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Integrating Agro-Morpho-Physiological Traits and SSR Markers for Detecting the Salt Tolerance of Advanced Spring Wheat Lines under Field Conditions

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Abstract: To successfully enhance the salt tolerance of genotypes, it is crucial to conduct field-based trials, establish effective screening criteria and analysis tools, evaluate salt tolerance at various growth stages, and integrate phenotypic assessment-based traits with molecular markers. This study aimed to assess the salt tolerance of 16 F8 recombinant inbred lines (RILs) and eight genotypes by analyzing 13 agro-morpho-physiological traits using various analysis tools and SSR markers under both control and high salinity levels (15 dS m^{-1}) in real field conditions. Analysis of variance (ANOVA), comparison of mean values, calculation of reduction percentage, and multivariate analysis were used to compare the assessed traits among genotypes and identify which traits are the most effective ones in describing the salt tolerance of these genotypes. A heatmap cluster analysis (HMCA) was also employed to categorize the salt tolerance of genotypes into different clusters based on the stress tolerance index (STI) for all traits. The ANOVA results revealed significant statistical differences ($p \leq 0.05$) between the genotypes and salinity levels for all assessed traits in each season and their combined data. Moreover, the 150 mM NaCl treatment led to decreases in the assessed traits by 10.2% to 36.9% when compared to the control treatments. Furthermore, the mean values of assessed traits for certain genotypes were approximately one to three times greater than those of other genotypes. Principal component analysis has identified plant dry weight, green leaf area, leaf area index, and grain yield per hectare as effective screening criteria for explaining the substantial variation observed among the genotypes. The HMCA successfully grouped genotypes into three distinct clusters and distinguished the salt-tolerant genotypes from the salt-sensitive and intermediate ones. The 24 genotypes/RILs were classified into three main groups according to the allelic data of 40 SSRs associated with salt-tolerant genes. A weak yet significant correlation was observed between the similarity coefficients of agro-morpho-physiological traits and SSR markers, as determined by the Mantel test (r = 0.13, $p < 0.03$, and alpha = 0.05). In conclusion, this study has successfully identified several traits, particularly those associated with SSR markers, that greatly contribute to our understanding of the phenotypic and genotypic basis influencing the salt tolerance of wheat genotypes in real field conditions. Consequently, assessing these traits for a large number of wheat plant materials in a rapid and cost-effective manner will be greatly importance in breeding programs aimed at improving salt stress tolerance in this vital food crop. This will be the main focus of our forthcoming research.

Keywords: chlorophyll content; mantel test; multivariate analysis; relative water content; saline water; stress tolerance index; yield components

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1. Introduction

Currently, numerous environmental stresses present a substantial threat to global food security. On the other hand, global food production needs to be increased by about

70% to ensure food security for the projected population of 9.7 billion people by 2050 [1]. Although the annual increase of 1.0–1.6% in the production of most major food crops, such as wheat, rice, and maize, the impact of environmental stresses on grain yield (GY) surpasses this growth rate. This situation will intensify the pressure on global food security in the coming years [2]. Salinity is widely recognized as one of the most prominent of these environmental stresses that greatly reduces GY for most food crops in many parts of the world. Under moderate salinity stress (40–80 mM NaCl), the average GY of most major food crops may be globally reduced by over 50% [3–5]. Statistically, salinity stress currently affects about 20% of cultivated areas. Furthermore, it is projected to reach 50% or more by 2050, as approximately 1.5 million hectares of cultivated land are salinized annually due to both natural and human factors. In addition, salinity stress significantly restricts the productivity of approximately 33% of irrigated lands, which are responsible for producing around one-third of the world's food supply [4,6]. Moreover, there is a loss of over USD 13 billion annually due to irrigation-induced salinity alone [7]. All these facts about salinity may explain why there is a close association between food crises and salinity issues in the agricultural sector, particularly in arid and semiarid countries.

Bread wheat (*Triticum aestivum* L.) is a moderately salt-tolerant crop with a salinity threshold of about 7.0 dS m^{-1} (70 mM NaCl). However, higher salinity concentrations such as 15.0 dS m^{-1} (150 mM NaCl) can cause a significant decrease in its potential yield of up to 60%, especially when grown in open-field conditions [8]. Therefore, the introduction of salt-tolerant genotypes that can produce satisfactory GY even in environments with high salt concentrations is recognized as the most beneficial approach for sustaining wheat production under salinity conditions [3,6,8]. Therefore, the primary goal of plant breeders is to exploit genetic variations in salt tolerance within wheat germplasm in order to identify potential donors with salt tolerance and incorporate them into breeding programs. The ultimate end of this goal is to create new wheat genotypes that exhibit greater tolerance to salt stress compared to others. Despite the simplicity of this goal, there has been little progress in developing high-yielding wheat genotypes that are tolerant to salinity. According to the literature, this lack of progress can be attributed to several reasons. Firstly, several screening experiments only assess the salt tolerance of genotypes during germination and the early vegetative phase without considering that the salt tolerance of genotypes can vary throughout different growth stages [9,10]. Thus, experiments that evaluate the performance of genotypes under salt stress conditions during both the vegetative and reproductive stages are urgently needed. Secondly, the majority of experiments evaluating the salinity tolerance of genotypes and understanding the mechanism of salt tolerance are conducted in controlled conditions, such as a greenhouse and growth chamber, using sand or hydroponics as growth media. However, these conditions do not accurately reflect the complexity of field conditions and thus do not provide a reliable proof of concept for plant performance in open-field conditions [4,11]. Thirdly, there is a lack of appropriate evaluation methods and screening criteria that can accurately assess the salt tolerance of genotypes under open-field conditions. Under real field conditions, the wheat plants are exposed to high variability in physical and chemical properties of soil, temporal and spatial variability in salt and water contents in the root zone at different growth stages, and fluctuations in air temperature and humidity [4,8,10]. Fourthly, although the mechanisms associated with salt stress responses in plants are complex and polygenic traits, there are few screening studies that evaluate the salt tolerance of genotypes based on multiple traits. It is known that the different components of salinity stress, including osmotic, ionic, and essential ion imbalance components, simultaneously cause many morphological, physiological, and metabolic changes [4,12,13]. Fifthly, the evaluation of salt tolerance is primarily focused on the GY criterion, even though direct selection for GY is often inefficient due to its low heritability. Therefore, evaluating the salt tolerance of genotypes based on secondary traits that are highly correlated with GY and have a high heritability can make the assessment of the salt tolerance of genotypes more accurate and efficient [14,15].

In general, the different components of salinity stress interact together and result in a notable reduction in several agro-morpho-physiological traits, including plant dry weight (PDW), leaf area (LA), total chlorophyll content (Chlt), and leaf water content (LRWC), as well as different yield components such as thousand-grain weight (TGW), spike length (SL), grain number per spike (GNPS), spike number per plant (SNPP), and harvest index (HI). It is worth noting that these traits show a reduction even at low salinity levels [3,8,16]. For instance, Munns et al. [3] reported that a low salinity level may not reduce wheat GY despite reducing PDW and LA. However, GY only decreases once a certain salinity threshold is reached. Similarly, Radi et al. [17] and Uzair et al. [18] showed that salt stress caused a significant reduction in wheat PDW, with a more pronounced effect on salt-sensitive genotypes compared to salt-tolerant ones. Importantly, PDW and LA exhibit high heritability coupled with a high expected genetic gain from selection and are strongly associations with GY under salinity conditions [15,19,20]. Therefore, PDW and LA traits can serve as effective screening criteria for distinguishing salt-tolerant genotypes from salt-sensitive ones in real field conditions.

As toxic ions build up in the leaf blade, the negative effect of salt stress on photosynthetic pigments can be detected before irreversible morphological damage becomes apparent [21]. Furthermore, Munns et al. [3] found that the wheat lines with high levels of Na^+ lose chlorophyll at a faster rate compared to those with low Na^+ levels. In addition, Omrani et al. [20] conducted a study using seven generations of wheat grown under normal and saline conditions in the field, and they reported a high broad-sense heritability for Chlt and SPAD values in saline conditions. Moreover, a strong positive correlation between Chlt and overall plant salinity tolerance was found in several field crops, including wheat, barley, rapeseed, and chickpea [22,23]. Considering this, the maintenance of photosynthetic pigments in saline conditions can be seen as a key physiological criterion for differentiating between salt-tolerant and salt-sensitive genotypes.

A high concentration of salt in the root zone can impair the plant's ability to absorb water, similar to the effects of drought stress. This subsequently results in an imbalance in the plants' water status, specifically their LRWC, which reflects the balance between water uptake and transpiration. A reduction in LRWC can affect and alter various physiological and metabolic processes [24]. Therefore, the ability of genotypes to maintain optimal water content levels in tissues is an important mechanism for counteracting the effects of salinity stress. Previous studies have also shown a moderate broad-sense heritability for RWC under salt stress conditions [24]. Accordingly, LRWC can be considered an important physiological criterion for evaluating the salt tolerance of genotypes.

Because wheat GY is significantly influenced by the environment and exhibits low heritability, especially under environmental stresses, plant breeders rely on yield-related traits, including TGW, SL, GNPS, and SNPP, as indirect screening criteria to evaluate genotypes and improve their yield under both normal and stress conditions [25,26]. In addition, HI, which represents the ratio of GY to biological yield (BY) and indicates the allocation of photosynthetic products between source and sink organs, is also an important indirect screening criterion. Therefore, the secondary traits with high heritability can be considered essential agronomic criteria for evaluating the salt stress tolerance of genotypes [14,27,28].

Although several agro-morpho-physiological traits have been identified as effective screening criteria, these traits are often affected by environmental conditions and are also dependent on the developmental growth stages. Furthermore, this becomes even more problematic in salt tolerance assessment because any change in the environment can alter the salt tolerance between genotypes [29]. Therefore, when agro-morpho-physiological traits are used alone, they show some restrictions in assessing genetic diversity in salt tolerance. Moreover, assessing the salt tolerance of genotypes based on conventional phenotypic variance is expensive and time-consuming. Fortunately, molecular markers have helped identify the genes responsible for the complex agro-morpho-physiological traits that confer salt tolerance in several field crops. This has resulted in a more efficient and cost-effective evaluation process [14,15,30]. Therefore, in order to enhance the precision of salt

tolerance evaluation in genotypes, it is crucial to integrate phenotypic assessment based on agro-morpho-physiological traits with molecular markers to identify the candidate genes involved in the variation of these traits. Molecular markers offer a vast number of markers that can be used to compare individual genotypes across different environmental conditions. Furthermore, they are not limited by crop growth stages, enabling the combination of different tolerance traits into a single efficient genotype. Additionally, they provide objective data that can be analyzed. Among the various molecular markers available for genetic characterization, simple sequence repeat (SSR) or microsatellite markers are extensively used for cultivar identification, germplasm characterization, genetic diversity, and molecular mapping due to their numerous advantages, including cost-effectiveness, the presence of multi-alleles, high levels of polymorphism, high-throughput capabilities, abundance, co-dominant, locus-specificity, informative, lack of bias, and repeatability [15,31–33]. Therefore, SSR markers can play a vital role with phenotypic traits in evaluating the salt tolerance of wheat genotypes and identifying the most salt-tolerant ones. Fortunately, there are several successful SSR primers, including Gwm 312, Xgwm312, and Xwmc170, that have been used to assess the salt tolerance of wheat genotypes. These primers are effective in identifying the Nax1 gene, which serves as an indicator of Na^+ exclusion [34–36]. Thus, SSR analysis of DNA polymorphism can be instrumental in identifying the key genes associated with salt tolerance.

The main objectives of this study were to (1) assess the salt tolerance of 16 F8 recombinant inbred lines (RILs) and eight genotypes grown in real field conditions, using different agro-morpho-physiological traits, (2) identify which traits can serve as effective screening criteria for distinguishing salt tolerance among genotypes, employing multivariate and cluster analysis, and (3) validate these screening criteria by examining the genetic basis using SSR markers, specifically by examining the association between the matrices of the traits and SSR data using Mantel test.

2. Materials and Methods

2.1. Plant Materials

The genetic plant materials used in this study consisted of 24 diverse bread wheat genotypes (*Triticum aestivum* L.). These genotypes comprised 8 varieties and 16 F8 recombinant inbred lines (RILs). The eight varieties included three parents (Sakha-93, Sakha-61, and Sids-1) and other five cultivars (Kharchia 65, Shandawel-1, Misr-1, Gemiza-9, and Kawz). Based on our previous evaluations in the pot experiment and actual saline field conditions, the three parents, Sakha-93, Sakha-61, and Sids-1, were considered to be salt-tolerant, salt-sensitive, and moderately salt-tolerant genotypes, respectively [8,12,37]. Kharchia 65 was also considered a salt-tolerant genotype and is used as a standard cultivar for evaluating wheat's salt tolerance [12,37–39]. Based on the study of Mansour et al. [40] under actual saline field growing conditions, Shandawel-1, Misr-1, and Gemiza-9 were considered to be salt-sensitive, moderately salt-sensitive, and moderately salt-tolerant genotypes, respectively. Among sixteen RILs developed at the Faculty of Agriculture, Suez Canal University, Ismailia, Egypt, and the College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia, five were derived from a cross between Sakha 93 and Sids 1 and eleven were derived from a cross between Sakha 93 and Sakha 61.

2.2. Experimental Site and Growth Conditions

All genetic plant materials were evaluated under open-field conditions at the Research Station (24°25′ N, 46°34′ E, 400 m a.s.l.) of the Department of Plant Production, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia (Figure 1), during the winter seasons of 2019/2020 (S1) and 2020/2021 (S2). According to data from the meteorological station located near the research station (approximately 500 m away from the field experiment) and during the wheat's growing stages (December to April), the minimum temperature ranged from 7 °C to 19.5 °C in S1 and 8.3 °C to 20 °C in S2. The maximum temperature ranged from 21.3 °C to 34.1 °C in S1 and from 22.3 °C to

35.5 °C in S2. Precipitation ranged from 0.0 mm to 6.25 mm in S1 and 0.32 mm to 3.66 mm in S2. Analysis of soil samples taken from the experimental farm at a depth of 0–30 cm showed that the soil texture is sandy loam (i.e., 14.9% clay, 28.4% silt, and 56.7% sand) and characterized by the following physicochemical properties: organic matter, 0.46%; Walkley–Black C, 0.34%; bulk density, 1.48 g cm^{-3}; electrical conductivity (EC), 1.12 dS m^{-1}; pH, 7.85; $CaCO_3$, 29.22%; available N, 3.98 g kg^{-1}; available K, 1.67 mg kg^{-1}; available P, 0.07 mg kg^{-1}; water holding capacity, 18.56%; and permanent wilting point, 7.21%.

Figure 1. Plot layouts of genotypes grown under control and salinity treatments in real field conditions.

2.3. Experimental Design, Agronomic Practices, and Treatments

The field experiment was conducted as a split-plot design with three replications. Salinity levels, including the control (≈0.35 dS m^{-1}) and a high salinity concentration of 15.0 dS m^{-1}, were referred to as the main plot, while twenty-four bread wheat RILs/genotypes were assigned at random to the subplots. Therefore, the field experiment included 144 experimental units (2 salinity levels × 24 RILs/genotypes × 3 replications). Each experimental unit had five rows of planting, with a length of 1.5 m, row spacing of 20 cm, and a space of 50 cm between experimental units. The replications and salinity levels were separated by buffer zones of 1 and 3 m, respectively.

In both growing seasons, the different genotypes were sown at a seeding rate of 15 g m^{-2} during the optimum period, which was the fourth week of November. All genotypes were fertilized with a recommended dose of nitrogen–phosphorus–potassium (NPK) fertilizer at a rate of 150, 100, and 90 kg ha^{-1}, respectively. The NPK fertilizers were applied in the form of ammonium nitrate (33.5% N), calcium superphosphate (18.5% P_2O_5), and potassium chloride (50% K_2O), respectively. Before sowing, the entire amount of P, half the dose of K, and one-third of the nitrogen (N) were applied. The second one-third of N was applied at the late tillering growth stage, while the second half dose of K and the last dose of N were applied at the late booting growth stage. The other recommended agronomic practices, such as removing weeds and protecting plants from pests and diseases, were carried out in a timely manner to raise a healthy crop.

All genotypes in both the control and salinity treatments were irrigated with fresh water for up to 21 days after sowing in order to achieve a high germination percentage and good seedling establishment. Subsequently, the genotypes in the high salinity treatment were irrigated with artificial saline water containing 8.8 g NaCl L^{-1} (150 mM NaCl), while in the control treatment, they continued to be irrigated with fresh water until the last irrigation. To control the amount of water delivered to each subplot, a low-pressure surface irrigation system was used. This system consisted of a 76 mm diameter main line, which delivered either saline or fresh water from a five cubic-meter water tank to the subplots. The main line was branched off to the sub-main hoses and equipped with a manual control valve at each subplot (Figure 1). The amount of irrigation water for each irrigation event and the irrigation frequency were determined based on the plant phenology and daily climatic data of the experimental site. Based on these data, the total amount of irrigation water applied for each treatment was 4800 m^3 ha^{-1}. To monitor the build-up of salinity concentration in the root zone of plants during the growing season, soil samples were collected from different places of the salinity treatment at a depth of 0–80 cm, and their electrical conductivity was measured. This monitoring approach ensured that the desired salinity level was consistently maintained throughout the duration of the experiment. Based on the EC analysis conducted on soil samples, the EC of these samples did not exceed 16.3 dS m^{-1}.

2.4. Phenotypic Characterization

Agro-Morpho-Physiological Characteristics

Phenotypic observations were recorded on 13 agro-morpho-physiological parameters at different growth stages. Observations of plant dry weight (PDW), green leaf area (GLA), and leaf area index (LAI) were recorded at 75 (booting stage) and 90 (anthesis stage) days after sowing on ten randomly selected plants from each experimental unit. The green leaf blades of 10 plants were separated and run through a leaf area meter (LI 3100; LI-COR Inc., Lincoln, NE, USA) to measure the GLA. Subsequently, the different parts of the ten plants (stems, leaves, and spikes) were oven-dried at 75 °C until their weight became constant to determine the PDW. The values of LAI were obtained by dividing the values of GLA per plant by the ground area per plant.

Observations of leaf relative water content (LRWC) and total chlorophyll content (Chlt) were recorded at anthesis stage on ten randomly selected fully expanded leaves from each experimental unit, with five leaves for each measurement. An area of approximately 0.20 cm^2 was excised from each of five leaves. These were immediately weighed, soaked in distilled water in the dark at 25 °C for 24 h, and then dried at 75 °C until their weight became constant. This allowed for the recording of the fresh weight (FW), turgid weight (TW), and dry weight (DW). The percentage of LRWC was then calculated using the following formula:

$$\text{LRWC} = (\text{FW} - \text{DW})/(\text{TW} - \text{DW}) \times 100 \quad (1)$$

Approximately 0.4 g of leaf tissue was cut from the other five leaves, washed with distilled water, and soaked in 80% (v/v) acetone at room temperature in darkness until the tissue was completely bleached. The extracted sap was then centrifuged for 5 min at 400 rpm and adjusted to a total volume of 15 mL with 80% acetone. The absorbance of the extract was measured spectrophotometrically at 645 nm (A645) and 663 nm (A663) using a UV/VIS spectrophotometer (UV-2550, Shimadzu, Tokyo, Japan). Finally, the concentrations of Chlt were calculated according to Arnon [41] and Lichtenthaler [42] using the following formula:

$$\text{Chlt (mg g}^{-1}\text{ FW}^{-1}) = [(20.21 \times \text{A645}) + (8.02 \times \text{A663})] \times \text{V}/(1000 \times \text{W}) \quad (2)$$

where V and W are the volume of the extract solution (15 mL) and the weight of the fresh weight of leaf tissue (0.4 g).

Observations of the number of grains per spike (GNPS), thousand-grain weight (TGW), biological yield (BY), grain yield (GY), and harvest index (HI) were recorded at the maturity stage, which was in the fourth week of April in both growing seasons. Observations of NGPS and TGW were recorded from fifty randomly selected spikes from each experimental unit, and observations of BY and GY (in ton ha^{-1}) were recorded from a 0.75 m^2 area of each experimental unit. The area was harvested by hand, air-dried for one week, and weighed to record the BY. The plants were then threshed, and the grains were collected, cleaned, adjusted to 14% moisture content, and weighed to record the GY. The values of HI were obtained by dividing the values of GY by BY.

2.5. Genotypic Characterization

DNA Isolation and SSR Marker Analysis

Genomic DNA was isolated from twenty-day-old seedlings using the Wizard Genomic DNA Purification Kit (PROMEGA Corporation Biotechnology, Madison, WI, USA). After isolation, the samples were treated with RNase and then immediately transferred to a −20 °C freezer. The concentration of the final isolated DNA was determined at 260 nm using a UV–visible spectrophotometer, while its quality was checked by running the isolated DNA on a 0.8% agarose gel. Subsequently, the quantified DNA stock was standardized to a final concentration of 25 ng μL^{-1}.

Sixty different SSR primers (markers) covering most of the chromosomes of the hexaploid wheat genomes were used to characterize the genetic diversity of 24 wheat germplasms in this study (Table S1). These markers were chosen because they have been reported in several previous studies as being associated with salt tolerance in wheat [15,34,43–46]. Sequences of these SSR markers can be found on the Grain Genes website (http://wheat.pw.usda.gov/ggpages/maps.shtml; accessed on 11 January 2022) and presented in Table 1. The polymerase chain reaction (PCR) was carried out in a 20 µL mixture with the following composition: 1.5 µL of DNA sample, 8 µL of nuclease-free water, 10 µL of Green Master Mix (Promega Corporation, Madison, WI, USA), and 0.5 µL of both forward and reverse primers. The temperature cycles of PCR profiles were programmed with an initial denaturation for 4 min at 94 °C, followed by 40 cycles of denaturation for 1 min at 94 °C, 1 min at the primer-specific annealing temperature (as mentioned in Table 1 for each SSR primer), 2 min at 72 °C for extension, and 10 min at 72 °C for a final extension before cooling to 4 °C. The PCR amplification products were analyzed via capillary electrophoresis using the QI Axcel Advanced System Device (Qiagen, Hilden, Germany). The SSR markers amplified bands of each amplified loci were scored visually for their presence or absence with each primer. The scores were obtained in the form of binary matrix with '1' and '0' indicating the presence and absence of bands in each genotype, respectively.

Table 1. Mean square values for the effects of the year, salinity, genotype, and their possible interactions by ANOVA on different agro-morpho-physiological traits of 24 wheat genotypes evaluated under two salinity levels for each year and the combined analysis of two years.

Source of Variance	df	PDW-1	GLA-1	LAI-1	PDW-2	GLA-2	LAI-2	LRWC	Chlt	GNPS	TGW	BY	GY	HI
							First Year							
Salinity (S)	1	86.5 ***	78,446.4 **	92.66 ***	288.5 ***	49,905.7 **	59.7 ***	1870.9 *	38.1 ***	1005.9 **	2111.0 **	963.4 **	147.8 ***	371.9 **
Genotype (G)	23	1.3 ***	358.0 ***	0.27 ***	3.3 ***	320.0 ***	0.25 ***	108.7 ***	1.64 ***	130.1 ***	61.6 ***	9.07 ***	1.09 ***	24.1 ***
G × S	23	0.55 ***	226.3 ***	0.17 **	2.08 ***	220.0 ***	0.16 ***	20.6 *	0.45 ***	24.7 *	15.31 ^{ns}	8.98 ***	0.61 ***	37.6 ***
							Second year							
Salinity (S)	1	73.3 **	41,957.6 **	37.5 **	282.3 ***	31,273.9 **	32.9 **	3701.1 **	36.6 **	746.7 **	768.03 *	942.8 ***	144.1 ***	387.3 **
Genotype (G)	23	0.99 ***	372.5 ***	0.414 ***	3.11 ***	369.0 ***	0.37 ***	39.6 ***	1.89 ***	90.63 ***	81.8 ***	9.79 ***	1.09 ***	23.13 ***
G × S	23	0.52 ***	374.1 ***	1.16 ***	1.85 ***	230.9 ***	0.59 ***	37.46 ***	0.60 ***	28.69 ***	13.57 **	8.94 ***	0.61 ***	30.18 ***
							Combined two years							
Year (Y)	1	0.54 *	25,267.5 **	6.85 ***	0.037 ^{ns}	18,842.1 **	4.72 ***	587.0 ***	3.37 ***	1268.4 *	6610.2 *	5.62 *	0.113 ^{ns}	9.86 ^{ns}
Salinity (S)	1	159.51 ***	117,572 **	124.07 *	570.85 *	80,095.9 **	90.59 **	5417.4 **	74.8 ***	1743.1 *	2712.6 *	1905.9 *	291.9 ***	759.1 ***
S ×Y	1	0.272 ^{ns}	2831.1 ***	6.13 ***	0.017 ^{ns}	1083.5 ^{ns}	1.99 ***	154.56 **	0.007 ^{ns}	9.64 ^{ns}	166.26 ***	0.057 ^{ns}	0.012 ^{ns}	0.078 ^{ns}
Genotype (G)	23	2.20 ***	590.9 ***	0.408 ***	6.359 ***	572.6 ***	0.369 **	105.15 ***	3.11 ***	189.9 ***	130.16 ***	18.38 ***	2.162 ***	45.13 ***
G × Y	23	0.05 ^{ns}	139.6 **	0.275 ***	0.075 ^{ns}	116.4 ***	0.255 **	43.16 ***	0.42 ***	30.79 ***	13.28 ^{ns}	0.465 ^{ns}	0.021 ^{ns}	2.00 ^{ns}
G × S	23	1.02 ***	380.6 ***	0.779 ***	3.88 ***	282.9 ***	0.414 **	43.39 ***	0.88 ***	32.56 ***	16.37 *	17.34 ***	1.19 ***	64.01 ***
G × S × Y	23	0.058 ^{ns}	219.8 ***	0.556 ***	0.046 ^{ns}	168.1 ***	0.328 **	14.69 *	0.17 **	20.83 **	12.51 ^{ns}	0.582 ^{ns}	0.020 ^{ns}	3.728 ^{ns}

Abbreviations of PDW, GLA, LAI, LRWC, Chlt, GNPS, TGW, BY, GY, and HI are plant dry weight (g plant^{-1}), green leaf area (cm^2 plant^{-1}), leaf area index, leaf relative water content (%), total chlorophyll content (mg g^{-1} FW), grain number per spike, thousand-grain weight (g), biological yield (ton ha^{-1}), grain yield (ton ha^{-1}), and harvest index (%), respectively. Values 1 and 2 represent measurements at 70 and 90 days after sowing, respectively. Those sharing the same letter for year, salinity, genotype, and their possible interactions in the same column do not differ significantly at the 0.05 level according to Tukey's test. ^{ns}, *, **, and *** indicate non-significant and significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

2.6. Statistical Analysis

The analysis of the phenotypic data involved several techniques. Initially, before analyzing the data of agro-morpho-physiological traits, the normal distribution and variance homogeneity of all traits were tested using the Shapiro–Wilk and Bartlett's chi-squared tests, respectively, from the tapply function of the base package in the statistical software R. Thereafter, an analysis of variance (ANOVA) was conducted, using SAS software (Version 9.2; Cary, NC, USA), to test for significant differences in morpho-physiological traits between salinity treatments and genotypes. The differences among the mean values of genotypes for each morpho-physiological trait under control or salinity conditions were compared using Tukey's HSD post hoc test at a 5% probability level. The level of correlation between different parameters across years, replications, and genotypes under control or salinity conditions was estimated using Pearson's correlation matrix. To reduce the dimensionality and complexity of data, detect interrelationships among multiple traits, and identify the parameters that contribute most to the variation in tested wheat genotypes, principal component analysis (PCA) was performed on the genotype-by-parameter matrix of means. A biplot was then made using the XLSTAT package. Furthermore, a heatmap cluster analysis based on the stress tolerance index (STI) for traits was performed to express the inter-relationships between traits and genotypes and to group genotypes according to their level of salt tolerance. The STI was calculated for each trait using the following formula suggested by [47]:

$$STI = (V_C \times V_S)/(V_C)^2 \quad (3)$$

where V_C and V_S are the values of the trait of each genotype under control and salinity conditions, respectively.

For the genotypic data analysis, the SSR data were scored visually to determine the presence or absence of each primer. The SSR bands were then scored as qualitative characters, representing "present" as 1 and "absent" as 0, resulting in the creation of a binary matrix. Based on the Jaccard dissimilarity coefficient, a dissimilarity matrix was generated to assess the pairwise genetic dissimilarity between genotypes. Within the same statistical package, the agglomerative hierarchical clustering (AHC) analysis was conducted using the unweighted pair group average method (UPGMA). The Mantel test was performed to assess the similarity between the Euclidean distance matrices based on morpho-physiological traits and the genetic distance matrices based on the SSR data [48].

Finally, a stepwise multiple linear regression (SMLR) analysis was performed to identify the most influential SSR markers associated with each morpho-physiological trait under both control and salinity conditions, as well as the STI for each trait. The molecular marker observations and morpho-physiological traits were considered as independent and dependent variables, respectively. The critical significance level of the coefficients of determination (R^2) was tested at a 5% probability level.

3. Results

3.1. Analysis of Variance

The results of the ANOVA showed statistically significant differences ($p \leq 0.001$) among the salinity levels (S), genotypes (G), and their interaction (G × S) for all agro-morpho-physiological traits in each year and in the combined analysis of two years (Table 1). Statistically significant differences ($p \leq 0.05$, 0.01, and 0.001) were also observed among the years (Y) for all traits, except for PDW at 90 days after sowing (DAS), GY, and HI. The interaction effect between S × Y and G × Y was highly significant for GLA and LAI at 70 DAS, LAI at 90 DAS, and LRWC. The G × Y interaction had a significant effect on GLA at 90 DAS, Chlt, and GNPS, whereas the S × Y interaction had no significant effect; the opposite was true for TGW (Table 1). The three-way interaction (G × S × Y) had a significant effect on GLA and LAI at 70 and 90 DAS, LRWC, Chlt, and GNPS (Table 1).

3.2. Genotypic Performance in Different Morpho-Physiological Traits under Control and Salinity Conditions

Generally, a high salinity level (150 mM NaCl) significantly decreased all agro-morpho-physiological traits compared to the control treatment. When averaged across the two years, the 150 mM NaCl treatment resulted in decreases of 25.9% in PDW, 28.5% in GLA, and 36.9% in LAI measured at 70 DAS. Additionally, it led to decreases of 34.0% in PDW, 28.6% in GLA, and 38.3% in LAI measured at 90 DAS. It also resulted in reductions of 11.1% in LRWC, 33.0% in Chlt, 10.6% in GNPS, 13.4% in TGW, 27.8% in BY, 34.9% in GY, and 10.2% in HI compared to the control treatment (Tables 2 and 3). Based on the aforementioned percentage reduction, LRWC, GNPS, TGW, and HI were the traits least affected by salinity stress. Conversely, the other traits were severely affected, with salinity stress causing reductions of more than 25% in the mean values of these traits. In addition, significant differences were observed among genotypes for most agro-morpho-physiological traits under both control and salinity conditions. The maximum values for these traits were approximately one to two times higher under control conditions and one to three times higher under salinity conditions compared to the minimum values (Tables 2 and 3). At 70 DAS, PDW, GLA, and LAI ranged from 4.4 to 7.1 g plant^{-1}, 129.5 to 155.6 cm^2 plant^{-1}, and 2.75 to 4.09 under control conditions, while, under salinity conditions, they ranged from 3.4 to 5.2 g plant^{-1}, 83.2 to 115.6 cm^2 plant^{-1}, and 1.71 to 2.79, respectively. At 90 DAS, PDW, GLA, and LAI ranged from 6.4 to 11.0 g plant^{-1}, 106.2 to 128.2 cm^2 plant^{-1}, and 2.31 to 3.35 under control conditions, while, under salinity conditions, they ranged from 4.3 to 6.4 g plant^{-1}, 63.9 to 98.2 cm^2 plant^{-1}, and 1.31 to 2.24, respectively (Table 2). Both physiological traits (LRWC and Chlt) ranged from 72.3 to 88.6% and 2.20 to 4.60 mg g^{-1} FW under control conditions and from 59.0 to 76.7% and 1.00 to 3.84 mg g^{-1} FW under salinity conditions, respectively (Table 3). The different yield components (GNPS, TGW, BY, GY, and HI) ranged from 37.4 to 54.8, 38.3 to 56.4 g, 14.6 to 21.8 ton ha^{-1}, 4.7 to 6.8 ton ha^{-1}, and 25.4 to 39.4% under control conditions, and from 33.7 to 48.3, 32.7 to 48.5 g, 9.4 to 15.5 ton ha^{-1}, 2.7 to 4.8 ton ha^{-1}, and 22.9 to 35.9% under salinity conditions, respectively (Table 3).

Table 2. Mean values of plant dry weight (PDW), green leaf area (GLA), and leaf area index (LAI) measured at 70 and 90 days after sowing of 24 wheat genotypes under control (C) and salinity (S) conditions over two years.

Genotypes	70 Days						90 Days					
	PDW		GLA		LAI		PDW		GLA		LAI	
	C	S	C	S	C	S	C	S	C	S	C	S
Sakha-93	6.23 cd	5.20 a	130.79 ef	111.78 ab	3.48 f–i	2.44 b–e	8.63 de	6.40 a	111.79 fgh	94.69 ab	2.97 e–i	2.07 ab
Sids-1	5.58 f–i	4.81 abc	134.94 c–f	94.4 ef	3.61 d–i	2.07 g–j	7.57 hi	5.59 cde	113.03 d–h	78.09 f–i	3.02 d–h	1.71 d–h
Sakha-61	5.91 def	3.59 kl	143.59 bc	83.23 g	3.74 b–g	1.71 k	8.53 def	4.76 ghi	112.07 e–h	63.87 k	2.93 e–i	1.31 i
Kharchia-65	5.29 ijk	4.83 abc	130.76 ef	106.49 abcd	3.56 d–i	2.38 b–g	6.97 jkl	5.89 a–d	112.10 e–h	89.30 a–e	3.05 b–g	2.0 a–d
Kawz	5.59 f–i	4.08 f–j	153.74 a	102.82 bcd	3.08 jkl	2.79 a	8.88 cd	5.72 b–e	121.39 a–d	86.18 b–f	2.5 jk	2.25 a
Gemiza-9	5.59 f–i	4.31 e–h	148.60 ab	103.85 bcd	3.11 jk	2.61 abc	7.77 gh	6.05 abc	124.10 abc	91.07 a–d	2.76 hij	2.05 ab
MISR-1	5.83 d–g	4.08 f–j	138.87 cde	105.91 bcd	2.75 mn	2.65 ab	7.13 ijk	5.27 efg	116.49 b–g	90.47 a–d	2.31 kl	1.98 a–e
Shandaweel-1	5.74 e–h	3.71 jkl	140.64 bcd	92.29 fg	2.83 klm	2.30 d–h	7.68 ghi	4.71 ghi	119.89 a–f	80.32 e–i	2.47 k	1.81 b–g
RIL-1	7.14 a	4.36 def	139.99 b–e	105.47 bcd	3.51 e–i	2.25 d–	10.97 a	5.67 bcde	112.19 e–h	87.54 b–e	2.81 ghi	1.86 b–g
RIL-2	5.42 g–j	4.78 a–d	154.42 a	115.61 a	4.01 ab	2.47 bcd	8.70 de	6.33 a	126.47 a	98.17 a	3.29 abc	2.10 ab
RIL-3	6.02 c–e	4.95 ab	137.94 c–f	110.7 ab	3.57 d–i	2.36 b–g	9.60 b	5.87 a–d	109.04 gh	90.76 a–d	2.82 ghi	1.94 b–f
RIL-4	5.53 f–i	4.38 def	154.94 a	110.93 ab	4.09 a	2.34 c–g	6.7 klm	5.55 cde	127.06 a	91.79 abc	3.35 a	1.94 a–f
RIL-5	6.11 cde	4.69 b–e	143.35 bc	111.64 ab	3.78 a–f	2.39 b–f	7.54 hij	5.73 b–e	115.14 c–h	94.66 ab	3.03 c–g	2.03 abc
RIL-6	4.42 n–q	3.41 l	155.64 a	110.70 ab	4.01 ab	2.46 b–e	6.81 klm	5.21 e–h	128.21 a	91.41 a–d	3.30 ab	2.03 abc
RIL-7	6.06 cde	4.83 abc	148.38 ab	100.03 c–f	3.73 b–h	2.17 d–i	10.50 a	6.25 ab	125.23 ab	83.27 c–h	3.15 a–e	1.81 b–g
RIL-8	5.34 h–k	4.14 f–i	148.25 ab	108.10 abc	3.94 abc	2.24 d–i	7.73 gh	5.2 e–h	123.77 abc	86.62 b–f	3.28 a–d	1.80 b–g
RIL-9	5.56 f–i	3.9 h–k	143.26 bc	91.66 fg	3.84 a–d	1.98 ijk	8.23 efg	4.92 fgh	115.91 c–g	72.25 ijk	3.11 a–f	1.56 ghi
RIL-10	5.86 def	4.35 d–g	148.61 ab	83.20 g	3.81 a–e	1.82 jk	9.38 bc	4.73 ghi	120.98 a–e	64.77 jk	3.10 a–f	1.42 hi
RIL-11	5.58 f–i	3.57 kl	129.47 f	94.28 ef	3.34 ij	2.01 h–k	8.91 cd	4.66 hi	106.18 hi	73.40 ij	2.73 ij	1.56 ghi
RIL-12	6.43 bc	4.05 f–j	134.73 c–f	110.98 ab	3.56 d–i	2.39 b–f	9.53 b	5.73 b–e	108.29 gh	85.28 c–g	2.86 f–i	1.84 b–g
RIL-13	5.73 e–h	4.27 e–h	131.87 def	98.22 def	3.44 ghi	2.12 f–j	8.03 fgh	5.36 def	108.28 gh	76.67 ghi	2.83 ghi	1.66 fgh
RIL-14	5.43 g–j	3.77 i–l	142.70 bc	98.02 def	3.63 d–i	2.18 d–i	7.46 hij	5.46 def	115.45 c–g	75.81 hi	2.93 e–i	1.68 e–h
RIL-15	6.72 ab	4.45 c–f	141.57 bc	103.91 bcd	3.64 c–i	2.15 e–i	8.87 cd	5.62 cde	113.57 d–h	82.67 d–h	2.93 e–i	1.72 c–h
RIL-16	5.05 –m	3.93 g–k	131.35 ef	84.35 g	3.43 hi	1.72 k	6.43 lmn	4.29 i	109.05 gh	66.15 jk	2.85 f–i	1.35 i

Those sharing the same letter in the same column do not differ significantly at the 0.05 level according to Tukey's test.

Table 3. Mean values of leaf relative water content (LRWC), total chlorophyll content (Chlt), grain number per spike (GNPS), thousand-grain weight (TGW), biological yield (BY), grain yield (GY), and harvest index (HI) of 24 wheat genotypes under control (C) and salinity (S) conditions over two years.

Genotypes	LRWC		Chlt		GNPS		TGW		BY		GY		HI	
	C	S	C	S	C	S	C	S	C	S	C	S	C	S
Sakha-93	78.9 b–g	73.82 abc	3.35 def	2.89 b	46.95 e–k	45.0 b–e	43.21 i–m	41.94 bc	17.76 ghi	13.58 c–g	5.58 fgh	4.03 cd	31.61 e–l	29.64 c–g
Sids-1	76.05 g–j	69.83 efg	3.34 def	1.50 k	43.61 l–p	39.95 h–k	44.25 f–l	40.12 b–e	21.75 a	13.37 d–h	5.53 ghi	3.71 def	25.5 v–y	27.74 f–i
Sakha-61	78.44 c–h	67.12 ghi	2.28 m–q	1.0 l	44.32 k–o	39.5 i–l	46.91 c–h	38.82 c–f	14.64 m–p	12.40 ghi	5.77 efg	3.07 h	39.62 a	24.77 kl
Kharchia-65	78.4 d–h	73.3 bcd	4.6 a	3.84 a	40.03 q–u	37.54 k–n	45.56 d–j	42.26 b	18.24 fgh	14.25 a–e	5.39 h–k	4.34 bc	29.59 j–r	30.56 b–e
Kawz	81.83 bc	70.37 d–g	3.08 fg	2.58 c	54.82 a	45.83 a–d	38.34 p–t	32.7 i	18.76 efg	12.36 ghi	6.41 b	3.69 ef	34.42 bcd	29.88 b–f
Gemiza-9	82.23 b	76.72 a	2.49 k–n	2.18 de	49.62 cde	44.15 c–f	45.05 e–k	34.57 hi	19.98 cde	13.82 b–f	5.89 ef	4.23 bc	29.57 j–r	30.71 bcd
MISR-1	80.42 b–e	75.75 ab	2.77 h–k	2.13 def	51.67 abc	47.94 ab	47.54 b–f	41.13 bcd	18.1 f–i	14.84 abc	6.04 de	4.24 bc	33.46 c–f	28.62 d–h
Shandaweel-1	75.22 h–l	68.5 e–h	3.05 fgh	1.69 jk	54.34 ab	45.36 a–d	41.79 k–o	36.94 e–h	20.42 bcd	12.61 fghi	6.43 b	3.48 fg	31.73 e–k	27.63 f–j
RIL-1	73.09 j–o	68.11 fgh	2.37 l–o	1.84 f–j	49.18 c–f	40.9 g–j	44.15 g–l	39.66 b–f	20.36 bcd	12.29 hi	6.24 bcd	3.49 fg	30.75 g–n	28.42 d–h
RIL-2	75.46 h–k	71.84 cde	2.73 ijk	2.0 e–i	47.14 e–k	45.54 a–d	46.73 c–h	41.17 bcd	20.96 abc	14.81 abc	6.33 bcd	4.79 a	30.28 g–p	32.38 b
RIL-3	78.03 d–h	70.81 cdef	3.25 ef	1.74 ijk	46.2 f–l	38.58 j–m	47.17 b–g	41.55 bc	19.21 def	13.39 d–h	5.62 fgh	3.76 def	29.43 k–r	28.14 d–i
RIL-4	77.84 d–h	68.99 e–h	3.31 def	1.98 e–j	43.64 l–p	39.64 i–l	46.44 c–i	42.01 bc	20 bcde	14.48 a–d	5.8 efg	3.81 de	29.09 l–t	26.34 h–k
RIL-5	77.6 d–h	70.97 c–f	3.59 bcd	2.32 cd	44.23 k–o	42.94 d–h	44.95 e–k	41.49 bc	18.86 efg	14.39 a–d	5.49 g–j	4.28 bc	29.31 k–s	29.78 b–f
RIL-6	75.85 g–j	70.87 c–f	2.57 j–m	2.07 d–h	51.23 bcd	48.34 a	42.45 j–n	37.97 d–g	18.86 efg	15.03 ab	6.09 cde	4.21 bc	32.29 d–i	28.0 e–i
RIL-7	76.99 f–i	68.99 e–h	3.47 cde	2.31 cd	45.62 h–m	44.02 c–g	47.59 b–f	42.79 b	19.62 de	14.65 abc	6.78 a	3.74 def	34.7 bcd	25.57 ijk
RIL-8	75.96 g–j	69.45 efg	2.26 n–q	2.03 d–i	48.68 c–h	46.34 abc	44.15 g–l	37.52 e–h	17.25 hij	13.76 c–f	6.39 bc	4.43 b	37.05 ab	32.19 bc
RIL-9	72.31 k–p	59.03 j	2.87 g–j	1.79 h–k	47.73 e–j	36.45 l–o	48.45 bcd	39.74 b–f	19.86 cde	9.36 k	5.71 fg	3.34 gh	28.81 m–u	35.9 a
RIL-10	76.17 g–j	63.91 i	2.2 n–r	1.82 g–j	45.98 f–m	39.92 h–k	48.91 bc	39.86 b–f	21.25 ab	12.33 ghi	6.54 ab	3.33 gh	30.89 f–m	27.02 g–k
RIL-11	88.57 a	69.05 e–h	2.64 i–l	1.89 e–j	48.9 c–g	42.95 d—h	49.71 bc	39.68 b–f	16.5 jk	10.94 j	5.22 i–l	2.74 i	31.66 e–l	25.04 jkl
RIL-12	79.02 b–g	68.97 e–h	3.58 bcd	2.06 d–h	37.37 u–	34.95 no	50.49 b	41.83 bc	16.43 jkl	15.5 a	5.17 jkl	3.55 efg	31.62 e–l	22.92 l
RIL-13	80.32 b–f	70.45 c–g	2.85 g–j	1.93 e–j	48.5 c–i	41.65 f–j	47.63 b–e	39.69 b–f	18.02 f–i	13.12 e–h	4.74 mn	3.6 efg	26.45 t–x	27.47 f–j
RIL-14	78.3 d–h	65.81 hi	3.66 bc	2.06 d–h	46.25 f–l	33.67 o	43.69 h–l	36.73 fgh	16.89 ij	13.67 c–f	5.01 lm	3.33 gh	29.71 i–q	24.44 kl
RIL-15	77.01 e–i	65.94 hi	4.38 a	2.11 d–g	37.48 u–x	35.48 mno	56.43 a	48.51 a	15.24 lmn	14.15 b–e	4.96 lm	3.74 def	32.62 d–g	26.75 h–k
RIL-16	80.43 bcd	67.68 fgh	3.45 cde	1.88 f–j	43.19 l–q	41.92 e–i	39.65 n–s	35.25 ghi	15.2 lmn	11.57 ij	5.17 kl	3.04 hi	33.99 cde	26.37 h–k

Those sharing the same letter in the same column do not differ significantly at the 0.05 level according to Tukey's test.

The relative changes in each agro-morpho-physiological trait for every genotype are presented in Table 4. These changes indicate the percentage reduction of each trait under high salinity levels compared to the control treatment. The different color shades in Table 4 represent the range of reduction percentages. The color gradient, ranging from dark blue to dark orange, reflects the gradient in reduction percentages of the trait from the minimum to the maximum. Generally, there was a wide range in the reduction percentages for all agro-morpho-physiological traits, except for LRWC, GNPS, and TGW, which exhibited a narrow range. The reduction percentages ranged from 8.7% to 39.2%, 14.5% to 44.0%, and 3.7% to 54.3% for PDW, GLA, and LAI at 70 DAS; 15.5% to 49.5%, 15.3% to 46.5%, and 10.0% to 55.2% for PDW, GLA, and LAI at 90 DAS; and 4.8% to 22.0%, 9.9% to 56.1%, 2.9% to 23.3%, 5.6% to 52.8%, 19.5% to 49.1%, and −24.8% to 37.2% for LRWC, Chlt, GNPS, TGW, BY, GY, and HI, respectively (Table 4). Additionally, the salt-tolerant genotypes Sakha 93 and Kharachia-65 exhibited the lowest reduction percentages for most agro-morpho-physiological traits, while the salt-sensitive genotypes Sakha 61 and Shandaweel-1 displayed the highest reduction percentages. Among the RILs, three out of eleven (RIL9, RIL10, and RIL11) from the crossing between Sakha 93 and Sakha 61 and all RILs (RIL12–RIL16) from the crossing between Sakha 93 and Sids1 had the highest reduction percentages for most agro-morpho-physiological traits. Conversely, the remaining RILs from the crossing between Sakha 93 and Sakha 61 (RIL1–RIL8) showed the opposite trend (Table 4). Kawz and Misr-1 exhibited the lowest reduction percentage in LAI at 70 and 90 DAS (3.7–14.5%). In contrast, the reduction percentage for this trait surpassed 30% in the majority of RILs and genotypes. The minimum reduction percentage in PDW and GLA at 70 and 90 DAS was observed in Sakha 93 and Kharachia-65. Although RIL12 and RIL15 had the lowest reduction percentage in BY (5.6% and 4.1%), they had an adequate reduction percentage in GY (31.4% and 24.5%) and HI (27.3% and 18.6%). Seven genotypes (Sids-1, Kharachia-65, Gemiza-9, RIL2, RIL5, RIL9, and RIL13) exhibited an increase in HI under salinity conditions compared to the control (Table 3). Consequently, the reduction percentages of HI in these genotypes were negative (Table 4).

Table 4. Reduction percentage of different agro-morpho-physiological traits under high salinity level relative to the control treatment for each genotype.

Genotypes	PDW-1	GLA-1	LAI-1	PDW-2	GLA-2	LAI-2	LRWC	Chlt	GNPS	TGW	BY	GY	HI
Sakha-93	16.6	14.5	29.7	25.9	15.3	30.3	6.4	13.9	4.1	2.9	23.5	27.9	5.7
Sids-1	13.8	30.0	42.5	26.1	30.9	43.3	8.2	55.0	8.4	9.3	38.5	32.9	−9.1
Sakha-61	39.2	42.0	54.3	44.2	43.0	55.2	14.4	56.1	10.9	17.2	15.3	46.8	37.2
Kharchia-65	8.7	18.6	33.2	15.5	20.3	34.3	6.5	16.5	6.2	7.2	21.9	19.5	−3.1
Kawz	26.9	33.1	9.6	35.6	29.0	10.0	14.0	16.3	16.4	14.7	34.1	42.4	12.6
Gemiza-9	22.9	30.1	16.0	22.1	26.6	25.8	6.7	12.3	11.0	23.3	30.9	28.2	−3.7
MISR-1	30.0	23.7	3.7	26.1	22.3	14.5	5.8	22.9	7.2	13.5	18.0	29.7	14.3
Shandaweel-1	35.4	34.4	18.7	38.7	33.0	26.5	8.9	44.8	16.5	11.6	38.2	45.9	12.5
RIL-1	38.9	24.7	36.0	48.3	22.0	33.8	6.8	22.3	16.8	10.2	39.7	44.1	7.4
RIL-2	11.9	25.1	38.5	27.3	22.4	36.4	4.8	26.5	3.4	11.9	29.3	24.3	−6.9
RIL-3	17.8	19.7	34.0	38.9	16.8	31.4	9.3	46.5	16.5	11.9	30.3	33.0	3.9
RIL-4	20.9	28.4	42.8	17.1	27.8	42.1	11.4	40.3	9.2	9.5	27.6	34.4	9.3
RIL-5	23.4	22.1	36.7	24.0	17.8	33.2	8.5	35.3	2.9	7.7	23.7	22.0	−2.1
RIL-6	22.9	28.9	38.7	23.5	28.7	38.5	6.6	19.2	5.7	10.6	20.3	30.8	13.2
RIL-7	20.3	32.6	41.7	40.5	33.5	42.5	10.4	33.5	3.5	10.1	25.3	44.8	26.1
RIL-8	22.4	27.1	43.1	32.7	30.0	45.3	8.6	9.9	4.8	15.0	20.2	30.7	13.2
RIL-9	29.9	36.0	48.6	40.3	37.7	49.9	18.4	37.4	23.6	18.0	52.8	41.5	−24.8
RIL-10	25.7	44.0	52.4	49.5	46.5	54.4	16.1	17.2	13.2	18.5	42.0	49.1	12.3
RIL-11	36.1	27.2	39.9	47.7	30.9	42.7	22.0	28.3	12.2	20.2	33.7	47.5	20.9
RIL-12	37.0	17.6	32.8	39.9	21.3	35.7	12.7	42.5	6.5	17.2	5.6	31.4	27.3
RIL-13	25.4	25.5	38.4	33.3	29.2	41.4	12.3	32.1	14.1	16.7	27.2	24.0	−4.3
RIL-14	30.6	31.3	39.9	26.8	34.3	42.6	15.9	43.9	27.2	15.9	19.1	33.5	17.8
RIL-15	33.8	26.6	41.0	36.7	27.2	41.2	14.4	51.8	5.3	14.0	7.1	24.5	18.6
RIL-16	22.2	35.8	49.9	33.3	39.3	52.7	15.9	45.5	2.9	11.1	23.9	41.1	22.6

Abbreviations of PDW, GLA, LAI, LRWC, Chlt, GNPS, TGW, BY, GY, and HI are plant dry weight (g plant^{-1}), green leaf area (cm^2 plant^{-1}), leaf area index, leaf relative water content (%), total chlorophyll content (mg g^{-1} FW), grain number per spike, thousand-grain weight (g), biological yield (ton ha^{-1}), grain yield (ton ha^{-1}), and harvest index (%), respectively. Values 1 and 2 represent measurements at 70 and 90 days after sowing, respectively. The color gradient, ranging from dark blue to dark orange, reflects the reduction percentages of the trait, gradually transitioning from the minimum to the maximum.

3.3. Correlation Matrix between Different Agro-Morpho-Physiological Traits under Control and Salinity Conditions

In general, the different agro-morpho-physiological traits showed stronger correlations with each other under salinity conditions compared to control conditions (Table 5). Under control conditions, the PDW, GLA, and LAI at 70 DAS exhibited a strong positive correlation with themselves at 90 DAS; that is, correlation coefficients (r) ranged from 0.72 to 0.96. Meanwhile, under salinity conditions, the three traits displayed a moderate to strong positive correlation with each other at both 70 and 90 DAS (r ranged from 0.41 to 0.96; Table 5). The LRWC showed a moderate negative correlation with LAI at 70 and 90 DAS under control conditions (r = −0.44). Meanwhile, under salinity conditions, this trait had a moderate to strong positive correlation with PDW, GLA, LAI at 70 and 90 DAS, Chlt, GNPS, BY, and GY (r ranged from 0.43 to 0.61; Table 5). Under control conditions, Chlt exhibited only significant correlations with GNPS (−0.62) and GY (0.50). However, under salinity conditions, Chlt had a moderate to strong positive correlation with all traits ((r ranged from 0.43 to 0.61), except TGW and HI (Table 5). Under salinity conditions, GY was substantially and positively correlated with all traits except TGW. However, under control conditions, GY was only positively correlated with GLA at 70 and 90 DAS, Chlt, GNPS, and BY. There was a negative correlation between BY and HI under both control and salinity conditions; however, this correlation was only significant under control conditions (Table 5).

Table 5. Pearson's correlation matrix of different agro-morpho-physiological traits under control (upper right) and salinity (lower left) conditions over two years (n = 104).

	1	2	3	4	5	6	7	8	9	10	11	12	13
PDW-1 (1)		−0.25	−0.16	0.72 ***	−0.38	−0.27	−0.15	0.12	−0.28	0.45 *	−0.04	−0.05	−0.01
GLA-1 (2)	0.44 *		0.39	0.001	0.90 ***	0.39	−0.34	−0.35	0.31	−0.13	0.38	0.64 **	0.22
LAI-1 (3)	0.25	0.79 ***		0.03	0.29	0.96 ***	−0.44 *	−0.01	−0.44 *	0.22	0.07	0.01	−0.03
PDW-2 (4)	0.75 ***	0.75 ***	0.65 **		−0.16	−0.08	−0.12	−0.18	−0.03	0.34	0.13	0.29	0.12
GLA-2 (5)	0.49 *	0.95 ***	0.86 ***	0.77 ***		0.41 *	−0.33	−0.28	0.36	−0.22	0.49 *	0.72 ***	0.15
LAI-2 (6)	0.41 *	0.85 ***	0.96 ***	0.75 ***	0.93 ***		−0.44 *	0.02	−0.39	0.14	0.17	0.08	−0.08
LRWC (7)	0.44 *	0.56 **	0.68 ***	0.51 **	0.67 ***	0.66 **		0.03	0.08	0.01	−0.43 *	−0.36	0.10
Chlt (8)	0.43 *	0.47 *	0.51 **	0.50 **	0.50 **	0.58 **	0.43 *		−0.62 **	0.17	−0.24	0.50 **	−0.26
GNPS (9)	−0.05	0.21	0.42 *	0.04	0.37	0.42 *	0.55 **	0.07		−0.55 **	0.36	0.53 **	0.10
TGW (10)	0.48 *	0.32	−0.08	0.34	0.22	0.001	−0.08	0.15	−0.35		−0.19	−0.30	−0.09
BY (11)	0.34	0.69 ***	0.55 **	0.60 **	0.66 **	0.56 **	0.60 **	0.32	0.16	0.35		0.56 **	−0.56 **
GY (12)	0.49 *	0.76 ***	0.69 ***	0.65 **	0.83 ***	0.75 ***	0.61 **	0.48 *	0.43 *	0.17	0.68 ***		0.37
HI (13)	0.25	0.24	0.30	0.20	0.34	0.36	0.05	0.27	0.30	−0.13	−0.24	0.54 **	

Abbreviations of PDW, GLA, LAI, LRWC, Chlt, GNPS, TGW, BY, GY, and HI are plant dry weight (g plant^{-1}), green leaf area (cm^2 plant^{-1}), leaf area index, leaf relative water content (%), total chlorophyll content (mg g^{-1} FW), grain number per spike, thousand-grain weight (g), biological yield (ton ha^{-1}), grain yield (ton ha^{-1}), and harvest index (%), respectively. Values 1 and 2 represent measurements at 70 and 90 days after sowing, respectively. *, **, and *** indicate significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

3.4. Principal Component Analysis for Agro-Morpho-Physiological Traits under Control and Salinity Conditions

The potential relationships between different agro-morpho-physiological traits and genotypes under control and salinity conditions were assessed using a biplot of a principal component analysis (PCA). The first four and three principal components (PCs) had eigenvalues greater than one and explained 80.24% and 78.97% of the total variation among traits and genotypes under control and salinity conditions, respectively (Figure 2). However, the first two components explained the highest percentages of variance: 51.86% and 68.82% of the total variability under control and salinity conditions, respectively. Therefore, the PCA-biplot was created using the first two PCs (Figure 3). Under control conditions, PC1 explained 30.60% of the total variability and was mostly associated with GLA at 70 and 90 DAS and GY. PC2 explained 21.26% of the total variability and was strongly associated with LAI at 70 and 90 DAS, GNPS, and TGW (Figure 2). Under salinity conditions, PC1 explained 53.47% of the total variability and was mainly influenced by GLA and LAI at 70 and 90 DAS, PDW at 90 DAS, and GY. PC2 explained 15.35% of the total variability and was mainly associated with TGW and GNPS (Figure 2). PC3 and PC4 accounted for 16.14% and 12.24% of the total variation in the data under control conditions and 10.15% and 5.77%

under salinity conditions, respectively. PC3 was strongly related to PDW at 70 and 90 DAS under control conditions and BY and HI under salinity conditions, while PC4 showed a strong association with BY and HI under control conditions and Chlt under salinity conditions (Figure 2). PC5 explained only 6.22% and 5.30% of the total variation under control and salinity conditions, respectively, and was strongly influenced by physiological parameters (LRWC and Chlt) under control conditions and by PDW at 70 DAS and LRWC under salinity conditions (Figure 2).

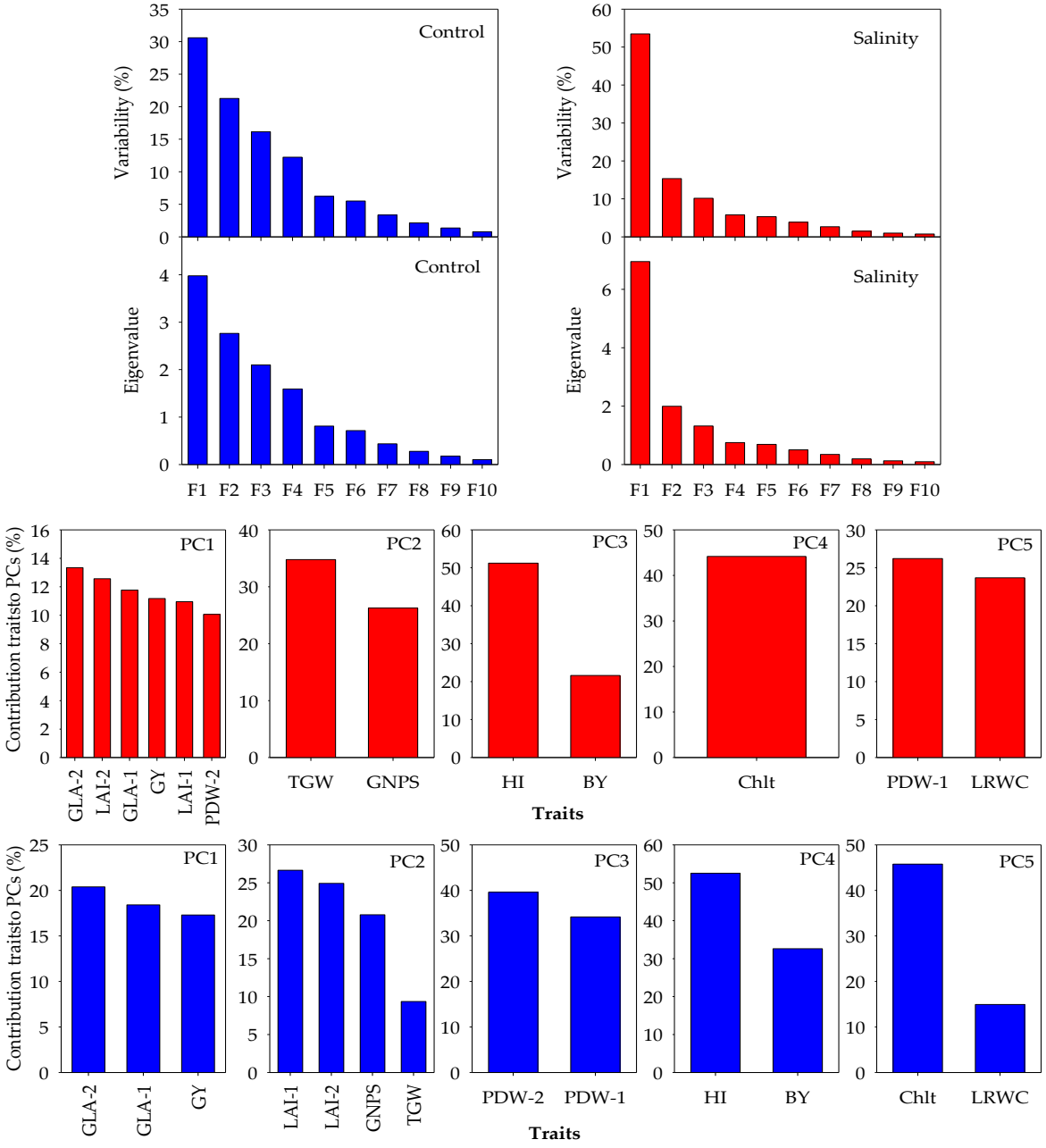

Figure 2. Eigenvalue and variability percentage of the first ten principal components (PCs) and contribution of the traits to first five PCs under control and salinity conditions. Abbreviations of PDW, GLA, LAI, LRWC, Chlt, GNPS, TGW, BY, GY, and HI are plant dry weight (g plant^{-1}), green leaf area (cm^2 plant^{-1}), leaf area index, leaf relative water content (%), total chlorophyll content (mg g^{-1} FW), grain number per spike, thousand-grain weight (g), biological yield (ton ha^{-1}), grain yield (ton ha^{-1}), and harvest index (%), respectively. Values 1 and 2 represent measurements at 70 and 90 days after sowing, respectively.

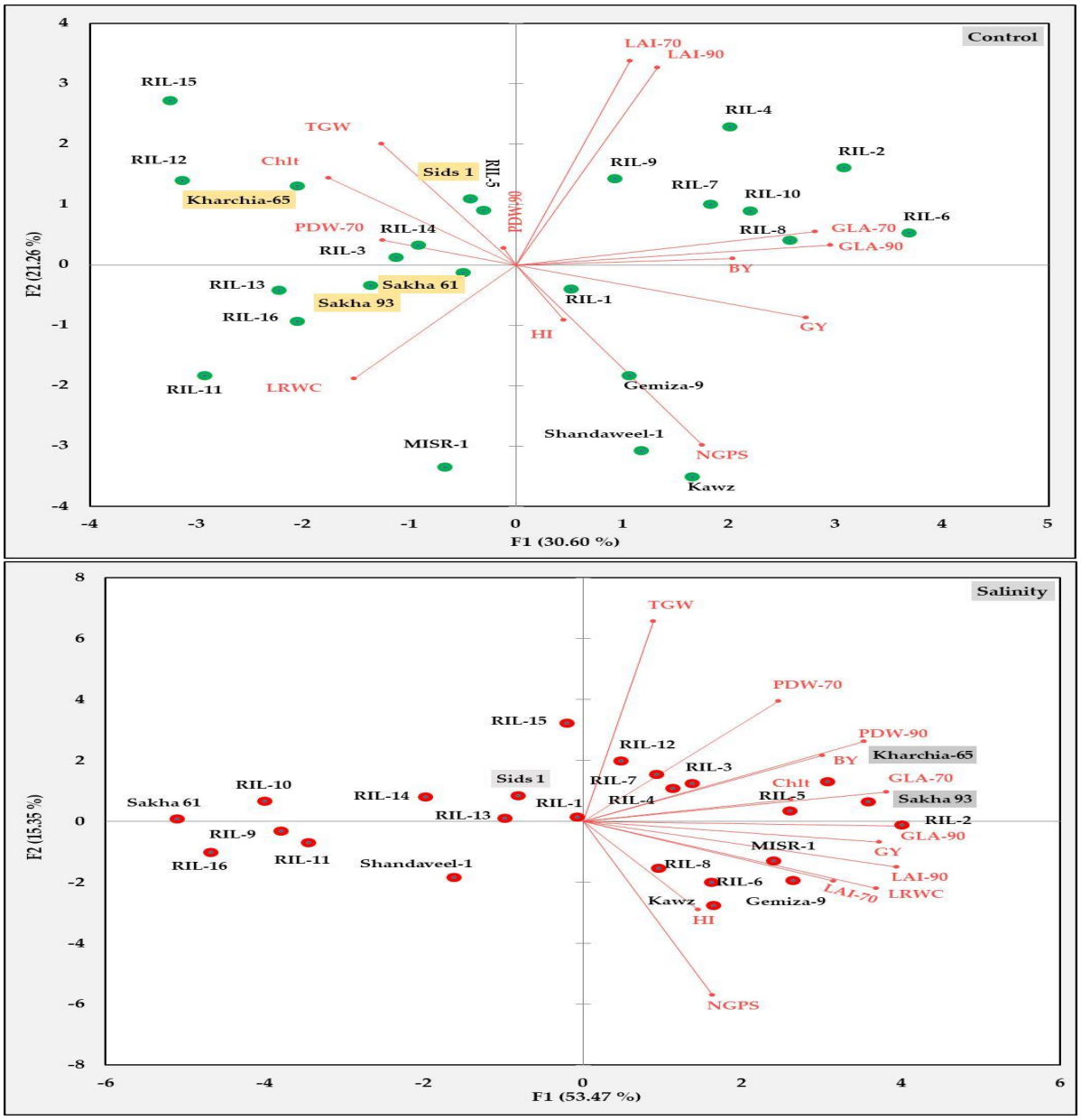

Figure 3. Principal component analysis biplot for genotypes and traits under control and salinity conditions. Abbreviations of PDW, GLA, LAI, LRWC, Chlt, GNPS, TGW, BY, GY, and HI are plant dry weight (g plant^{-1}), green leaf area (cm^2 plant^{-1}), leaf area index, leaf relative water content (%), total chlorophyll content (mg g^{-1} FW), grain number per spike, thousand-grain weight (g), biological yield (ton ha^{-1}), grain yield (ton ha^{-1}), and harvest index (%), respectively. Values 1 and 2 represent measurements at 70 and 90 days after sowing, respectively.

According to the PCA biplot (Figure 3), the thirteen agro-morpho-physiological traits discriminated the different genotypes into four main groups under control conditions and three main groups under salinity conditions. Under control conditions, RILs 2, 4, 6, 7, 8, 9, and 10 were found to be located in the positive region of PC1 and PC2. They showed a strong association with GLA and LAI at 70 and 90 DAS and a moderate association with BY. The genotypes located in the negative region of PC1 and PC2 were the salt-tolerant genotype Sakha 93, the salt-sensitive genotype Sakha 61, Misr-1, and RILs 11, 16, and 19. These genotypes were closely correlated with LRWC. The third group included the salt-tolerant genotype Kharachia-65, the moderately salt-tolerant genotype Sids 1, and RILs 3, 5, 12, 14, and 15. These genotypes were highly correlated with TGW and Chlt,

moderately correlated with PDW at 70 DAS, and weakly correlated with PDW at 90 DAS. Gemeza 9, Shendaweel-1, Kawz, and RIL1 were located in the positive region of PC1 and the negative region of PC2 and were closely correlated with GY and GNPS and weakly correlated with HI (Figure 3).

Under salinity conditions, the two salt-tolerant genotypes Sakha 93 and Kharachia-65 were grouped together with RILs 2, 6, and 8 and situated along the positive region of PC1 and PC2. These genotypes showed a strong correlation with PDW at 70 and 90 DAS, GLA at 70 DAS, Chlt, and BY (Figure 3). Gemeza 9, Kawz, Misr 1, and RILs 2, 6, and 8 were located in the positive region of PC1 and the negative region of PC2. They were closely correlated with the remaining traits. Salt-sensitive genotype Sakha 61, Sids 1, Shendaweel-1, and RILs 1, 9, 10, 11, 13, 14, 15, and 16 leaned towards the negative region of PC1 and positive or negative region of PC2. These genotypes did not show any relationship with morpho-physiological traits (Figure 3).

3.5. Clustering of Genotypes and Traits Based on Stress Tolerance Index

The two-way clustering was performed using the salt tolerance index (STI) for each agro-morpho-physiological trait. This was carried out to group genotypes into different sub-clusters based on their similarities and to assess the contribution of each trait in response to salt stress. Based on this analysis, the genotypes and traits were grouped into three column clusters and three row clusters, respectively (Figure 4). Clusters 1, 2, and 3 comprised 4, 8, and 12 genotypes, respectively, as well as two, three, and eight traits. The salt-sensitive genotype Sakha 61 and three RILs (9, 10, and 11) were grouped together in Cluster 1 (Figure 4). They exhibited a low STI (0.49–0.89) for all agro-morpho-physiological traits (Figure 5). The opposite was true of the genotypes in Cluster 2, which included two salt-tolerant genotypes, Kharachia-65 and Sakha 93, a moderately salt-tolerant genotype, Sids-1, and five RILs (2, 3, 4, 5, and 13) (Figure 4). These genotypes exhibited a high STI value (0.63–1.01) for all traits (Figure 5). The remaining genotypes (Gemeza 9, Shendaweel-1, Kawz, and Misr 1) and RILs were grouped together in Cluster 3 (Figure 4) and exhibited higher STI values than Cluster 1 and lower STI values than Cluster 2 for all traits (0.57–0.92), with a few exceptions (Figure 5). Therefore, the genotypes and RILs in Clusters 1, 2, and 3 can be considered salt-sensitive (S), salt-tolerant (T), and intermediate (I) genotype groups, respectively.

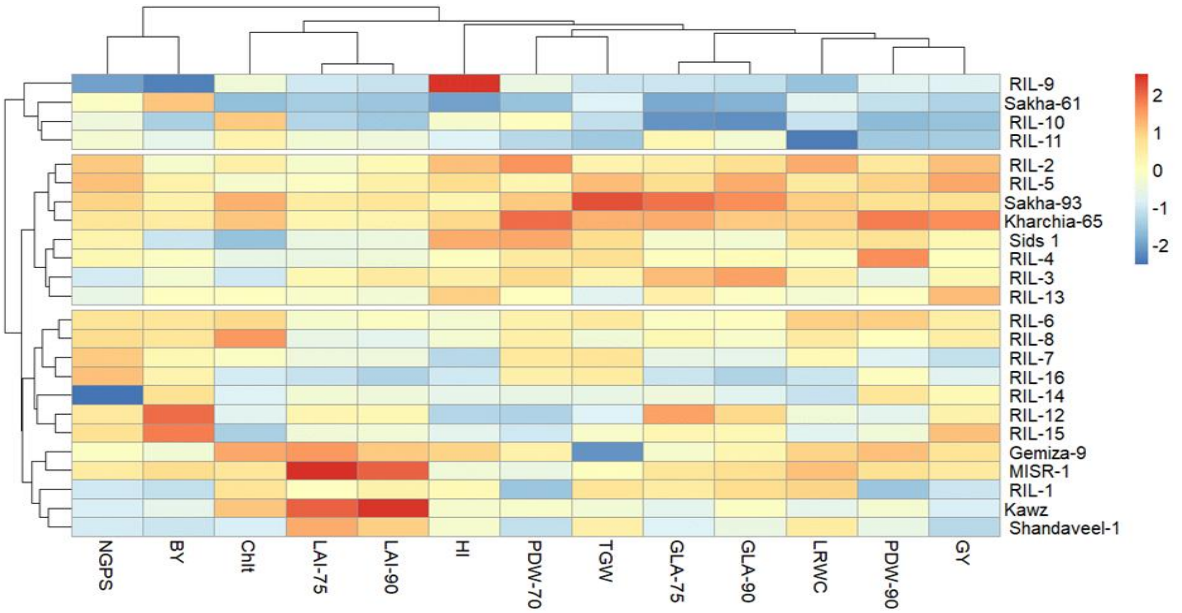

Figure 4. Heatmap cluster analysis displaying the associations among 24 wheat genotypes based on salt tolerance index (STI) of different traits. The different colors and densities were adjusted based on

associations between genotypes and STI for each trait. The darker red indicates higher values, while the darker blue indicates lower values. Abbreviations of PDW, GLA, LAI, LRWC, Chlt, GNPS, TGW, BY, GY, and HI are plant dry weight (g plant^{-1}), green leaf area (cm^2 plant^{-1}), leaf area index, leaf relative water content (%), total chlorophyll content (mg g^{-1} FW), grain number per spike, thousand-grain weight (g), biological yield (ton ha^{-1}), grain yield (ton ha^{-1}), and harvest index (%), respectively. Values 1 and 2 represent measurements at 70 and 90 days after sowing, respectively.

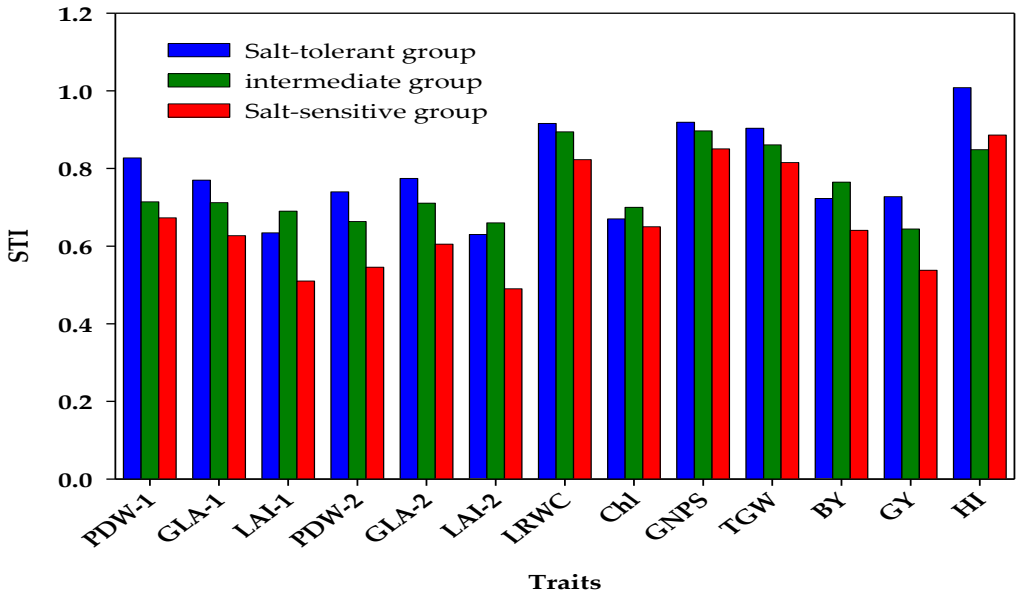

Figure 5. Salt tolerance index (STI) of measured traits for salt-tolerant (T), intermediate (I), and salt-sensitive (S) genotypes groups. Abbreviations of PDW, GLA, LAI, LRWC, Chlt, GNPS, TGW, BY, GY, and HI are plant dry weight (g plant^{-1}), green leaf area (cm^2 plant^{-1}), leaf area index, leaf relative water content (%), total chlorophyll content (mg g-1 FW), grain number per spike, thousand-grain weight (g), biological yield (ton ha^{-1}), grain yield (ton ha^{-1}), and harvest index (%), respectively. Values 1 and 2 represent measurements at 70 and 90 days after sowing, respectively.

The GNPS and BY traits were grouped into Cluster 1, and the STI for the former trait was higher in the T group, followed by the I and S groups, while the STI for the latter trait was higher in the I group, followed by the T and S groups (Figures 4 and 5). The STI values for the traits of Cluster 2 (LAI at 70 and 90 DAS and Chlt) were highest in the I group, followed by the T group. The S group had the lowest STI values for these traits. For the traits in Cluster 3, the T group consistently had higher STI values, followed by the I and S groups (Figures 4 and 5). Additionally, the lowest STI values were observed for the Chlt, LAI at 70 DAS, GLA, and PDW at 70 and 90 DAS, and GY. The STI values for these traits in the S group decreased by 18.8%, 22.1%, 18.6%, 21.9%, 26.2%, 18.6%, and 26.1%, respectively, compared to those in the T group (Figure 5).

The results in Table 6 show that, under control conditions, both the I and S groups achieved higher mean values for most morpho-physiological traits compared to the T group. However, under salinity conditions, the opposite was observed. Under control conditions, both the I and S groups attained higher mean values for all traits compared to the T group, except for LAI at 70 and 90 DAS for the I group and Chlt and BY for both groups. The T group exhibited higher values for these traits compared to the I and S groups. Meanwhile, under salinity conditions, both the I and S groups displayed a reduction in the mean values of various agro-morpho-physiological traits by 1.7–13.6% and 2.8–28.5%, respectively, compared to the T group (Table 6). Furthermore, the S group was significantly more affected by salinity stress than the T and I groups. The mean values of different agro-morpho-physiological traits under salinity conditions decreased by 8.4–37.1%, 10.6–36.0%,

and 15.0–50.7% in the T, I, and S groups, respectively, when compared to the control conditions (Table 6).

Table 6. Mean values of different traits for salt-tolerant (T), intermediate (I), and salt-sensitive (S) genotypes groups under control (C) and salinity (S) conditions.

	T-Group			I-Group			S-Group		
	C	**S**	**%Change**	**C**	**S**	**%Change**	**C**	**S**	**%Change**
PDW-1	5.74	4.74	17.5	5.78	4.09	29.1	5.73	3.85	32.7
PDW-2	7.97	5.84	26.7	8.31	5.43	34.7	8.76	4.77	45.6
GLA-1	139.88	107.47	23.2	143.70	102.20	28.9	141.23	88.09	37.6
GLA-2	115.36	89.26	22.6	118.13	83.90	29.0	113.79	68.57	39.7
LAI-1	3.69	2.32	37.1	3.43	2.32	32.3	3.68	1.88	49.1
LAI-2	3.04	1.93	36.7	2.84	1.85	35.1	2.97	1.46	50.7
LRWC	77.83	71.25	8.4	78.03	69.76	10.6	78.87	64.78	17.9
Chlt	3.38	2.28	32.6	3.09	2.08	32.8	2.49	1.63	34.8
NGPS	45.04	41.36	8.2	47.45	42.41	10.6	46.73	39.71	15.0
TGW	45.74	41.28	9.8	45.11	38.80	14.0	48.49	39.52	18.5
BY	19.35	13.92	28.0	18.09	13.69	24.4	18.06	11.26	37.7
GY	5.56	4.04	27.3	5.88	3.76	36.0	5.81	3.12	46.3
HI	28.80	28.98	-0.6	32.57	27.57	15.4	32.66	28.18	13.7

Abbreviations of PDW, GLA, LAI, LRWC, Chlt, GNPS, TGW, BY, GY, and HI are plant dry weight (g plant^{-1}), green leaf area (cm^2 plant^{-1}), leaf area index, leaf relative water content (%), total chlorophyll content (mg g^{-1} FW), grain number per spike, thousand-grain weight (g), biological yield (ton ha^{-1}), grain yield (ton ha^{-1}), and harvest index (%), respectively. Values 1 and 2 represent measurements at 70 and 90 days after sowing, respectively.

3.6. Clustering of Genotypes Based on SSR Markers and Their Association with Those Derived from Agro-Morpho-Physiological Traits Using Mantel Test

The 24 genotypes/RILs were divided into three distinct clusters based on the allelic data of 40 SSRs linked to salt-tolerant genes (Figure 6). In general, the SSR data separated the salt-sensitive check genotype Sakha 61 from the two salt-tolerant check genotypes Sakha 93 and Kharachia-65. Cluster 1 included two, two, and one genotype(s)/RIL(s) from the S, T, and I groups, respectively. Cluster 2 included two, three, and four genotypes/RILs from the S, T, and I groups, respectively. Cluster 3 did not include any genotypes/RILs from the S group, while it included three and seven genotypes/RILs from the T and I groups, respectively (Figure 4).

The clustering pattern of 24 genotypes/RILs based on their STI of all agro-morpho-physiological traits (phenotypic distance) was associated with those derived from 40 SSRs linked to salt-tolerant genes (genetic distance) using the Mantel test. The Mantel test showed a significant positive correlation (r = 0.13, $p < 0.03$, and alpha = 0.05) between agro-morpho-physiological traits and SSR data, which indicates that the molecular and phenotypic classifications are somewhat correlated. These positive correlations were necessary, as agro-morpho-physiological traits are typically used as an efficient way of routinely accessing several genotypes in a breeding program without the use of molecular markers.

3.7. Association of SSR Markers with Morpho-Physiological Traits and Their Salt Tolerance Index

The stepwise linear regression was used to identify the most influential SSR markers associated with different agro-morpho-physiological traits under both control and salinity conditions, as well as with an STI. In general, the different markers showed significant association with all agro-morpho-physiological traits under both control (R^2 ranged from 0.24 to 0.88) and salinity (R^2 ranged from 0.38 to 0.78) conditions. They also demonstrated association with the STIs of all traits (R^2 ranged from 0.25 to 0.85), except for PDW at 70 and 90 DAS under control conditions, TGW under salinity conditions, and the STI for BY (Table 7). Two markers, Wmc154 and Wmc367, were significantly associated with PDW at 70 DAS under salinity conditions ($R^2 = 0.51$) and with the STI of this trait ($R^2 = 0.50$). Five markers (Gwm55, Wmc154, Wmc405, Barc110, and Cfd18) and three markers (Wmc154,

Barc182, and Wmc419) exhibited highly significant associations with PDW at 90 DAS under salinity conditions (R^2 = 0.76) and with the STI of this trait (R^2 = 0.69), respectively. The marker Gmw350 showed a significant association with GLA at 70 and 90 DAS under control conditions, whereas the marker WMC154 displayed a significant association with the STI for both traits. Under salinity conditions, markers Gmw350 and Wmc154 exhibited a highly significant association with GLA at 70 DAS (R^2 = 0.59), while Barc44 and Cfd9 markers had a highly significant association with GLA at 90 DAS (R^2 = 0.68). The combination of two markers and three markers accounted for 51.0% and 66.0% of the total variations in LAI at 70 DAS among genotypes. Similarly, the combination of five markers and three markers accounted for 88.0% and 77.0% of the total variation in LAI at 90 DAS among genotypes under both control and salinity conditions, respectively. However, when it came to the STI of LAI at 70 and 90 DAS, the combinations of two markers and three markers justified 60.0% and 81.0% of the total variation, respectively (Table 7).

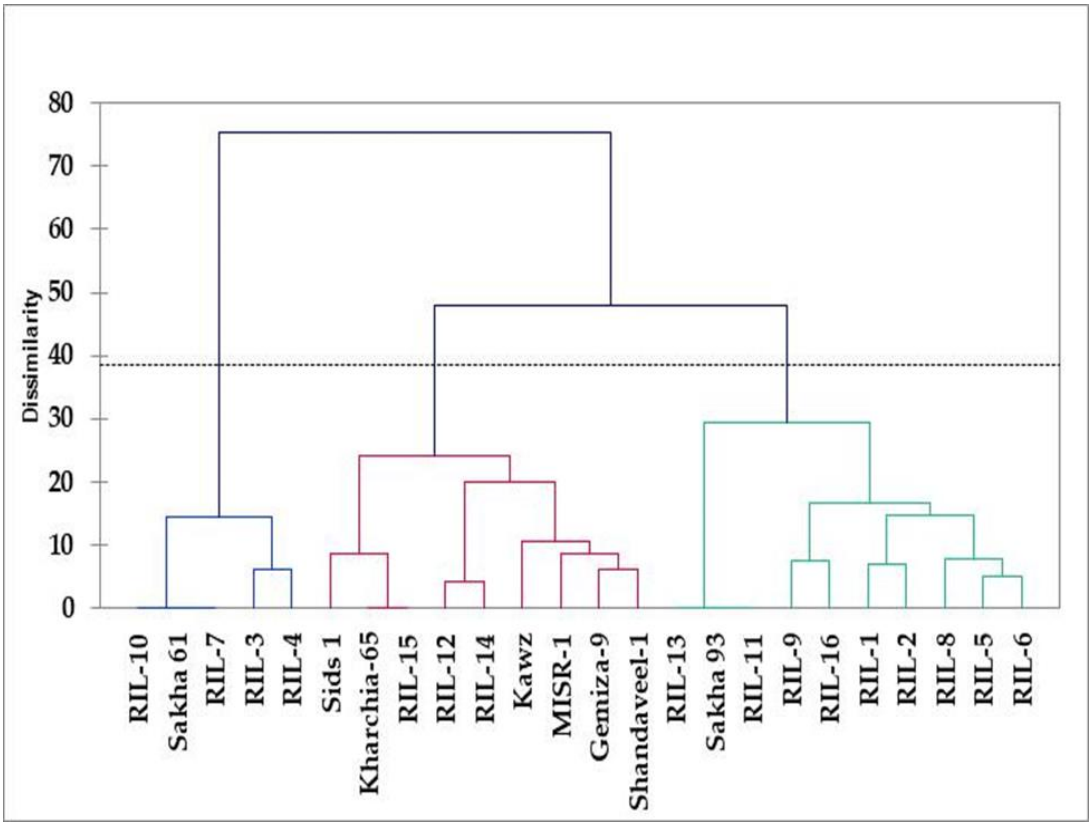

Figure 6. Genotypic clustering of 24 tested genotypes based on 40 simple sequence repeat (SSR) markers linked to salt-tolerant genes using unweighted clustering trees.

Regarding the two physiological traits (LRWC and Chlt), the markers showed a significantly stronger association with both traits under salinity conditions (R^2 = 0.54 and 0.74) and with the STI for both traits (R^2 = 0.85 and 0.47) compared to control conditions (R^2 = 0.24 and 0.37). One and two markers accounted for more variation in LRWC and Chlt among genotypes under control conditions. Two and three markers accounted for more variation in LRWC and Chlt among genotypes under salinity conditions. Three and four markers accounted for more variation in STI for LRWC and Chlt among genotypes, respectively.

Table 7. Associating the most influential SSR markers with different agro-morpho-physiological traits under control and salinity conditions as well as with a stress tolerance index (STI).

Control				Salinity				STI			
Trait	**Markers**	**R^2 Par.**	**R^2 Cum**	**Trait**	**Markers**	**R^2 Par.**	**R^2 Cum**	**Trait**	**Markers**	**R^2 Par.**	**R^2 Cum**
GLA-1	Gwm350	0.32 **	0.32	PDW-1	Wmc154	0.31 ***	0.51	PDW-1	Wmc154	0.34 ***	0.50
GLA-2	Gwm350	0.31 **	0.31		Wmc367	0.20 *			Wmc367	0.16 *	
LAI-1	Wmc661	0.37 ***	0.51	PDW-2	Gwm55	0.26 ***	0.76	PDW-2	Wmc154	0.45 ***	0.69
	Gwm55	0.14 **			Wmc154	0.19 **			Barc 182	0.11 ***	
LAI-2	Gwm210	0.29 ***	0.88		Wmc405	0.12 *			Wmc419	0.13 ***	
	Barc34	0.25 ***			Barc110	0.12 **		GLA-1	Wmc154	0.31 ***	0.31
	Wmc18	0.22 ***			Cfd18	0.07 *		GLA-2	Wmc154	0.25 **	0.25
	Gwm296	0.09 **		GLA-1	Gwm350	0.33 **	0.59	LAI-1	Cfd18	0.46 ***	0.60
	Cfd49	0.03 ***			Wmc154	0.26 **			Gwm410	0.14 **	
LRWC	Wmc11	0.24 *	0.24	GLA-2	Barc44	0.39 ***	0.65	LAI-2	Cfd18	0.41 ***	0.81
Chlt	Barc110	0.29 ***	0.37		Cfd9	0.26 **			Gwm249	0.13 ***	
	Cfd18	0.08 *		LAI-1	Cfd1	0.29 ***	0.66		Wmc405	0.14 *	
GNPS	Barc34	0.29 **	0.45		Cfd9	0.27 ***			Wmc245	0.13 ***	
	Barc167	0.16 *			Wmc405	0.10 ***		LRWC	Barc109	0.61 ***	0.85
TGW	Barc34	0.21 ***	0.62	LAI-2	Barc44	0.39 **	0.77		Wmc154	0.14 ***	
	Gwm410	0.20 **			Cfd9	0.28 ***			Wmc177	0.10 ***	
	Gwm335	0.12 *			Wmc405	0.10 ***		Chlt	Barc167	0.29 **	0.47
	Wmc367	0.09 **		LRWC	Barc44	0.36 ***	0.54		Cfd9	0.18 **	
BY	Cfd9	0.21 **	0.42		Wmc11	0.18 *		NGPS	Barc112	0.26 ***	0.61
	Wmc177	0.11 **		Chlt	Wmc245	0.24 *	0.74		Gwm539	0.16 ***	
	Gwm55	0.10 **			Cfd9	0.15 ***			Barc110	0.20 ***	
GY	Barc167	0.49 ***	0.49		Cfd9	0.35 ***		TGW	Gwm210	0.54 ***	0.82
HI	Cfd9	0.18 *	0.41	GNPS	Gwm335	0.38 ***	0.38		Wmc503	0.09 ***	
	Wmc503	0.23 *		BY	Wmc154	0.16 *	0.58		Wmc405	0.12 ***	
					Cfd66	0.18 *			Gwm174	0.08 **	
					Wmc154	0.13 **		GY	Wmc154	0.30 ***	0.69
					Gwm314	0.11 *			Cfd66	0.22 *	
				GY	Barc44	0.37 ***	0.66		Wmc367	0.09 **	
					Cfd9	0.15 *			Barc110	0.08 *	
					Barc34	0.14 *		HI	Gwm174	0.36 ***	0.68
				HI	Gwm55	0.31 ***	0.78		Cfd18	0.17 **	
					Wmc154	0.16 ***			Cfd49	0.14 **	
					Barc58	0.22 ***					
					Gwm296	0.09 *					

Abbreviations of PDW, GLA, LAI, LRWC, Chlt, GNPS, TGW, BY, GY, and HI are plant dry weight (g plant^{-1}), green leaf area (cm^2 plant^{-1}), leaf area index, leaf relative water content (%), total chlorophyll content (mg g^{-1} FW), grain number per spike, thousand-grain weight (g), biological yield (ton ha^{-1}), grain yield (ton ha^{-1}), and harvest index (%), respectively. Values 1 and 2 represent measurements at 70 and 90 days after sowing, respectively. *, **, and *** indicate significant at $p \leq 0.05$, 0.01, and 0.001, respectively.

Regarding the yield and yield component traits, the markers demonstrated a stronger association with STI for GNPS (R^2 = 0.61) compared to GNPS under control (R^2 = 0.45) and salinity (R^2 = 0.38) conditions. Specifically, two markers accounted for a greater amount

of variation in GNPS under control conditions, one marker under salinity conditions, and three markers for STI in this trait (Table 7). Additionally, the markers exhibited a stronger correlation with GY and HI under salinity conditions (R^2 = 0.66 and 0.78) and with STI for both traits (R^2 = 0.69 and 0.68) compared to GY and HI under control conditions (R^2 = 0.49 and 0.41). Only one marker explained more variation in GY, while two markers explained more variation in HI among genotypes under control conditions. However, under salinity conditions and for STI in both traits, more than two markers explained the variation in GY and HI among genotypes (Table 7). There were no markers that showed a significant association with TGW under salinity conditions and STI for BY. However, TGW under control conditions and the STI for this trait were significantly associated with four markers. These four markers accounted for 62.0% and 82.0% of the total variation among genotypes, respectively. Regarding the BY, three and four markers accounted for 42.0% and 58.0% of the total variation for this trait among genotypes under control and salinity conditions, respectively (Table 7).

4. Discussion

Salinity stress is one of the most well-known abiotic stressors that significantly decreases wheat production in many arid and semiarid countries. Therefore, it is crucial to provide farmers with salt-tolerant genotypes. This approach is not only the most beneficial but also the most economical way to sustain wheat production in saline conditions. However, the tolerance of genotypes to salinity stress is influenced by a multitude of intricate and interconnected mechanisms and polygenic traits. As a result, genotypes display a wide range of responses to salinity stress at different levels, including morphological, physiological, biochemical, and molecular. Furthermore, the acquisition of salt tolerance in genotypes necessitates a significant amount of genetic variability across multiple traits. Furthermore, it is of utmost importance to evaluate the salt tolerance of different genotypes in real field conditions, where plants are exposed to a range of environmental factors. Therefore, it is essential to gain a comprehensive understanding of how various traits respond to salinity stress in different genotypes at different stages of growth under real field conditions. This knowledge is crucial for the success of breeding programs aiming to develop salt-tolerant varieties [4,9,10,49]. In this study, we investigated the impact of salt stress on multiple morphological, physiological, and agronomic traits in different wheat genotypes cultivated under actual field conditions. The ANOVA results revealed significant statistical differences between the genotypes and salinity levels for all traits examined, both within each year and when the data from both years were combined (see Table 1). Additionally, certain genotypes exhibited average trait values that were approximately one to three times higher than those of the other genotypes, irrespective of whether they were exposed to control or salinity conditions (Tables 2 and 3). Additionally, there was a wide range among genotypes in the percentage reduction of the tested traits under high salinity levels compared to the control treatment (Table 4). All of these findings indicate that there is genetic variation among the tested genotypes in terms of salt tolerance. Additionally, the assessed traits have proven to be effective screening criteria for distinguishing between salt-tolerant and salt-sensitive genotypes. The results presented in Table 4 further support these findings, as they demonstrate that the genotypes Sakha 93 and Kharachia-65, as well as the majority of the RILs from the cross between Sakha 93 and Sakha 61, exhibited the lowest reduction percentages for the majority of the agro-morpho-physiological traits. This confirms the salinity stress tolerance of these RILs/genotypes. Conversely, the genotypes Sakha 61 and Shandaweel-1, which are salt-sensitive, displayed the opposite trend.

Under salinity stress conditions, various agro-morpho-physiological traits are negatively affected due to the combined stresses of osmotic and ionic toxicity, as well as a deficiency in essential nutrients. These three factors contribute to a decrease in cell division and elongation, cell membrane stability, leaf turgidity, leaf water content, biomass accumulation, photosynthetic capacity, metabolic functions, chlorophyll pigments, light interception, protein synthesis, and source–sink activity. Additionally, salinity stress induces

the production of high levels of reactive oxygen species. As a result, these detrimental effects of salinity stress lead to a significant reduction in plant growth indicators such as PDW, LA, and LAI, as well as physiological indicators like LRWC and Chl content, and agronomic indicators including GY, BY, HI, TGW, and GNPS [20,23,25,26,28,40]. Therefore, these various indicators are essential attributes that play a significant role in evaluating genotypes for their capacity to endure salinity conditions. In this study, LWRC, GNPS, and TGW demonstrated the least susceptibility to salinity stress. However, other traits exhibited reductions exceeding 25% in their average values due to salinity stress (Tables 2 and 3). These findings indicate that genotypes have a substantial impact on the diversity observed in LWRC, GNPS, and TGW, thereby making these characteristics unique to each genotype. Nevertheless, it is important to consider these traits in conjunction with other agro-morpho-physiological traits when evaluating salt tolerance based on effective screening criteria. Similarly, previous reports have indicated that PDW, LA, Chlt, GNPS, and GY are essential screening criteria for assessing the salinity tolerance of wheat genotypes [3,16,40]. These findings can be attributed to the significant decrease in these characteristics, even when exposed to low and medium salinity stresses. Moreover, traits like PDW, LA, and Chlt can act as reliable indicators of the plant's overall response to salt stress throughout different growth stages. It is crucial to acknowledge that the final crop production (GY) is greatly influenced by the strong correlation between GY and the growth and physiological traits that take place at different growth stages [15,49]. The results of this study indicated a noteworthy and positive correlation between GY and the growth traits (PDW, LA, and LAI) assessed at 70 and 90 DAS. Additionally, physiological traits (LRWC and Chlt) exhibited a significant association with GY under salinity conditions (Table 5). Therefore, these traits have been recognized as essential for evaluating wheat genotypes in terms of their salt tolerance. As a result, it can be inferred that the identification of traits closely associated with salt stress tolerance can serve as a benchmark for effectively differentiating between salt-tolerant and salt-sensitive genotypes.

The main goal of PCA, a type of multivariate analysis, is to illustrate the relationships among traits themselves and the correlations between traits and genotypes. As a result, it enables the assessment of salt tolerance in genotypes by considering all the examined traits. Additionally, it assists in identifying the most influential trait that contributes the most to the overall variance and effectively distinguishes between salt-tolerant and sensitive genotypes [9,50,51]. The PCA results in this study successfully identified the screening criteria that effectively evaluate the salt tolerance of wheat genotypes under real field conditions. This study revealed that the PDW at 90 DAS, GLA, and LAI at 70 and 90 DAS, GY, TGW, and GNPS played a significant role in describing the greatest variation observed among different genotypes in saline conditions (Figure 2). Furthermore, the thirteen morpho-agro-physiological traits were able to successfully differentiate the various genotypes into three distinct groups when exposed to salinity conditions. Additionally, they effectively separated the salt-tolerant genotypes (Sakha 93 and Kharachia-65) from the salt-sensitive ones (Sakha 61 and Shendaweel-1), with the most salt-tolerant genotypes positioned on the right side and the most sensitive ones on the left side under salinity conditions. However, when grown under normal control conditions, the salt-tolerant and salt-sensitive genotypes were grouped together and placed on the left side (Figure 3). These results indicate that PCA has the potential to distinguish between genotypes based on their salt tolerance in real field conditions by utilizing multiple traits. Likewise, PCA has been widely and successfully used by several studies to accurately evaluate the salt tolerance of genotypes using multiple parameters [4,9,52–54].

Generally, when plants are exposed to high levels of salt, their growth tends to decrease. This decrease in growth is attributed to a reduction in the activity of the plant's cellular metabolic pathways, leading to a decrease in the synthesis of important compounds. Additionally, the plant's energy and metabolic resources are redirected towards activating mechanisms that help the plant's ability to tolerate salinity stress rather than being allocated towards the growth of various plant organs and the production of plant biomass. As a

result, the plant's ability to accumulate biomass, also known as PDW, is negatively affected as a consequence. Moreover, the simultaneous existence of osmotic and ionic stresses caused by salinity can cause substantial changes in leaf structure. This leads to cell death, necrosis, and senescence of leaves, ultimately leading to a noticeable reduction in both GLA and LAI [4,6,40,55]. The ultimate GY is significantly impacted by different plant traits, particularly those related to biomass distribution and the capture and transformation of sunlight. Furthermore, these traits are primarily established during critical growth stages throughout the crop's life cycle. As a result, GY serves as a comprehensive indicator of the plant's overall lifespan and highlights the magnitude and importance of the negative impacts caused by salinity stresses experienced by the plants [56,57]. The explanations mentioned above offer support for the important role of PDW, GLA, LAI, and GY in explaining the considerable variation observed among different genotypes. The results of Figures 1 and 2 validate these findings and demonstrate that PC1, which accounted for 53.47% of the total variability and was able to isolate the salt-tolerant genotypes, exhibited a strong correlation with these four traits. Additionally, there is a strong acute angle between their vectors under salinity conditions.

To categorize the genotypes according to their salt tolerance and identify those that simultaneously exhibit high trait values and potential for salt tolerance, we utilized hierarchical cluster analysis-based STI for all traits. In this study, the heatmap clustering pattern based on STI of all traits successfully grouped genotypes into three distinct clusters and distinguished the salt-tolerant genotypes (T) from the salt-sensitive (S) and intermediate (I) ones (Figure 4). Additionally, the three clusters of genotypes exhibited significant variations in STI for all traits. The T group displayed the highest values of STI for almost all traits, followed by the I group. However, the S group had the lowest values for all traits except HI (Figure 5). These findings indicate that using hierarchical cluster analysis in combination with STI of multiple traits is an effective method for assessing the salt tolerance of wheat genotypes in real field conditions. In this study, this approach effectively differentiated between salt-tolerant and salt-sensitive genotypes, irrespective of growth stages and salinity levels. Several previous studies have confirmed the effectiveness of this approach in categorizing genotypes based on their tolerance to various abiotic stresses, including salinity and drought. However, it is worth noting that the majority of research evaluating the salt tolerance of genotypes through this approach was conducted in controlled environments such as greenhouses and growth chambers [15,52,58,59].

While the aforementioned approach successfully distinguishes the salt tolerance of genotypes, it is important to note that most agro-morpho-physiological traits are typically influenced by environmental conditions. Therefore, any changes in the environment can potentially influence salt tolerance among genotypes. Additionally, assessing the salt tolerance of genotypes based on multiple agro-morpho-physiological traits can be costly and time-consuming. As a result, when solely relying on phenotypic traits, there are limitations in accurately assessing the genetic diversity in salt tolerance. Therefore, there is an urgent need for a complementary approach to accurately evaluate the genetic diversity in salt tolerance of genotypes in a rapid and cost-effective manner. One prominent approach in molecular breeding involves selecting genotypes using molecular markers that are linked to quantitative trait loci (QTLs) responsible for key agro-morpho-physiological traits under salinity stress. This approach is particularly useful in cases where candidate genes are unavailable. Among these markers, SSR markers serve as a potential tool for assessing the degree of genetic variation in salt tolerance in several crops [15,31–33,46]. This approach offers numerous advantages that have already been mentioned in the Introduction section. In this study, cluster analysis based on SSR data successfully grouped the salt-tolerant genotypes and salt-sensitive genotypes into separate main clusters (Figure 6). Furthermore, the clustering pattern of the tested genotypes, based on the hierarchical cluster analysis approach in combination with STI of multiple traits, was found to be associated with the clusters derived from the cluster analysis based on SSR data. This association was demonstrated through a positive and significant correlation observed in Mantel's test

(r = 0.13, $p < 0.03$, and alpha = 0.05). However, this association was weak, indicating a lack of agreement between the phenotypic and molecular aspects. Despite the weak association, there were significant correlations between phenotypic traits and molecular characteristics. Therefore, this association is crucial as agro-morpho-physiological traits are an effective approach for regularly evaluating several genotypes in a breeding program, even in the absence of molecular markers. Previous studies have also reported a weak correlation between the clustering of genotypes, determined by phenotypic traits, and their clustering based on SSR marker data [60,61]. However, other studies have found a strong correlation between the clustering of genotypes based on agro-morpho-physiological traits and their clustering based on SSR marker data [15,46,62,63]. There are multiple reasons that may explain the weak correlation between phenotypic and molecular data in this study. Firstly, the measurements of different agro-morpho-physiological traits were conducted under complex and environmentally influenced field conditions. This complexity may have introduced variability and affected the accuracy of the data. Secondly, agro-morpho-physiological traits are influenced by polygenes, which allow plants to adapt to diverse environmental conditions through phenotypic plasticity. On the other hand, microsatellite variability is primarily neutral, which makes it a reliable tool for providing an unbiased representation of diversity and accurately distinguishing stress tolerance among closely related genotypes. Lastly, the limited number of markers and genotypes used in this study may have contributed to the weak correlation observed. With a larger sample size and more markers, a stronger correlation might have been detected.

Given the importance of markers associated with various agro-morpho-physiological traits and their role in salt tolerance, we have identified 18 and 19 markers that are linked to different traits under salinity conditions (with R^2 values ranging from 0.38 to 0.78) and STI (with R^2 values ranging from 0.25 to 0.85), respectively (Table 7). These findings further suggest that the thirteen agro-morpho-physiological traits measured in this study can be used as valuable indicators for evaluating the genetic diversity of salt tolerance in wheat. Moreover, they offer valuable insights into the mechanisms that contribute to salt tolerance in different wheat genotypes under real field conditions. Among the effective SSR markers used in this study, the marker known as cfd 9 from the D genome [64] was amplified under salinity conditions and showed a significant association with various traits, including GLA-2, LAI-1, LAI-2, Chlt, and GY. The D genome primarily regulates salt tolerance in hexaploid wheat, which validates the phenotypic assessment of genotypes [65]. El-Hendawy et al. [46] conducted a study that revealed a strong correlation between Wmc 154 and water absorption in the presence of 60 and 120 mM NaCl. The current study validates the observed correlation, as the amplification of wmc 154 was solely observed with PDW-1, PDW-2, GLA-1, GLA-2, LRWC, and GY under salinity conditions and with STI. In contrast, no correlation with wmc 154 was observed under control conditions. It is not surprising that multiple markers exhibit correlations with more than two variables, considering their polygenic background. In our study, we found that certain SSR markers, which are associated with desirable traits, were only observed in wheat crops under salinity stress conditions. These markers hold significant potential for future breeding programs focused on improving salinity tolerance in wheat crops grown in field conditions.

5. Conclusions

This study has yielded important findings on the agro-morpho-physiological traits that can be used as screening criteria, along with their correlation with SSR markers, to evaluate the salt tolerance of wheat genotypes in real field conditions. Our results indicate that PDW, GLA, and LAI measured at different growth stages and GY are effective traits for evaluating the salt tolerance of wheat genotypes grown in field conditions. Clustering genotypes based on STI for all traits or based on SSR data successfully grouped the tested genotypes into three distinct categories and distinguished the salt-tolerant genotypes from the sensitive ones. Therefore, a significant association between agro-morpho-physiological traits and SSR markers data was detected. However, this association was found weak on the

Mantel test. The traits identified in this study can be recommended as valuable screening criteria for evaluating the salt tolerance of different wheat plant materials grown in field conditions. However, there is an urgent need for a rapid, cost-effective, and large-scale phenotypic identification tool to detect these traits in a large number of wheat materials under field conditions. This will be the focus of our future study.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agriculture13112135/s1. Table S1: List of SSR markers, their sequence, and annealing temperature that were used in this study across 24 wheat genotypes.

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