotypes (HS; <20%); Cluster 2 = susceptible genotypes (S; 20–40%); Cluster 3 = moderately tolerant genotypes (MT; 40–60%); Cluster 4 = tolerant genotypes (T; 60–80%); Cluster 5 = highly tolerant genotypes (HT; 80–100%). Four wheat genotypes including, EC576356, IC533596, IC279230, and IC290188 performed better over the salt-tolerant check than KRL 210 (Figure 1).

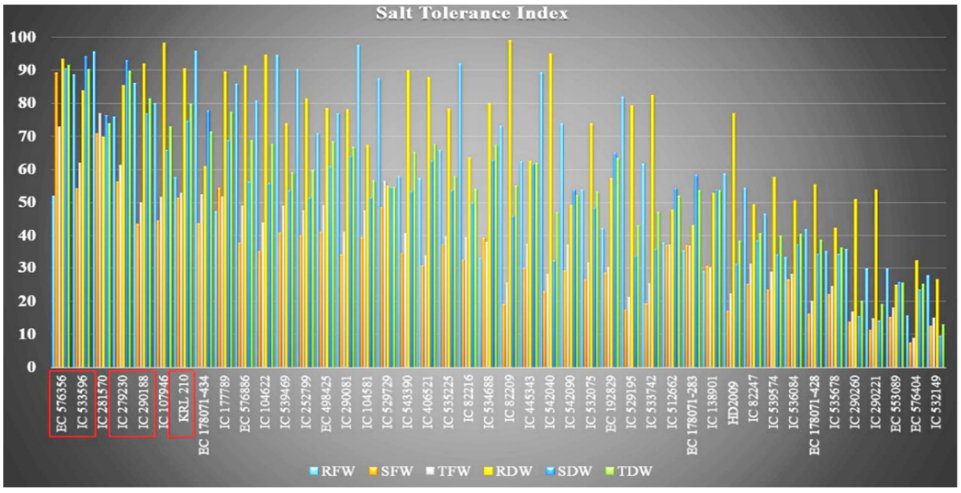

Figure 1. Bar plot of STI values of the 44 wheat accessions based on biomass traits (RFW: root fresh weight, SFW: shoot fresh weight, TFW: total fresh weight, RDW: root dry weight, SDW: shoot dry weight, and TDW: total dry weight) tested in 150 mM NaCl concentration. Tolerant genotype is indicated with a red underline.

3.3. Salt Tolerance Index and Correlation between Traits under Salt Stress

In order to find the key traits contributing to salt tolerance, we estimated correlation among traits that were inspected under salt stress (Figure 2). Pearson's correlation coefficient analysis revealed that, out of 197 trait combinations, 120 pairs were positive, and 77 pairs were negatively associated. Pearson's correlation coefficients between STI (relative value between salt and control condition of total dry weight) and various inspected traits is an important criterion to determine the role of respective traits in imparting salt tolerance to wheat genotypes. The STI (salt tolerance index) was positively correlated with CHL ($r = 0.209$), MSI ($r = 0.239$), RDW ($r = 0.252$), TDW ($r = 0.184$), and RL ($r = 0.371$). Among the ionic traits assessed, STI displayed strong positive correlation with shoot K^+ content ($r = 0.600$) and shoot K^+/Na^+ ratio ($r = 0.533$) and root Ca^{2+} ($r = 0.31$); however, it was negatively associated with shoot Na^+/Ca^{2+} ($r = -0.178$), root Na^+/Ca^{2+} ratios ($r = -0.361$), shoot RWC ($r = -0.252$), and root RWC ($r = -0.329$) (Table 2 and Figure S2). The positive correlations of shoot K^+ content, shoot K^+/Na^+ ratio, and root Ca^{2+} with STI suggested that these traits might enable plants to withstand salt stress. Therefore, these three ionic traits, as well as three morpho-physiological traits RL, MSI, and RDW, which showed strong positive correlations with STI, should be considered key parameters for assessing salt tolerance in wheat genotypes and can be used as important indirect selection criteria for the selection of salt tolerance lines in wheat breeding programs. Furthermore, we observed that higher

shoot Na^+ content under the salt stress conditions was negatively correlated with CHL ($r = -0.431$), MSI ($r = -0.214$), SFW ($r = -0.287$), RFW ($r = -0.285$), SDW ($r = -0.355$), RDW ($r = -0.317$), SL ($r = -0.264$), and PS ($r = -0.213$), showing that these phenotypes were the most affected traits with excess accumulation of shoot Na^+ content (Table S3). This shows that excess accumulation of excess Na^+ in shoot tissues can have a detrimental effect on various plant growth parameters and thus genotypes with effective Na exclusion mechanisms are better able to withstand salinity stress than those with less effective Na exclusion mechanisms.

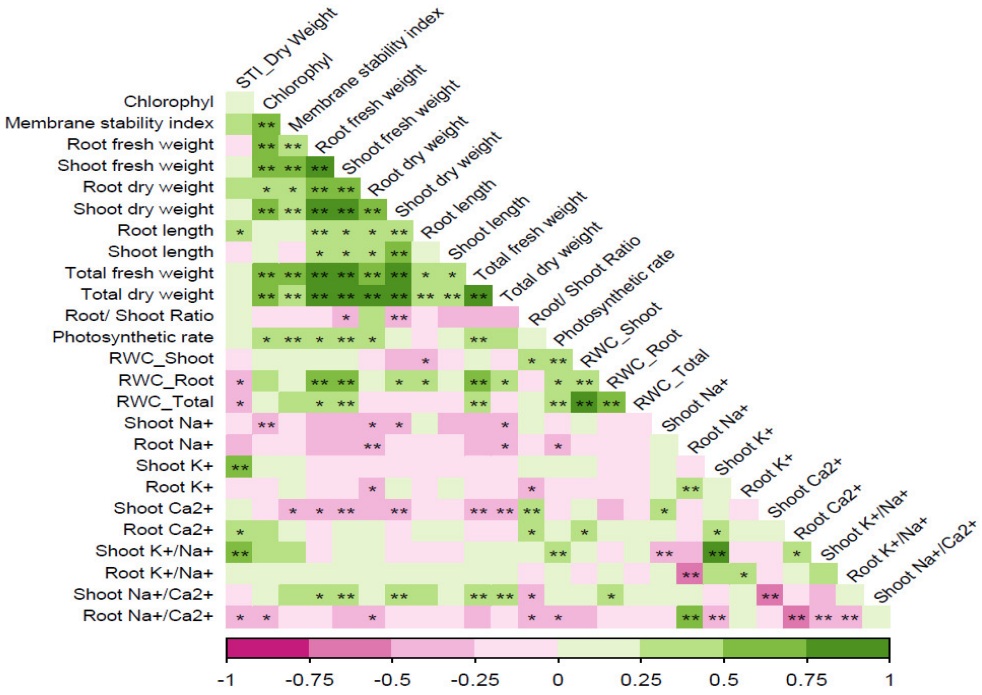

Figure 2. Correlation matrix of 24 morpho-physiological traits along with salt tolerance index (STI) evaluated at 150 mM NaCl (* and ** represent significance at $p \leq 0.05$, $p \leq 0.01$ respectively).

Table 2. Coefficients of correlation (r) between ionic traits of the shoot and root plant and salt tolerance index (STI), total dry weight (TDW), shoot dry weight (SDW), and root dry weight (RDW) after 15 days of 150 mM NaCl salt stress condition.

Trait	Treatment	STI	TDW	SDW	RDW
Shoot Na^+	Salt	NS	−0.371 **	−0.355 **	−0.317 **
	Control		−0.64 **	−0.622 **	−0.528 **
Root Na^+	Salt	−0.244 *	−0.317 **	−0.233 *	−0.443 **
	Control		−0.224 *	NS	−0.445 **
Shoot K^+	Salt	0.60 **	NS	−0.197 *	NS
	Control		−0.536 **	−0.562 **	−0.271 *
Root K^+	Salt	NS	NS	NS	−0.30 **
	Control		0.436 **	0.473 **	NS
Shoot Ca^{2+}	Salt	NS	−0.442 **	−0.473 **	−0.26*
	Control		−0.632 **	−0.649 **	−0.379 **
Root Ca^{2+}	Salt	0.31 **	NS	−0.189 *	NS
	Control		NS	NS	NS
Shoot K^+/Na^+	Salt	0.533 **	NS	NS	NS
	Control		NS	NS	NS
Root K^+/Na^+	Salt	NS	NS	NS	NS
	Control		0.51 **	0.491 **	0.443 **

Table 2. *Cont.*

Trait	Treatment	STI	TDW	SDW	RDW
Shoot Na^+/Ca^{2+}	Salt	−0.178 *	0.40 **	0.459 **	NS
	Control		0.245 *	0.29 *	NS
Root Na^+/Ca^{2+}	Salt	−0.361 **	NS	NS	−0.351 **
	Control		−0.226 *	−0.181 *	−0.344 **

* and ** represents significance at $p \leq 0.05$ and $p \leq 0.01$ respectively.

3.4. Principal Component Analysis (PCA)

In order to find out the major traits contributing to the response of salinity stress, and also to develop a better understanding of traits' efficacy and reduction of traits' dimensionality, PCA was performed (Figure S2). The first five principal components, explaining 71.54% of the total variance, were considered good representatives of them all. The PC1 accounted a significant portion of the total variance with 29.69%, and the second was 15.37%. The eigenvalues of PC1 and PC2 were 7.72 and 3.99, respectively. Biomass-related traits, such as SDW, RDW, TDW, shoot K^+/Na^+, and root K^+/Na^+, showed a positive contribution towards PC1. In contrast, shoot Na^+, root K^+, shoot Ca^{2+}, shoot Na^+, root Na^+, root Na^+/Ca^{2+}, and R/S showed a negative contribution towards PC2. Therefore, biomass-related traits, such as SDW, RDW, TDW, and ionic traits, such as shoot K^+/Na^+ and root K^+/Na^+, seem to be important for providing higher levels of salt tolerance in wheat genotypes.

3.5. Ionic Concentrations among the Wheat Genotypes

Plants under salinity stress are exposed to too much Na^+, which is very harmful to their growth and development. Moreover, excess Na^+ in soil can also interfere with the uptake and transport of other nutrients including K^+ and Ca^{2+}. However, wheat genotypes differ in their ability to uptake and transport these ions and thus also have varied responses to salinity stress.

The effects of the salinity treatment on ionic concentrations were significant for Na^+, K^+, and Ca^{2+} in both shoots and roots. From 44 wheat accessions, 20 genotypes were selected for ionic interactions within the cluster groups ($n \pm$ SE) data presented in Figure 3 (Cluster 1 = highly susceptible; Cluster 2 = susceptible; Cluster 3 = moderately tolerant; Cluster 4 = tolerant; Cluster 5 = highly tolerant) (n = 4). After 17 days of the salt treatment, a higher accumulation of Na^+ was observed in the shoot (106.36 mg g^{-1}) compared to the root (67.34 mg g^{-1}) regarding moderate to highly tolerant genotypes. In the salt stress condition, the tolerant wheat check (KRL 210) had a lower shoot Na^+ content (105.1 mg g^{-1}) than the sensitive check genotype (176.5 mg g^{-1}), but there was little difference in the root Na^+ content of the tolerant and sensitive genotypes. Shoot Ca^{2+} concentrations were found similar to the tolerant (36.01 mg g^{-1}) and susceptible genotypes (37.30 mg g^{-1}), but completely reciprocal observations were made in the case of the root. The interactions were perceived between root Na^+ and Ca^{2+} in tolerant genotypes, and four times higher uptake of K^+ was detected in shoot comparison to root under salinity stress. A higher value of K^+/Na^+ ratio in tolerant genotypes than the susceptible genotypes infers that K^+/Na^+ ion concentration plays a vital role in developing tolerance against salinity.

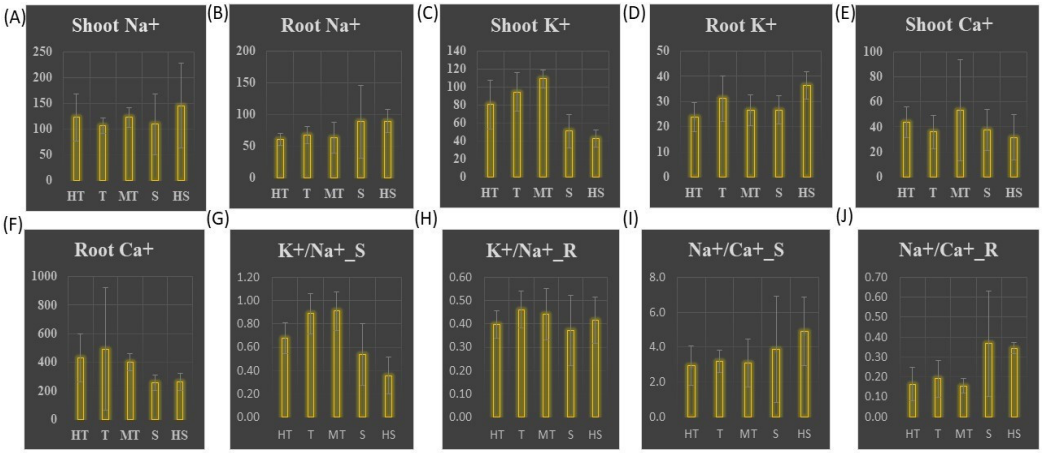

Figure 3. Mean ionic content of selected 20 wheat genotypes clustered according to their salt tolerance index (n = 4). Cluster 1 = HS: highly susceptible (<20%); Cluster 2 = S: susceptible (20–40%); Cluster 3 = MT: moderately tolerant (40–60%); Cluster 4 = T: tolerant (60–80%); Cluster 5 = HT: highly tolerant (80–100%).

3.6. Contribution of Traits in Salt Tolerance

To access the contribution of each trait in the cumulative response of salinity stress, screening clustering among the traits was performed using the mean value of all traits (Figure 4 and Table S4). The traits, including shoot K^+, MSI, RWC_R, root Ca^{2+}, root Na^+/Ca^{2+}, root K^+, photosynthetic rate (PS), root and shoot ratio (R/S), RWC_T, along with STI_TDW showing significant contribution in response to salt stress, were clustered into three distinct groups, as depicted in Figure 4. Further, four major traits, including shoot K^+ (19.1%), MSI (12.4%), RWC_R (10.3%), and root Ca^{2+} (9.3%), were the most contributed traits in response to salinity stress determining salt tolerance of wheat genotypes.

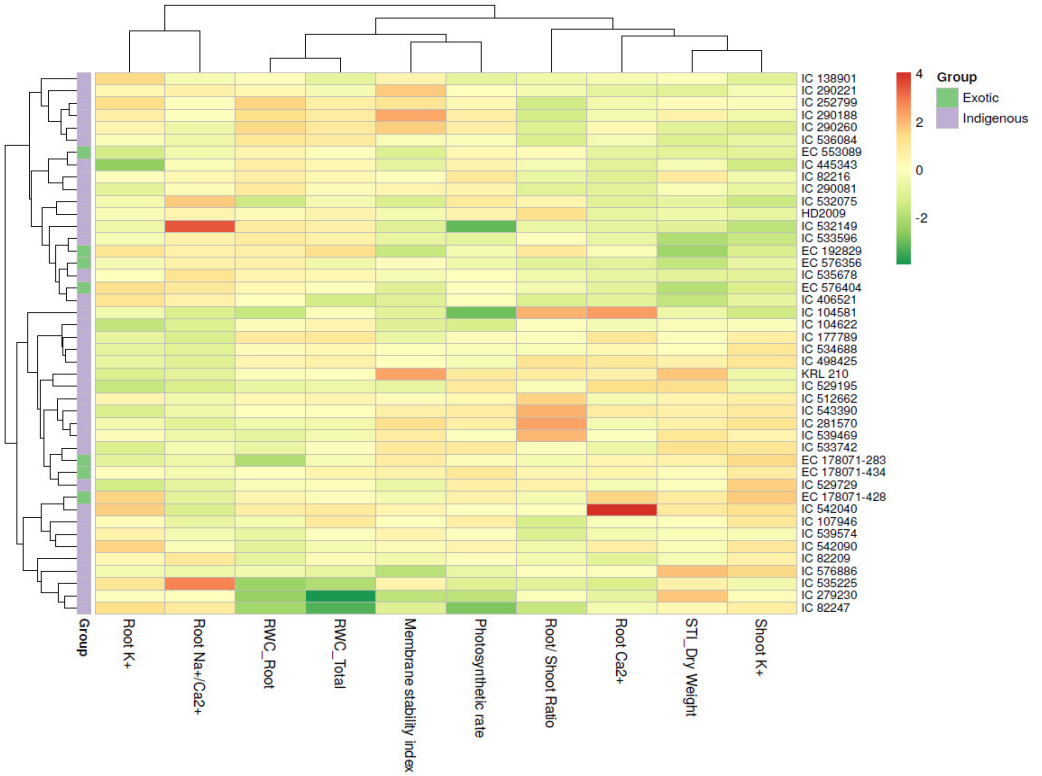

Figure 4. Clustering of wheat genotypes based on most contributing traits under salt stress condition (150 mM NaCl).

4. Discussion

4.1. Phenotypic Trait Variation

Among the major abiotic stresses, salinity is considered to have a huge impact on global wheat production. To overcome the impact of salinity stress, salt-tolerant cultivars may be one of the most effective alternatives, although developing the genotypes having the potential to sustain salt stress is a difficult task [47]. Accessing the genetic diversity in wheat germplasm could be a valuable strategy for identifying the donors for salt tolerance. In our study, high genetic variability was observed for all the 25 traits, quantified as the coefficient of variation of seedling growth parameters, ranged from 2 to 86.04%. Few traits recorded on wheat seedlings, such as leaf chlorophyll content, photosynthetic rate, shoot dry weight, root dry weight, and plant height, were negatively affected by salinity in studied wheat accessions. Similar findings were also reported in previous studies [8,25,48,49]. Wheat genotypes significantly differ for Na^+, K^+, and Ca^{2+} accumulation in shoot and root. Trait variation for Na^+ concentration ranged from 61.01 to 261.61 mg g^{-1} in shoot and from 34.2 to 173.21 mg g^{-1} in the root. The excess Na^+ accumulation in cytosol of leaves might affect cellular and morphological processes, since a higher Na^+ content in the shoot negatively affected CHL, MSI, SFW, RFW, SDW, RDW, SL, and PS in wheat accessions and finally adversely affected plant growth. Similar findings were also reported by several researchers [25–27,50–52]. Further, we observed relatively higher heritability estimates for seedling growth-related traits under salinity stress, indicating that these traits are under genetic control, and phenotypic selection can be utilized for enhancing the salt tolerance in wheat. In previous studies, a similar range of heritability for CHL, RFW, RDW, and root Na^+ has been reported in barley [53].

4.2. Na^+ and Ca^{2+} Interactions

Excess sodium accumulation in plant tissues inhibits Ca^{2+} activities and disrupts the binding sites of Ca^{2+} within a plant organ. Therefore, the Na^+ and Ca^{2+} interaction plays a key role in regulating the Na^+ toxicity tolerance in plants [54–57]. Previous studies have also demonstrated that high salt concentration restricted Ca^{2+} uptake in crops such as tomato [32], aloe vera [58], *Vigna unguiculata* [59], and durum wheat [60]. Previous studies concluded that salt-tolerant genotypes retaining a lower Na^+/Ca^{2+} ratio acquired less membrane damage, and these criteria can be applied to screen plant genotypes for salt stress tolerance [58,61]. The calcium concentrations under salt stress can be variable according to crops and genotypes [35]. Our experimental findings also indicate that high Ca^{2+} uptake in salinity stress prohibits Na^+ ion accumulation in the roots of the tolerant genotypes. Conversely, Ca^{2+} uptakes were almost similar in shoots of tolerant and susceptible genotypes. The experimental findings reaffirmed that an increased level of cytosolic Ca^{2+} concentration and decreased Na^+/Ca^{2+} ratio in the roots can be considered a selection criterion for screening of the bread wheat genotypes for salt stress tolerance.

4.3. Shoot K^+/Na^+ Response to Salt Stress

Higher Na^+ concentration in the cytosol and competition for binding sites by Na^+ or K^+ under salt stress conditions, damage cytosolic enzymes, chlorophyll, and carotenoids, which ultimately leads to inhibition of photosynthesis, and accelerates leaf senescence and premature leaf death [20,40,51,62–64]. In a previous study [65], increased levels of Na^+ automatically decreased K^+ levels in the plant's tissues under salt stress conditions. Maintenance of K^+/Na^+ ratio in the shoot has been considered one of the key features to determine salt tolerance in crop plants by several researchers [40,62,66–68]. In our experiments too, the shoot K^+/Na^+ ratio in the genotypes was highly correlated with salt stress tolerance, as reported in the previous studies on bread wheat [69], durum wheat [70], and barley [71]. In wheat genotypes, shoot K^+ content accumulated in the range of 26.17 mg g^{-1} to 125.78 mg g^{-1} under salt stress conditions, and it indicated plants delivering K^+ to shoots for retention in the mesophyll rather than exclusion for balancing the Na^+ toxicity in the cytosol of leaves. Therefore, it is stated that K^+ has a major role

in the maintenance of the overall shoot K^+/Na^+ ratio in salt-tolerant wheat genotypes. Previously, [72] also reported that salt-tolerant wheat genotypes maintained higher K^+/Na^+ ratios under saline conditions. In our study, salt-tolerant wheat genotypes also maintain a relatively lower level of Na^+ content and a higher level of K^+/Na^+ ratios, which can provide valuable genetic resources to understand the genetic and molecular mechanism of salt tolerance in wheat and donors for the improvement program.

4.4. Important Traits for Salt Tolerance Evaluation

Several studies demonstrate that plant growth is one of the prominent components, which is associated with grain yield, and determines the salt tolerance in various crops [12,25,41,73]. Salt tolerance index (STI) based on biomass reduction under salinity stress is one of the most reliable indexes to assess the potential of tolerances within the genotypes [74]. In the present investigation, several morpho-physiological traits were examined for screening of salt tolerance, such as chlorophyll, membrane stability index, plant dry weight, root length, relative water content, shoot K^+, root Ca^{2+}, and shoot K^+/Na^+ (Table 1). The estimated STI based on plant biomass was positively associated with these parameters, especially with chlorophyll, plant dry weight, shoot K^+, root Ca^{2+}, and shoot K^+/Na^+. Multiple parameters showed different types of response on wheat genotypes under salt stress conditions, revealing complications during salt tolerance assessment. In our study, biomass, shoot K^+, root Ca^{2+}, and shoot K^+/Na^+ were identified as effective parameters for salt tolerance screening in bread wheat. Furthermore, relative water content was a considerable parameter for salt tolerance evaluation. Generally, relative water content is used to describe a plant's water status, hence the increasing salinity stress causes a reduction in water content of salt-sensitive genotypes compared to tolerance. Similarly, less reduction in RWC, CHL, and PS was observed under salt stress in comparison to the control condition. Previous studies also reported that the water status of plants has a crucial role in plants because the reduced turgor potential of the leaf directly affects leaf enlargement, stomata opening, and leaf photosynthesis [75–78]. Further, experimental findings also hinted at less reduction in CHL under salt stress compared to control conditions, and the possible reason is that the salt content increases the leaf chlorophyll content in thick small leaves in salt-tolerant plants [49]. Finally, experimental observations indicated that these parameters can be efficient screening markers for salt tolerance and further studies related to wheat improvement programs.

5. Conclusions

The study systematically characterized and investigated the salt tolerance of wheat using a multiple trait approach. Based on the empirical evidence, it is concluded that six parameters out of 25, i.e., biomass, relative water content, shoot K^+ content, root Ca^{2+}, shoot K^+/Na^+, and root Na^+/Ca^{2+}, are the most suitable parameters for salinity stress tolerance screening in bread wheat. These selected parameters might be regularly used for screening wheat genotypes at the seedling stage. Further, the experimental results revealed the high phenotypic variations among the 25 morpho-physiological traits using 44 wheat accessions under salt stress and control conditions. Salt tolerance index (STI) estimated for biomass traits indicate that four genotypes (EC576356, IC533596, IC279230, and IC290188) showed higher salt tolerance. These could be used as valuable genetic resources in wheat improvement programs and future genetic and molecular studies for salt tolerance. Further, the interactions between Na^+ and Ca^{2+} were also observed to play an important role in wheat in response to salinity stress.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12111765/s1, Figure S1: Frequency distribution histogram of 25 traits measured at vegetative stage among the 44 wheat genotypes; CHL: Chlorophyll, MSI: Membrane stability index, RFW: Root fresh weight, SFW: Shoot fresh weight, RDW: Root dry weight, SDW: Shoot dry weight, TFW: Total fresh weight, TDW: Total dry weight, R/S: Root/shoot ratio, RWC_S: Relative water content of shoot, RWC_R: Relative water content of root, RWC_T: Total relative water content, RL: Root length, SL: Shoot length, PS: Photosynthetic rate, Shoot Na^+, Root Na^+, Shoot K^+, Root K^+, Shoot Ca^{2+}, Root Ca^{2+}, K^+/Na^+_S, K^+/Na^+_R, Na^+/Ca^{2+}_S, Na^+/Ca^{2+}_R, Figure S2: Principal components analysis of 25 salt tolerance traits. The arrow represents the direction of the trait, and the gradient colors represent the contribution of each trait to the components, Figure S3: The correlations between STI (salt tolerance index) based on total dry weight (TDW) and shoot K^+, root Ca^{2+}, shoot K^+/Na^+ ratio, as well as root Na^+/Ca^{2+} ratio under salt stress (150 mM, 32 days). (A) Correlation between STI and shoot K^+ content; (B) correlation between STI and root Ca^{2+} content; (C) correlation between STI and shoot K^+/Na^+ ratio; (D) correlation between STI and root Na^+/Ca^{2+} ratio, Table S1: Details of germplasm material of the 44 wheat accessions used in this study, Table S2: Descriptive statistics of 25 traits measured at vegetative stage among the 44 wheat genotypes; CHL: Chlorophyll, MSI: Membrane stability index, RFW: Root fresh weight, SFW: Shoot fresh weight, TFW: Total fresh weight, RDW: Root dry weight, SDW: Shoot dry weight, TDW: Total dry weight, R/S: Root/shoot ratio, RL: Root length, SL: Shoot length, PS: Photosynthetic rate, RWC_S: Relative water content of shoot, RWC_R: Relative water content of root, RWC_T: Total Relative water content, Shoot Na^+, Root Na^+, Shoot K^+, Root K^+, Shoot Ca^{2+}, Root Ca^{2+}, K^+/Na^+_S: Shoot K^+/Na^+, K^+/Na^+_R: Root K^+/Na^+, Na^+/Ca^{2+}_S: Shoot Na^+/Ca^{2+}, Na^+/Ca^{2+}_R: Root Na^+/Ca^{2+}, Table S3: Coefficients of correlation (r) between the fifteen morpho-physiological traits and nine ionic traits along with salt tolerance index (STI) evaluated at 150 mM NaCl, Table S4: Prediction screening showing the contribution of different traits among the wheat genotypes under salt stress condition.

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Abbreviations

STI	Salt tolerance index based on TDW
CHL	Chlorophyll (SPAD-values)
MSI	Membrane stability index
RFW	Root fresh weight (g)
SFW	Shoot fresh weight (g)
RDW	Root dry weight (g)
SDW	Shoot dry weight (g)
TFW	Total fresh weight (g)
TDW	Total dry weight (g)
R/S	Root shoot ratio based on TDW
RWC_S	Relative water content of shoot (%)
RWC_R	Relative water content of root (%)
RWC_T	Total relative water content (%)

RL	Root length (cm)
SL	Shoot length (cm)
PS	Photochemical efficiency of PSII (μmols m^{-2} s^{-1})
Shoot Na^+	Na^+ content of shoot (mg g^{-1})
Root Na^+	Na^+ content of root (mg g^{-1})
Shoot K^+	K^+ content of shoot (mg g^{-1})
Root K^+	K^+ content of root (mg g^{-1})
Shoot Ca^{2+}	Ca^{2+} content of shoot (mg g^{-1})
Root Ca^{2+}	Ca^{2+} content of root (mg g^{-1})
K^+/Na^+_S	K^+/Na^+ ratio of shoot
K^+/Na^+_R	K^+/Na^+ ratio of root
Na^+/Ca^{2+}_S	Na^+/Ca^{2+} ratio of shoot

References

1. Raman, H.; Stodart, B.J.; Cavanagh, C.; Mackay, M.; Morell, M.; Milgate, A.; Martin, P. Molecular diversity and genetic structure of modern and traditional landrace cultivars of wheat (*Triticum aestivum* L.). *Crop. Pasture Sci.* **2010**, *61*, 222–229. [CrossRef]
2. Shewry, P.R.; Lafiandra, D.; Bedo, Z. Improving the nutritional quality and health benefits of wheat. *Qual. Assur Saf. Crop. Foods* **2012**, *4*, 136. [CrossRef]
3. Ramadas, S.; Kumar, T.K.; Singh, G.P. Wheat Production in India: Trends and Prospects. In *Recent Advances in Grain Crops Research*; Shah, F., Khan, Z., Iqbal, A., Turan, M., Olgun, M., Eds.; IntechOpen: Rijeka, Croatia, 2019.
4. FAO. The Special Challenge for Sub-Saharan Africa. In *How to Feed the World 2050*; FAO: Rome, Italy, 2009.
5. Zeeshan, M.; Lu, M.; Sehar, S.; Holford, P.; Wu, F. Comparison of biochemical, anatomical, morphological, and physiological responses to salinity stress in wheat and barley genotypes deferring in salinity tolerance. *Agronomy* **2020**, *10*, 127. [CrossRef]
6. Qadir, M.; Quillérou, E.; Nangia, V.; Murtaza, G.; Singh, M.; Thomas, R.J.; Drechsel, P.; Noble, A.D. Economics of salt-induced land degradation and restoration. *Nat. Resour. Forum* **2014**, *38*, 282–295. [CrossRef]
7. Kumar, A.; Kumar, A.; Mann, A.; Devi, G.; Sharma, H.; Singh, R.; Sanwal, S.K. Phytoamelioration of the salt-affected soils through halophytes. In *Ecophysiology, Abiotic Stress Responses and Utilization of Halophytes*; Springer: Singapore, 2019; pp. 313–326.
8. Munns, R.; Tester, M. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* **2008**, *59*, 651–681. [CrossRef]
9. Munns, R.; Gilliham, M. Salinity tolerance of crops—What is the cost? *New Phytol.* **2015**, *208*, 668–673. [CrossRef] [PubMed]
10. Hauser, F.; Horie, T. A conserved primary salt tolerance mechanism mediated by HKT transporters: A mechanism for sodium exclusion and maintenance of high K^+/Na^+ ratio in leaves during salinity stress. *Plant Cell Environ.* **2010**, *33*, 552–565. [CrossRef] [PubMed]
11. Roy, S.J.; Negrao, S.; Tester, M. Salt resistant crop plants. *Curr. Opin. Biotechnol.* **2014**, *26*, 115–124. [CrossRef] [PubMed]
12. Pires, I.S.; Negrao, S.; Oliveira, M.M.; Purugganan, M.D. Comprehensive phenotypic analysis of rice (*Oryza sativa*) response to salinity stress. *Physiol. Plant* **2015**, *155*, 43–54. [CrossRef]
13. Singh, A.K.; Singh, R.; Kumar, R.; Chaurasia, S.; Yadav, S.; Kumar, S.; Wankhede, D.P. Genomics technologies for improving salt tolerance in wheat. In *Engineering Practices for Management of Soil Salinity Agricultural, Physiological, and Adaptive Approaches*; Gupta, S.K., Goyal, M., Singh, A., Eds.; Apple Academic Press: Waretown, NJ, USA, 2018.
14. Singh, A.K.; Chaurasia, S.; Kumar, S.; Singh, R.; Kumari, J.; Yadav, M.C.; Singh, N.; Gaba, S.; Jacob, S.R. Identification, analysis and development of salt responsive candidate gene based SSR markers in wheat. *BMC Plant Biol.* **2018**, *18*, 249. [CrossRef] [PubMed]
15. El-Hendawy, S.E.; Hu, Y.C.; Yakout, G.M.; Awad, A.M.; Hafiz, S.E.; Schmidhalter, U. Evaluating salt tolerance of wheat genotypes using multiple parameters. *Eur. J. Agron.* **2005**, *22*, 243–253. [CrossRef]
16. Deng, Y.Q.; Bao, J.; Yuan, F.; Liang, X.; Feng, Z.T.; Wang, B.S. Exogenous hydrogen sulfide alleviates salt stress in wheat seedlings by decreasing Na^+, content. *Plant Growth Regul.* **2016**, *79*, 391–399. [CrossRef]
17. Neves-Piestun Beatriz, G.; Bernstein, N. Salinity-induced inhibition of leaf elongation in maize is not mediated by changes in cell wall acidification capacity. *Plant Physiol.* **2001**, *125*, 1419–1428. [CrossRef] [PubMed]
18. Ashraf, M.; Harris, P.J.C. Potential biochemical indicators of salinity tolerance in plants. *Plant Sci.* **2004**, *166*, 3–16. [CrossRef]
19. Jaleel, C.A.; Gopi, R.; Manivannan, P.; Panneerselvam, R. Antioxidative potentials as a protective mechanism in *Catharanthus roseus* (L.) plants under salinity stress. *Turk. J. Bot.* **2007**, *31*, 245–251.
20. Qiu, L. Evaluation of salinity tolerance and analysis of allelic function of HvHKT1 and HvHKT2 in Tibetan wild barley. *Theor. Appl. Genet.* **2011**, *122*, 695–703. [CrossRef]
21. Meneguzzo, S.; Navari-Izzo, F.; Izzo, R. NaCl effects on water relations and accumulation of minerals in shoots, roots and cell sap of wheat seedling. *J. Plant Physiol.* **2000**, *156*, 711–716. [CrossRef]
22. Ma, L.Q.; Zhou, E.F.; Huo, N.X.; Zhou, R.H.; Wang, G.Y.; Jia, J.Z. Genetic analysis of salt tolerance in a recombinant inbred population of wheat (*Triticum aestivum* L.). *Euphytica* **2007**, *153*, 109–117. [CrossRef]
23. James, R.A.; Blake, C.; Byrt, C.S.; Munns, R. Major genes for Na^+ exclusion, Nax1 and Nax2 (wheat HKT1;4 and HKT1;5), decrease Na^+ accumulation in bread wheat leaves under saline and waterlogged conditions. *J. Exp. Bot.* **2011**, *62*, 2939–2947. [CrossRef]
24. Munns, R.; James, R.A.; Xu, B.; Athman, A.; Conn, S.J.; Jordans, C.; Byrt, C.S.; Hare, R.A.; Tyerman, S.D.; Tester, M.; et al. Wheat grain yield on saline soils is improved by an ancestral Na^+ transporter gene. *Nat. Biotechnol.* **2012**, *30*, 360–364. [CrossRef]

25. Oyiga, B.C.; Sharma, R.C.; Shen, J.; Baum, M.; Ogbonnaya, F.C.; Leon, J.; Ballvora, A. Identification and characterization of salt tolerance of wheat germplasm using a multivariable screening approach. *J. Agron. Crop. Sci.* **2016**, *202*, 472–485. [CrossRef]
26. Chaurasia, S.; Singh, A.K.; Songachan, L.S.; Sharma, A.D.; Bhardwaj, R.; Singh, K. Multi-locus genome-wide association studies reveal novel genomic regions associated with vegetative stage salt tolerance in bread wheat (*Triticum aestivum* L.). *Genomics* **2020**, *112*, 4608–4621. [CrossRef]
27. Chaurasia, S.; Singh, A.K.; Kumar, A.; Songachan, L.S.; Yadav, M.C.; Kumar, S.; Kumari, J.; Bansal, R.; Sharma, P.C.; Kuldeep Singh, K. Genome-wide association mapping reveals key genomic regions for physiological and yield-related traits under salinity stress in wheat (*Triticum aestivum* L.). *Genomics* **2021**, *113*, 3198–3215. [CrossRef] [PubMed]
28. Ghogdi, E.A.; Izadi-Darbandi, A.; Borzouei, A. Effects of salinity on some physiological traits in wheat (*Triticum aestivum* L.) cultivars. *Indian J. Sci. Technol.* **2012**, *5*, 1901–1906. [CrossRef]
29. Tao, R.; Ding, J.; Li, C.; Zhu, X.; Guo, W.; Zhu, M. Evaluating and screening of agro-physiological indices for salinity stress tolerance in wheat at the seedling stage. *Front. Plant Sci.* **2021**, *12*, 646175. [CrossRef]
30. Quan, X.; Liang, X.; Li, H.; Xie, C.; He, W.; Qin, Y. Identification and Characterization of Wheat Germplasm for Salt Tolerance. *Plants* **2021**, *10*, 268. [CrossRef] [PubMed]
31. Uzair, M.; Ali, M.; Fiaz, S.; Attia, K.; Khan, N.; Al-Doss, A.A.; Khan, M.R.; Ali, Z. The Characterization of Wheat Genotypes for Salinity Tolerance Using Morpho-Physiological Indices under Hydroponic Conditions. *Saudi J. Biol. Sci.* **2022**, *29*, 103299. [CrossRef] [PubMed]
32. Kaya, C.; Higgs, D. Calcium nitrate as a remedy for salt-stressed cucumber plants. *J. Plant Nutr.* **2002**, *25*, 861–871. [CrossRef]
33. Cheong, Y.H.; Kim, K.N.; Pandey, G.K.; Gupta, R.; Grant, J.J.; Luan, S. CBL1, a calcium sensor that differentially regulates salt, drought, and cold responses in Arabidopsis. *Plant Cell* **2003**, *15*, 1833–1845. [CrossRef]
34. White, P.J.; Broadley, M.R. Calcium in plants. *Ann. Bot.* **2003**, *92*, 487–511. [CrossRef] [PubMed]
35. Shabala, S.; Demidchik, V.; Shabala, L.; Cuin, T.A.; Smith, S.J.; Miller, A.J.; Davies, J.M.; Newman, I.A. Extracellular Ca^{2+} ameliorates NaCl-induced K^+ loss from Arabidopsis root and leaf cells by controlling plasma membrane K^+ permeable channels. *Plant Physiol.* **2006**, *141*, 1653–1665. [CrossRef]
36. Tuna, A.L.; Kaya, C.; Ashraf, M.; Altunlu, H.; Yokas, I.; Yagmur, B. The effects of calcium sulfate on growth, membrane stability and nutrient uptake of tomato plants grown under salt stress. *Environ. Exp. Bot.* **2007**, *59*, 173–178. [CrossRef]
37. Zhao, M.G.; Tian, Q.Y.; Zhang, W.H. Nitric oxide synthase-dependent nitric oxide production is associated with salt tolerance in Arabidopsis. *Plant Physiol.* **2007**, *144*, 206–217. [CrossRef] [PubMed]
38. Tattini, M.; Traversi, M.L. Responses to changes in Ca^{2+} supply in two Mediterranean evergreens, Phillyrea latifolia and Pistacia lentiscus, during salinity stress and subsequent relief. *Ann. Bot.* **2008**, *102*, 609–622. [CrossRef]
39. Dubcovsky, J.; Santa Maria, G.; Epstein, E.; Luo, M.C.; Dvořák, J. Mapping of the K^+/Na^+ discrimination locus Knal in wheat. *Theor. Appl. Genet.* **1996**, *92*, 448–454. [CrossRef] [PubMed]
40. Zhu, M.; Shabala, S.; Shabala, L.; Fan, Y.; Zhou, M.X. Evaluating predictive values of various physiological indices for salinity stress tolerance in wheat. *J. Agron. Crop. Sci.* **2016**, *202*, 115–124. [CrossRef]
41. Feng, K.; Licao, C.; Shuzuo, L.; Jianxin, B.; Wang, M.; Song, W.; Xiaojun, N. Comprehensive evaluating of wild and cultivated emmer wheat (*Triticum turgidum* L.) genotypes response to salt stress. *Plant Growth Regul.* **2018**, *84*, 261–273. [CrossRef]
42. Kulshreshtha, N.; Kumar, A.; Kumar, A.; Lata, C. Genetic Interventions to Improve Salt and Microelement Toxicity Tolerance in Wheat. In *New Horizons in Wheat and Barley Research*; Springer: Singapore, 2022.
43. Goudarzi, M.; Pakniyat, H. Evaluation of wheat cultivars under salinity stress based on some agronomic and physiological traits. *J. Agric. Soc. Sci.* **2008**, *1*, 35–38.
44. Sairam, R.K. Effect of moisture stress on physiological activities of two contrasting wheat genotypes. *Indian J. Exp. Biol.* **1994**, *3*, 584–593.
45. Vogel, A.I. *A Text-Book of Quantitative INORGANIC analysis: Theory and Practice*; Longmans Green, and Co.: London, UK, 1951.
46. Nyquist, W.E.; Baker, R.J. Estimation of heritability and prediction of selection response in plant populations. *Crit. Rev. Plant Sci.* **1991**, *10*, 235–322. [CrossRef]
47. Chandna, R.; Azooz, M.; Ahmad, P. Recent advances of metabolomics to reveal plant response during salt stress. In *Salt Stress in Plants*; Springer: New York, NY, USA, 2014; pp. 1–14.
48. Gomes-Filho, E.; Lima, C.R.F.M.; Costa, J.H.; da Silva, A.C.M.; Lima, M.D.G.S.; de Lacerda, C.F.; Prisco, J.T. Cowpea ribonuclease: Properties and effect of NaCl-salinity on its activation during seed germination and seedling establishment. *Plant Cell Rep.* **2008**, *27*, 147–157. [CrossRef]
49. Shah, S.H.; Houborg, R.; McCabe, M.F. Response of chlorophyll, carotenoid and SPAD-502 measurement to salinity and nutrient stress in wheat (*Triticum aestivum* L.). *Agronomy* **2017**, *7*, 61. [CrossRef]
50. Davenport, R.; James, R.A.; Zakrisson-Plogander, A.; Tester, M.; Munns, R. Control of sodium transport in durum wheat. *Plant Physiol.* **2005**, *137*, 807–818. [CrossRef] [PubMed]
51. James, R.A.; Davenport, R.J.; Munns, R. Physiological characterization of two genes for Na^+ exclusion in durum wheat, Nax1 and Nax2. *Plant Physiol.* **2006**, *142*, 1537–1547. [CrossRef] [PubMed]
52. Genc, Y.; Oldach, K.; Verbyla, A.P.; Lott, G.; Hassan, M.; Tester, M.; Wallwork, H.; McDonald, G.K. Sodium exclusion QTL associated with improved seedling growth in bread wheat under salinity stress. *Theor. Appl. Genet.* **2010**, *121*, 877–894. [CrossRef]

53. Long, N.V.; Dolstra, O.; Malosetti, M.; Kilian, B.; Graner, A.; Visser, R.G.; van der Linden, C.G. Association mapping of salt tolerance in barley (*Hordeum vulgare* L.). *Theor. Appl. Genet.* **2013**, *126*, 2335–2351. [CrossRef]
54. Buschmann, P.H.; Vaidyanathan, R.; Gassmann, W.; Schroeder, J.I. Enhancement of Na^+ uptake currents, time-dependent inward-rectifying K^+ channel currents, and K^+ channel transcripts by K^+ starvation in wheat root cells. *Plant Physiol.* **2000**, *122*, 1387–1398. [CrossRef]
55. Reid, R.J.; Smith, F.A. The limits of sodium/calcium interactions in plant growth. *Funct. Plant Biol.* **2000**, *27*, 709–715. [CrossRef]
56. Cramer, G.R. Sodium-calcium interactions under salinity stress. In *Salinity, Environment-Plants-Molecules*; Lauchli, A., Luttge, U., Eds.; Kluwer: Dordrecht, The Netherlands, 2002; pp. 205–227.
57. Nedjimi, B.; Daoud, Y. Ameliorative effect of $CaCl_2$ on growth, membrane permeability and nutrient uptake in Atriplex halimus subsp. schweinfurthii grown at high (NaCl) salinity. *Desalination* **2009**, *249*, 163–166. [CrossRef]
58. Jin, Z.M.; Wang, C.H.; Liu, Z.P.; Gong, W.J. Physiological and ecological characters studies on Aloe vera under soil salinity and seawater irrigation. *Process Biochem.* **2007**, *42*, 710–714. [CrossRef]
59. Murillo-Amado, B.; Jones, H.G.; Kaya, C.; Aguilar, R.L.; Garcia-Hernandez, J.L.; Troyo-Di'eguez, E.; Avila-Serrano, N.Y.; Rueda-Puente, E. Effects of foliar application of calcium nitrate on growth and physiological attributes of cowpea (*Vigna unguiculata* L. Walp.) grown under salt stress. *Environ. Exp. Bot.* **2006**, *58*, 188–196. [CrossRef]
60. Allen, G.J.; Wyn-Jones, R.G.; Leigh, R.A. Sodium transport in plasma membrane vesicles isolated from wheat genotypes with differing K^+/Na^+ discrimination traits. *Plant Cell Environ.* **1995**, *18*, 105–115. [CrossRef]
61. Hadi, M.R.; Khoush, K.S.N.; Khavarinezhad, R.A.; Khayam, N.S. The effect of elements accumulation on salinity tolerance in seven genotype durum wheat (*Triticum turgidum* L.) collected from the of middle east. *Iran J. Biotechnol.* **2008**, *21*, 1–15.
62. Tester, M.; Davenport, R. Na^+ tolerance and Na^+ transport in higher plants. *Ann. Bot.* **2003**, *91*, 503–527. [CrossRef] [PubMed]
63. Anschutz, U.; Becker, D.S. Going beyond nutrition: Regulation of potassium homoeostasis as a common denominator of plant adaptive responses to environment. *J. Plant Physiol.* **2014**, *171*, 670–687. [CrossRef] [PubMed]
64. Cherel, I.; Lefoulon, C.; Boeglin, M.; Sentenac, H. Molecular mechanisms involved in plant adaptation to low K^+ availability. *J. Exp. Bot.* **2014**, *65*, 833–848. [CrossRef] [PubMed]
65. Kanawapee, N.; Sanitchon, J.; Lontom, W.; Threerakulpisut, P. Evaluation of salt tolerance at the seedling stage in rice genotypes by growth performance, ion accumulation, proline and chlorophyll content. *Plant Soil.* **2012**, *358*, 235–249. [CrossRef]
66. Munns, R. Comparative physiology of salt and water stress. *Plant Cell Environ.* **2002**, *25*, 239–250. [CrossRef] [PubMed]
67. Cuin, T.A.; Betts, S.A.; Chalmandrier, R.; Shabala, S. A root's ability to retain K^+ correlates with salt tolerance in wheat. *J. Exp. Bot.* **2008**, *59*, 2697–2706. [CrossRef] [PubMed]
68. Yang, C.W. Evolution of physiological responses to salt stress in hexaploid wheat. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 11882–11887. [CrossRef]
69. Genc, Y.; McDonald, G.K.; Tester, M. Reassessment of tissue Na(+) concentration as a criterion for salinity tolerance in bread wheat. *Plant Cell Environ.* **2007**, *30*, 1486–1498. [CrossRef]
70. Munns, R.; James, R.A. Screening methods for salinity tolerance: A case study with tetraploid wheat. *Plant Soil* **2003**, *253*, 201–218. [CrossRef]
71. Shen, Q.; Fu, L.; Qiu, L.; Xue, F.; Zhang, G.P.; Wu, D. Time-course of ionic responses and proteomic analysis of a Tibetan wild barley at early stage under salt stress. *Plant Growth Regul.* **2016**, *81*, 11–21. [CrossRef]
72. Shabala, S.; Shabala, S.; Cuin, T.A.; Pang, J.; Percey, W.; Chen, Z.; Conn, S.; Eing, C.; Wegner, L.H. Xylem ionic relations and salinity tolerance in barley. *Plant J.* **2010**, *61*, 839–853. [CrossRef] [PubMed]
73. Farissi, M.; Faghire, M.; Bargaz, A.; Bouizgaren, A.; Makoudi, B.; Sentenac, H.; Growth, G.C. Nutrients concentrations, and enzymes involved in plants nutrition of alfalfa populations under saline conditions. *J. Agric. Sci. Technol.* **2014**, *16*, 301–314.
74. Talei, D.; Valdiani, A.; Yusop, M.K.; Abdullah, M.P. Estimation of salt tolerance in Andrographis paniculata accessions using multiple regression model. *Euphytica* **2013**, *189*, 147–160. [CrossRef]
75. Çiçek, N.; Çakirlar, H. The effect of salinity on some physiological parameters in two maize cultivars. *Bulg. J. Plant Physiol.* **2002**, *28*, 66–74.
76. Hossain, A.A.; Halim, M.A.; Hossain, F.; Maher Niger, M.A. Effect of NaCl salinity on some physiological characters of wheat (*Triticum aestivum* L.). *Bangladesh J. Bot.* **2006**, *35*, 9–15.
77. Asfaw, K.G. Effects of salinity on seedling biomass production and relative water content of twenty sorghum (*Sorghum biolor* L. Moench) accessions. *Asian J. Agric. Sci.* **2011**, *3*, 242–249.
78. Win, K.T.; Oo, A.Z.; Hirasawa, T.; Ookawa, T.; Yutaka, H. Genetic analysis of *Myanmar vigna* species in responses to salt stress at the seedling stage. *Afr. J. Biotechnol.* **2011**, *10*, 1615–1624.