RESEARCH ARTICLE

Candidate genes controlling alkaline-saline tolerance in two different growing stages of wheat life cycle

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Abstract

Background Alkaline-saline (AS) stress threats crop development and productivity. Understanding the genetic control of AS tolerance in wheat is important to produce wheat cultivars that outstand such a severe stress condition.

Methods A set of 48 cultivars were tested under controlled and AS stress conditions at seedling and maturity stages. The effect of AS on seedlings and kernel traits was measured to select tolerant and high-yielding genotypes. Single-marker-analysis (SMA) and gene enrichment were conducted to understand the genetic control of AS tolerance in both growth stages.

Results AS stress decreased all kernel traits and most of the seedling traits. High correlations were found between the studied traits in each growth stage. The correlation between the traits related to both stages was non-significant. SMA identified a total of 292 and 52 markers significantly associated with the studied traits under controlled and AS stress conditions. Seven and 20 gene models were identified to control AS tolerance in each stage. Gene enrichment analysis identified one and six networks that control AS tolerance. Four genotypes were selected as superior genotypes.

Conclusion The genetic control of the studied traits differs under control and AS conditions. Two genetic systems control AS tolerance in each growth stage. This study is the first one that unlocked the genetic control of AS tolerance in seedling and mature growth stages and identified the biological process that lead to this tolerance. Four genotypes were

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selected for crossing in future breeding programs to improve AS tolerance in spring wheat.

Introduction

Across their life cycle, plants are exposed to a variety of abiotic stresses that threats plant development, growth, and productivity. Due to their sessile nature, plants can't escape or avoid them completely. Soil salinity-alkalinity is among the most important factors limiting agricultural yield all over the world (Liu et al. 2010; Qadir et al. 2014). Globally, about 80 million hectares of irrigated soil are damaged by salinity or alkalinity stress, representing about 40% of the total irrigated area, and over half of them are alkaline (Abdel Latef et al. 2019). With the continuously growing human population, the degradation of the ecosystem increased, resulting in more areas affected by soil salinity-alkalinity. Numerous unwisdom cultivation practices in agriculture, coupled with the aggravation of environmental pollution, increase soil salinization–alkalization (De Pascale et al. 2012). It is expected that by 2050, soil salinization will damage up to 50% of agricultural land (Qadir et al. 2014). Both salinity and alkalinity negatively affect plant growth. Salinity mainly reduces plant growth owing to the osmotic inhibition of water absorption and ion toxicity (Munns and Tester 2008). Alkalinity's deleterious effects on plant growth are attributed to high pH, which exerts ion imbalance and reduces the availability of micronutrients in soils. Alkalinity reduces the solubility of nutrients like phosphorus (P), iron (Fe), zinc (Zn), and other micronutrients (FAO 2000). Salinity stress is primarily attributed to neutral salts such as NaCl and Na_2SO_4, whereas alkalinity stress is exerted by alkaline salts such as $NaHCO_3$ and Na_2CO_3. When salinized soil contains high concentrations of alkaline salts, the soil pH increases, and the plants suffer from both saline and alkaline stresses. The combined effect of soil salinity-alkalinity could be more severe than soil salinization alone (Yang et al. 2008a). Several studies reported that saline-alkaline stress exerted significant changes in the growth of plants including ionic stress, osmotic stress, oxidative stress, and high pH stress (Guo et al. 2009; Li et al. 2010; Magistad, 1945; Zhang et al. 2020). Alkaline soils may be more stressful than saline soils (Tanji 2002; Wang et al. 2008). Salinity stress research focused on NaCl (Munns and Tester 2008) and less attention has been paid to alkaline stress (Zhang et al. 2020).

Wheat (*Triticum aestivum* L.) is one of the most important stable food crops. Wheat grain is being used to feed 40% of the world's population (Han et al. 2018). In terms of socio-economic importance, wheat dominates the remaining crops with 17% of the global crop area, feeding 40% of the world's population and insuring 20% of the total diet calories. To meet this high demand, wheat production needs to be increased by 2% annually (Al-Ashkar et al. 2020). Therefore, monitoring its planting areas is crucial to ensure food supply all over the world. Wheat was classified as moderately salt-tolerant relative to other crops, including cereals (Maas and Hoffman 1977). When soil salinization reaches 100 mM, both wheat development and growth are drastically affected (Munns et al. 2006). Salinization–alkalization effect on seedling stage and vegetative development-related parameters has been intensively investigated. Several traits such as root length, shoot length, seedling length, fresh biomass and dry biomass were measured in many plant species including sunflower (*Helianthus annuus* L.) (Liu et al. 2010), alfalfa (*Medicago sativa* L.) (Li et al. 2010; Liu et al. 2013), Lettuce (*Lactuca sativa*) (Roosta 2011), Indian mustard (*Brassica juncea* L.) (Javid et al. 2012), maize (*Zea mays* L.) (Xie et al. 2015; Cao et al. 2020; Fu et al. 2017, 2021; Fatima et al. 2021;), Sorghum (*Sorghum bicolor* L.) (Zhao et al. 2014), rice (*Oryza sativa* L.) (Lv et al. 2013; Li et al. 2019a, b; Li et al. 2020; Liu et al. 2021), artichoke (*Cynara scolymus* L.) (Dawood et al. 2021), tomato (*Solanum lycopersicum* L.) (Hu et al. 2012; Li et al. 2015; Capula-Rodríguez et al. 2016; Xu et al. 2022), strawberry (*Fragariaananasa* L.) (Malekzadeh Shamsabad et al. 2021; Roosta 2014), soybean (*Glycine max* L.) (Cao et al. 2021) and wheat (*Triticum aestivum* L.) (Guo et al. 2010, 2015; Lin 2012; Zhang et al. 2021a, b).

Except for the progress achieved in alkalinity stress studies, the effect of alkalinity-salinity stress on grain-related traits lags behind the study of its effect on germination, seedling, and vegetative stages. Some studies focused on the effect of alkalinity-salinity

stress on grain-related traits and yield-attributes in rice (Rao et al. 2008, 2013), sunflower (Li et al. 2021), cotton (*Gossypium barbadence* L.) (Zein et al. 2020), and wheat (Sharma et al. 2010b). In north-east China, soil salinization–alkalization decreased wheat productivity, reviewed by Zhang et al. (2020). Nevertheless, some saline-alkaline tolerant cultivars were found (Zhang et al. 2020).

A lot of drawbacks are coupled with the improvement of abiotic stress. For example, washing saline-alkaline soil artificially using excess fresh water decreases soil fertility by leaching fertilizers and minerals downwardly. Improvement of high saline-alkaline soils needs more manpower, materials, and financial resources (Liu et al. 2007). This artificial approach is expensive, environmentally unfriending, and unsustainable. The biological approach depends on selecting and developing more abiotic stress-resilient varieties, which are more reasonable and sustainable.

In terms of the association between abiotic stress tolerance and the plant growth stage, it has been demonstrated that plant resistivity to certain abiotic stresses varies from one growth stage to another. Thus, the current study focused on elucidating the genotypic variation of wheat under two stages; the seedling stage and the mature stage, in order to get comprehensive insights into wheat tolerance to alkalinity-salinity stress. As a result of the low number of studies that focused on the combined effect of alkalinity-salinity stress relative to salinity-stress or alkalinity-stress, as well as the low number of studies that dealt with the effect of such stress at different growth stages, the current study has been conducted to elucidate the effect of alkaline-saline stress on wheat at two different growth stages: the seedling stage and the adult stage.

Only in the last ten years, few studies dealt with the genetic control of the alkalinity-salinity either in model plants such as *Arabidopsis* (Almira Casellas et al. 2023) and alfalfa (An et al. 2020), as well as in some crop species such as rice (Liang et al. 2015; Krishnamurthy et al. 2016; Qian et al. 2023), soybean (Zhang et al. 2014), and maize (Zhang et al. 2018). Likewise, in wheat, the genetic factors that control the alkalinity-salinity stress are unclear up to now.

The objectives of this study are to: 1) study the effect of alkalinity-salinity stress on wheat development at seedling and maturity stages, 2) understand the genetic control of alkaline-saline tolerance in wheat plants in the two different growth stages, and 3) select the best high yielding and genetically distant parents to be incorporated in the breeding programs to improve wheat yield under AS stress.

Materials and methods

Plant material

In this study, a set of 48 spring wheat genotypes were evaluated for alkaline-saline (AS) tolerance in seedling and mature stages. This set consisted of 13 Egyptian cultivars, four Egyptian breeding lines, and 31 cultivars from additional eleven different countries (Table S1). Non-Egyptian genotypes were evaluated for several growing seasons in Egyptian fields and were highly adapted to Egyptian conditions (Mourad et al. 2020). Seeds of the non-Egyptian tested genotypes were obtained from the USDA-ARS, United States. The Egyptian genotypes were developed by the Egyptian governorate and Egyptian breeding lines were generated by Prof. Dr. Qadry Omara, Genetics Department, Faculty of Agriculture, Assiut University. These genotypes were reported to be highly diverse in their response to the different abiotic stresses including salt tolerance (Ghazy et al. 2021; Ahmed et al. 2022; Mohamed et al. 2023). Hence they could provide more information on saline-alkaline tolerance in wheat.

Experimental design and treatment application

Seedling stage experiment

To study the effect of AS treatment on the early wheat growth stage, the seedling experiment was conducted in the Plant Genetics Lab, Genetics Department, Faculty of Agriculture, Assiut University, Egypt using small trays. The room temperature of the lab was between 23°C to 25 °C during the experiment. The size of each tray was $35 \times 21 \times 4.4$ cm, and each contains 60 holes. Each hole was filled with 50 gm of field soil (collected from Assiut research station). The soil type is 5 clay: 3 silt: 2 sand. Before seed sowing, for the controlled treatment (C), 50 ml of tap water was added for each tray, and for Alkaline-saline treatment (AS), 50 ml of 150 mM NaCl: Na_2SO_4:

NaHCO$_4$:Na$_2$CO$_3$ at a proportion of 1:9:9:1, respectively was added for each hole (Dawood et al. 2021). In each hole, two seeds were sown from each genotype/replication. The experimental design was a randomized complete block design (RCBD) using three replications for each treatment. Irrigation with 50 ml tab water was applied for all trays when required until the end of the experiment. After two weeks, the seedlings were gently removed from the soil and cleaned for further measurements.

Mature stage experiment

To study the effect of AS on yield attributes (kernel-related-traits) and identify the most tolerant and high-yielding genotypes, the 48 genotypes were evaluated at the Research Farm of Agronomy Department, Faculty of Agriculture, Assiut University, Egypt for one season (2019/2020). Under field conditions, a pot experiment was conducted in a randomized complete block design (RCBD) with three replications per genotype. The size of the pots was 30 cm×20 cm×20 cm and each pot was filled with 7.5 kg of field-soil (collected from the research station, the same soil was used for the seedling stage experiment). Before seed sowing, for controlled treatment (C), 300 ml tap water was applied per pot, and for alkaline-saline treatment (AS) 300 ml of 150mM NaCl: Na$_2$SO$_4$: NaHCO4:Na$_2$CO$_3$ at a proportion of 1:9:9:1 was applied per pot. In each pot, eight seeds from each genotype/replication were sown. Until the end of the experiment, manual irrigation was applied to all pots when required.

Three soil samples were collected from both trays (in the seedling experiment) and pots (mature experiment) and three replications were prepared for each sample. The pH and electrical conductivity (EC) were measured by means of pH and conductivity meters. Sodium (Na$^+$) and potassium (K$^+$) were determined by flame photometers (Sujatha and Reddy 2003; Farghly et al. 2020). Ca$_2^+$ and Mg$_2^+$ contents were determined by the Ethylene Diamine Tetra-acetic Acid (EDTA) titration method. The chlorine ion (Cl$^-$) content was determined using the silver nitrate titration method (Adimalla and Taloor 2020; He et al. 2020). The saturation percentage (SP) equals the weight of water required to saturate the pore space divided by the weight of the dry soil (Aali et al. 2009). Sodium absorption ratio (SAR) was calculated as described in the following formula:

$$SAR = \frac{\text{Na}}{\sqrt{\frac{Ca+Mg}{2}}}$$

Measurement and scoring of the phenotypic traits

Seedling traits

After two weeks each seedling was removed and carefully cleaned from the soil. The following phenotypic traits were measured for each individual plant in each replication; 1) biological weight (BW, gm), 2) root weight (RW, gm), 3) the ratio of root fresh weight to the shoot fresh weight (RW/SW), 4) the number of roots (NR), 5) shoot length (SL, cm), 6) root length (RL, cm), 7) leaf width (LW, cm), and 8) leaf area (LA, cm^2). BW, SW, and RW were measured using a sensitive digital balance. SL, RL, LW, and LA were measured using ImageJ software (Schneider et al. 2012).

Kernel traits

The kernel-related-traits, kernel length (KL, cm), kernel width (KW, mm), kernel diameter (KD, mm), and thousand kernel weight (TKW, gm) were measured at the end of the field experiment as described in (Mourad et al. 2021). In brief, five random seeds were selected from each genotype in each replication and measured their KL, KW, and KD then an average value was used. TKW was measured for all kernels produced from each individual plant.

Changes in the studied seedling and kernel traits under the alkaline-saline effect

Using the average of the three replications for each studied trait, percentage changes (increase or decrease) of the studied seedling and kernel traits due to AS effect were calculated according to the following formula:

$$\text{CTH} - \text{trait} = \frac{\text{Xc} - \text{X}as}{\text{Xc}} x100$$

where CTH-trait is the changes in the studied trait, X_c and X_{as} are the mean performance of each studied genotype under control and AS stress, respectively. Based on this formula, an increase in the studied trait will produce a negative CTH-trait value, and a

positive value will result on decreasing in the studied trait.

Statistical analysis of seedling and kernel traits PLABSTAT software (Utz 2011) was used to calculate the analysis of variance (ANOVA) for each studied trait following two different models as follows:

$$Y_{ij} = \mu + r_j + g_i + gr_{ij} + e_{ij} \tag{1}$$

where Y_{ij} is an observation of genotype i in replication j, μ is the general mean; g_i, r_j are the main effects of genotypes and replications, respectively; eij is the error. Genotypes were considered as fixed effect while replications were considered as random effect. This model was used for each treatment separately to identify the differences between the tested genotypes under each condition.

$$Y_{ijk} = \mu + r_j + t_k + g_i + tg_{ik} + tgr_{ijk} + e_{ijk} \tag{2}$$

where Y_{ijk} is an observation of genotype i in replication j under treatment k, μ is the general mean; g_i, r_j, t_k are the main effects of genotypes, replications, and treatment, respectively; e_{ijk} is the error. Experiment was considered as fixed while the remaining factors were considered as random. This model was used to identify the differences between the two treatments, control and AS, on each of the studied traits.

In addition, broad-sense heritability was calculated for all traits using the following model

$$H^2 = \sigma_G^2 / (\sigma_G^2 + \frac{\sigma_R^2}{r})$$

where σ_G^2 and σ_R^2 are the variance of the lines and the residuals, respectively. r is the number of replicates.

Phenotypic variation and phenotypic correlation under each treatment at both stages were calculated and visualized using SRPlot online database available at http://www.bioinformatics.com.cn/srplot.

Genotyping of the tested panel, single marker analysis for seedling and kernel traits under alkaline-saline stress, and gene models controlling these traits

The tested panel was genotyped using 25K Infinium iSelect SNP array. This marker set was generated by SGS Institute Fresenius GmbH TraitGenetics

Section (Gatersleben, Germany) (Esmail et al. 2023; Mourad et al. 2023a). In total, 21,093 markers were obtained after filtration to minor allele frequency (MAF > 0.05) and maximum missing sites per genotype < 10%. This marker set covers all the wheat chromosomes.

To understand more about the genetic control of the studied traits under AS stress, a single marker analysis was conducted for each significant trait using the available marker data for all 48 genotypes. This was done using PowerMarker software v 3.25 (Liu and Muse 2005) using the following model

$$Y = \mu + f \, (marker) + error, \; where$$

Y is trait value, μ is population mean, and f (marker) is a function of the significant markers.

The marker-trait associations were tested against Bonferroni corrections at a significant level of 5% (Mourad et al. 2018). The phenotypic variation explained by marker (R^2) as well as the allele effect was estimated for each significant marker using TASSEL 5.0 software (Bradbury et al. 2007). Moreover, gene models harboring the identified significant markers were investigated by comparing gene models with the same position of the significant marker using *EnsemblePlants* (https://plants.ensembl.org/Triticum_aestivum/Info/Index). The functional annotation of these gene models was detected using International Wheat Genome Sequencing Consortium (IWGSC) V.1.0. The genetic base of these gene models concerning abiotic stress tolerance was investigated and presented as a network using the KnetMiner database (Hassani-Pak et al. 2021).

Gene enrichment and biological process pathways of the identified gene models

To understand more about the genetic control of the studied seedling and kernel traits under alkaline-saline stress, gene enrichment based on the biological process pathways of the identified gene models was investigated using ShinyGo 0.76 database (Ge et al. 2022). Furthermore, a cutoff value of false discovery rate (FDR) *p*-value < 0.05 was applied. The most significant pathways were visualized as an enrichment plot using the SRPLOT database available at (https://www.bioinformatics.com.cn/en). The

biological process networks were visualized using NetwrokD3 v.4.0, R package (Allaire et al. 2017).

Selection of superior genotypes for alkaline-saline stress tolerance

To select the superior genotypes, the stress tolerance index (STI) was calculated for thousand kernel weight (TKW) as it is the most important yield trait. The best ten genotypes were detected based on the iPASTIC toolkit average of sum ranks (ASR) (Pour-Aboughadareh et al. 2019). Furthermore, the genetic distance among the selected genotypes was calculated using 'ade4' R package (Dray and Dufour, 2007; R Core Team 2017). A phylogeny tree representing the genetic distance was performed using iTOL database (Letunic and Bork 2021).

Results

The analysis of soil before and after adding the AS solution is presented in Table 1. Based on soil analysis results, the pH of the AS treatment soils increased to 8.60 compared with 7.18 in the control soils. Furthermore, soil content of Na^+, K^+,

Table 1 The analysis of the experimental soils represents soil pH, electrical conductivity (EC), the content of important cations; Sodium (Na^+), Potassium (K^+), Calcium (Ca^{2+}), Magnesium (Mg^{2+}), and Chloride (Cl^-), and sodium adsorption ratio (SAR) in the control and alkaline-saline treatment

Property	Control soil	Alkaline-Saline soil
pH (1:5)	7.18	8.60
EC (dS/m)	2.51	5.08
Cation (mg/Kg soil)		
Na^+	937.5	2500
K^+	1012	1128
Ca_2^+	510	525
Mg_2^+	315	550
Anion (mg/Kg soil)		
Cl^-	710	11,094
Saturation capacity (%)	54.4	53.6
SAR	3.59	8.11

Ca_2^+, Mg_2^+, and Cl^- was increased as an effect of the alkaline-saline solution. The soil EC was almost duplicated while saturation capacity slightly decreased.

Genetic variation of seedling traits

The analysis of variance revealed highly significant differences among the tested genotypes for all the eight-studied-seedling traits under both controlled and AS conditions (Table S2 and S3). Moreover, significant ($p < 0.05$) and highly significant differences ($p < 0.01$) between the two treatments (control and AS treatments) were found for all the studied traits except BW, NR, and SL (Table 2). However, high genetic variation was found among the tested genotypes for these three traits (BW, NR, and SL) under each condition (Fig. S1). Furthermore, significant genotype x treatment interaction was found for SW, SL, RL, LW, and LA (Table 2). High values of broad-sense heritability were observed for all the studied traits with values ranging from 0.63 for RW/SW to 0.95 for LA.

Compared to the controlled conditions, the alkalinity-salinity stress reduced all seedling traits except RW and RW/SW, the variation and distribution for all seedling traits are represented in Fig. 1, Fig. S1 and Table S1. The roots of the tested genotypes under AS were found to be shorter than root length under controlled conditions with an average of 10.06 cm and 8.52 cm under controlled and AS conditions, respectively (Fig. 1a). The average values of RW/SW ratio were 0.73 under controlled conditions while it was 0.78 under AS (Fig. 1b). The average of RW was 0.11 gm and 0.12 gm under controlled and AS conditions, respectively (Fig. 1c). Under controlled conditions, LW and LA average was 0.32 cm and 204.55 cm^2 for each trait, respectively. On the other hand, under AS conditions LW and LA average was 0.30 cm and 164.10 cm^2 for LW and LA, respectively (Fig. 1d and e).

Genetic variation of kernel traits

Based on the ANOVA results, highly significant differences were found among the tested genotypes for all the four-studied-kernel-traits under both controlled and AS conditions (Table S2 and S3). Moreover, highly significant differences and highly significant G×T interactions were found between the two

Table 2 Analysis of variance (ANOVA) for 48 spring wheat seedling traits (upper table), and kernel traits (lower table) under control and alkaline-saline treatment

Seedling Traits

Source of variance	BW d.f.	BW M.S.	RW d.f.	RW M.S.	RW/SW d.f.	RW/SW M.S.	NR d.f.	NR M.S.	SL d.f.	SL M.S.	RL d.f.	RL M.S.	LW d.f.	LW M.S.	LA d.f.	LA M.S.
Treatment (T)	1	0.00	1	0.01*	1	0.54*	1	0.02	1	0.26	1	163.06**	1	0.04**	1	118,474.9**
Replications (R)	2	0.00	2	0.00	2	0.02	2	0.66	2	1.90	2	4.70	2	0.00	2	1306.361
Genotypes (G)	47	0.03**	47	0.01**	47	0.15**	47	2.78**	47	60.35**	47	14.31**	47	0.01**	47	25,714.44**
GT	47	0.00	47	0.00	47	0.10	47	0.31	47	23.94**	47	9.49**	47	0.004**	47	7799.65**
GR	94	0.00	94	0.00	94	0.06	94	0.35	94	8.26	94	2.32	94	0.00	94	1388.7
GTR	86	0.00	86	0.00	83	0.07	88	0.32	86	8.90	85	1.97	85	0.00	85	1578.718
Total	277	–	277	–	274		279	–	277	–	276	–	276	–	276	–
H^2	0.90		0.86		0.63		0.87		0.86		0.84		0.91		0.95	

Kernel Traits

Source of variance	KW d.f.	KW M.S.	KL d.f.	KL M.S.	KD d.f.	KD M.S.	TKW d.f.	TKW M.S.
Treatment (T)	1	298.05**	1	341.12**	1	0.43**	1	41,412.61**
Replications (R)	2	0.10**	2	0.02	2	0.00	2	0.89
Genotypes (G)	47	0.16**	47	0.55**	47	0.02**	47	251.15**
GT	47	0.07**	47	0.26**	47	0.01**	47	148.18**
GR	94	0.012	94	0.02	94	0.00	94	4.08
GTR	92	0.01	91	0.02	96	0.00	96	4.79
Total	283	–	282	–	287	–	287	–
H^2	0.92		0.89		0.96		0.98	

$*P<0.05$ $**P<0.01$

1) Biological weight (BW, gm), 2) Root weight (RW, gm), 3) The ratio of root fresh weight to the shoot fresh weight was computed as RW/SW, 4) Number of root (NR): the number of roots was counted manually, 5) Shoot length (SL, cm), 6) Root length (RL, cm), 7) Leaf width (LW, cm), 8) Leaf area (LA, cm^2)

Kernel Traits: 9) Kernel weight (KW), 10) Kernel length (KL), 11) Kernel diameter (KD), 12) Thousand kernel weight (TKW)
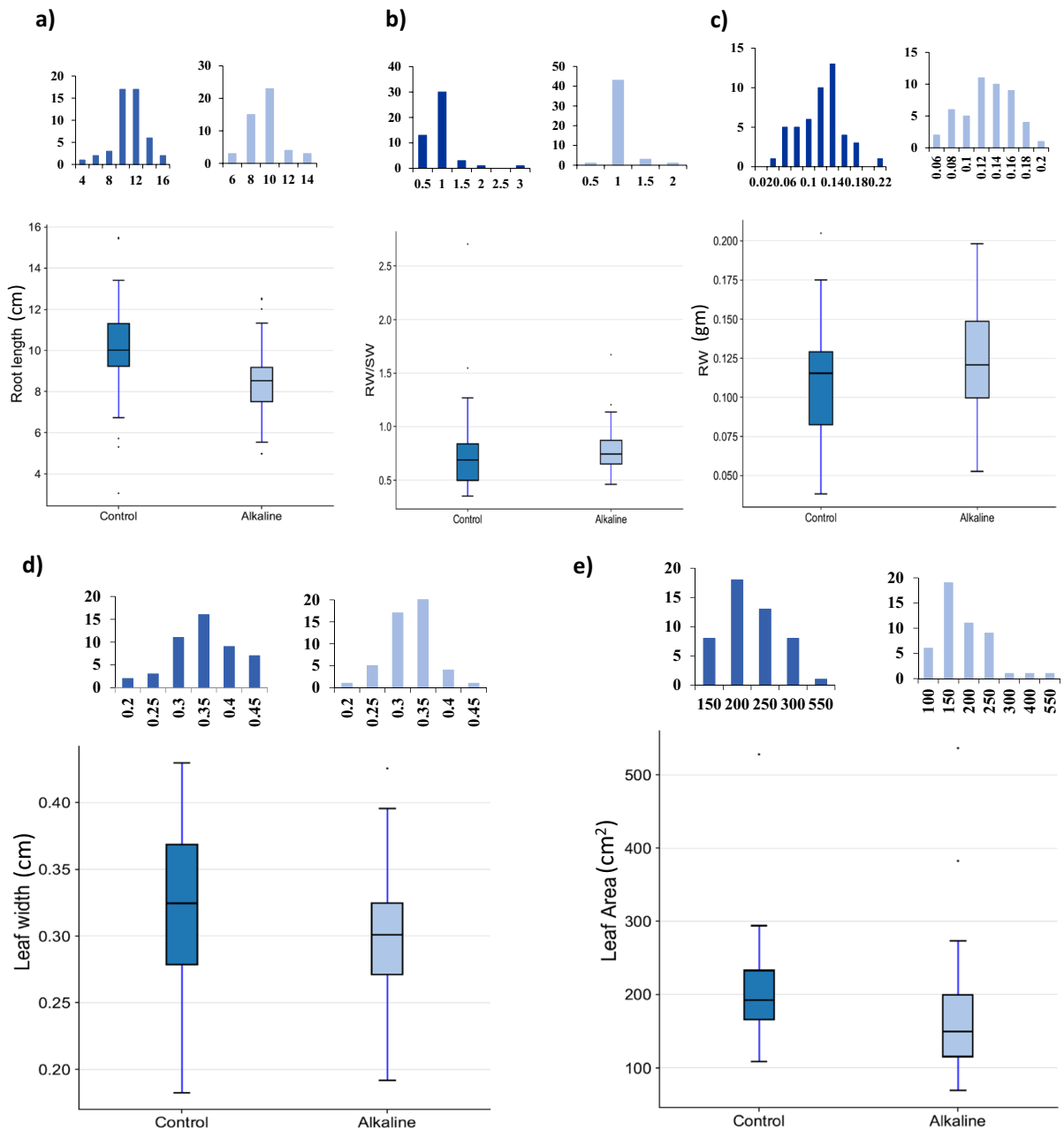
Fig. 1 Box plots and histograms representing the distribution of (**a**) root length (RL), (**b**) root weight/shoot weight (RW/SW), (**c**) root weight (RW), (**d**) leaf width (LW), and **e**) leaf area (LA) under control (blue) and alkaline-saline (light blue)

studied treatments (control and AS) for all the studied kernel traits (Table 2). High values of broad-sense heritability (H^2) were found for all the studied traits with values ranging from 0.89 for KL to 0.98 for TKW.

A great reduction was observed in all kernel traits under AS conditions compared with controlled conditions. The average of KW was 3.22 mm under controlled conditions, while it was 1.19 mm under AS conditions (Fig. 2a and Table S1). The average of KL
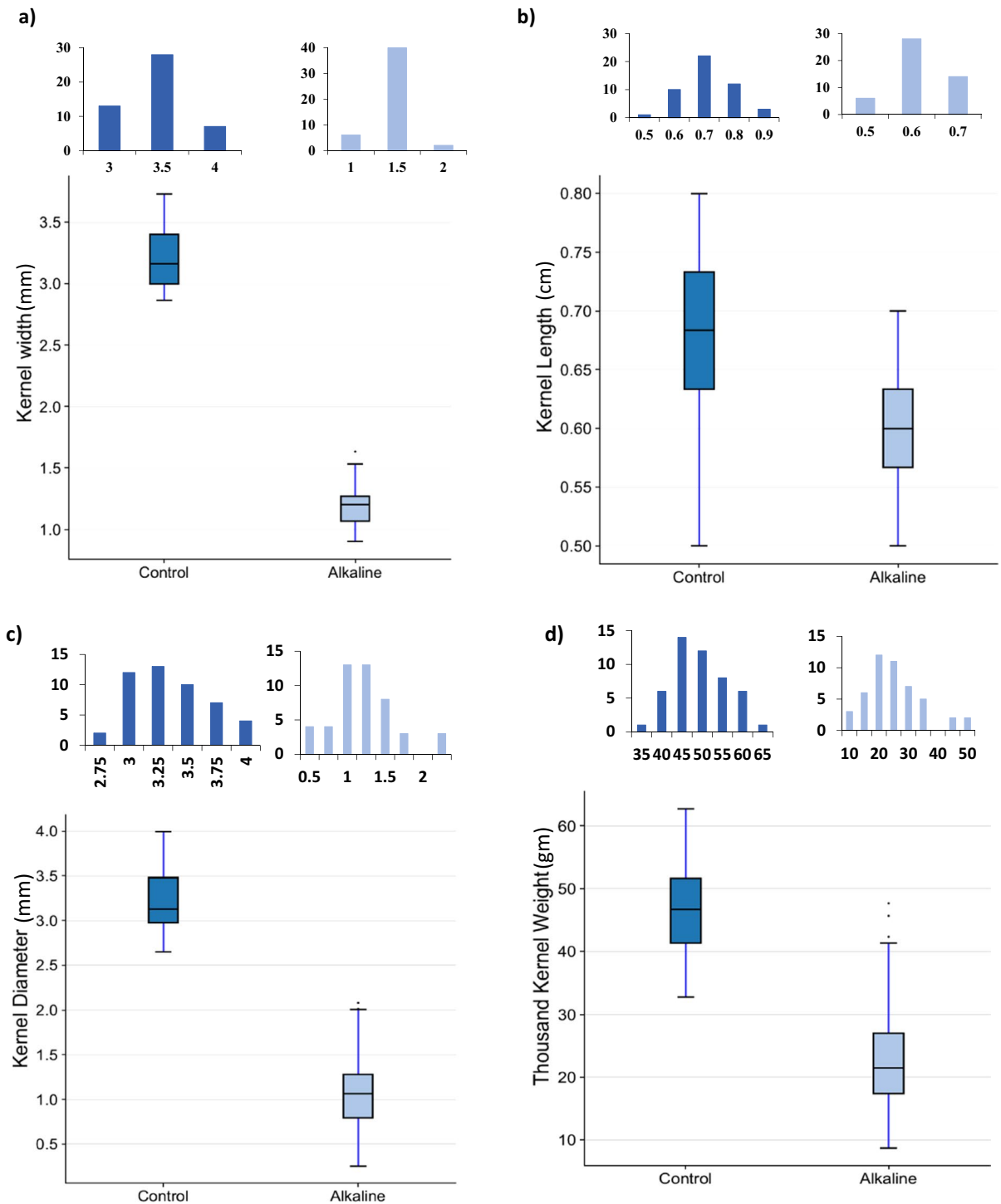
Fig. 2 Box plots and histograms representing the distribution of (**a**) kernel width (KW), (**b**) kernel length (KL), (**c**) kernel diameter (KD), and (**d**) thousand kernel weight (TKW) under control (blue) and alkaline-saline (light blue)

was 0.68 cm under controlled conditions, while it was 0.60 cm under AS conditions (Fig. 2b). Under controlled conditions, KD average was 3.24 mm while it was 1.06 mm under AS conditions (Fig. 2c). The average values of TKW under control conditions were 46.89 gm, while it was 22.91 gm under AS conditions (Fig. 2d).

Changes in the seedling and kernel traits as an effect of AS treatment

To estimate the magnitude of AS stress on seedling traits as well as the kernel traits, the percentage changes of all significant traits were computed (Fig. 3). For seedling traits, it was observed that AS
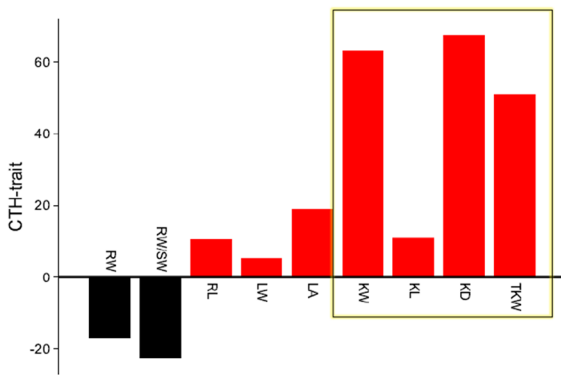
Fig. 3 Average percentage changes in the studied seedling and kernel traits due to alkaline-saline (AS) stresses. Positive values indicate a reduction in the studied parameter compared with its value under controlled conditions while negative values indicate an increase in the values

reduced LW, RL, and LA with values of 5.18%, 9% and 19.04% for each trait, respectively. Contrarily, an increase in RW and RW/SW was observed with values of 16.71% and 22.48%, respectively. For the kernel traits, a reduction in all traits was found with a percentage ranging from 10.79 to 67.23% for KL and KD, respectively.

Phenotypic correlation analysis for seedling and kernel traits under both conditions

To further understand the relationship between the studied seedling and kernel traits, correlations between each pair of the studied traits were calculated under each condition separately. Under controlled conditions, significant and highly significant positive correlations were observed between each pair of the studied seedling traits. The highest positive and significant correlations were observed between BW and SW ($r = 0.91^{**}$) (Fig. 4a). Oppositely, RW/SW showed the highest negative and significant correlations with SL ($r = -0.23^{**}$), SW ($r = -0.34^{**}$), and LA with $r = -0.37^{**}$. For the kernel traits, all correlations were positive and highly significant. The highest correlation was between KD and KW with $r = 0.73^{**}$. Interestingly, the correlation between the seedling and kernel traits varied from low to moderate values with many negative or nonsignificant correlations. The highest positive and significant correlation was between TKW and RL with $r = 0.44^{**}$. The highest negative and significant correlation was between KD and LA with $r = -0.53^{**}$ (Fig. 4a).

Fig. 4 The correlation between each pair of the studied traits under (**a**) control conditions and (**b**) alkaline-saline stress conditions. Highlighted part is the correlation between studied traits in the different growth stages
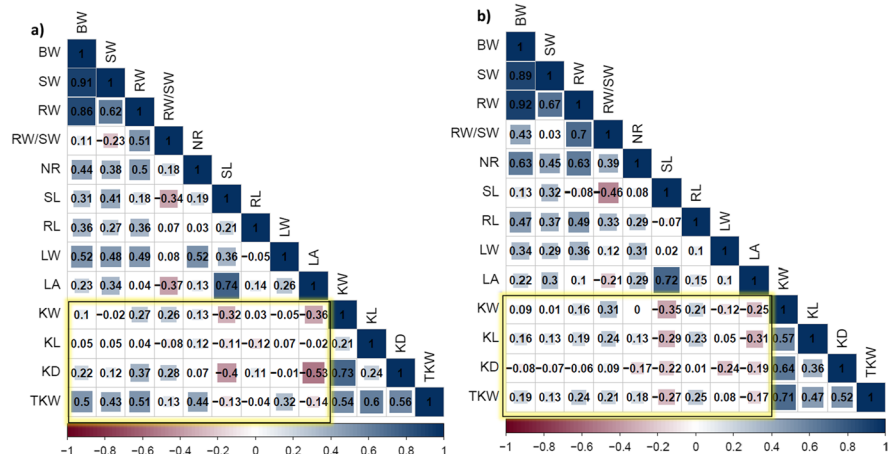
Similarly, under AS stress significant and highly significant correlations were observed between each pair of the studied seedling traits. BW showed the highest positive and significant correlations with SW and RW with *r* values of 0.89** and 0.92**, respectively (Fig. 4b). RW/SW showed the highest negative and significant correlations with SL and LA with *r* values of -0.46** and -0.21**, respectively. For the kernel traits, all correlations were positive and highly significant with the highest *r* value of 0.71** between TKW and KW (Fig. 4b). Under AS condition, no highly significant correlations were found between TKW and any of the studied seedling traits. However, some significant correlations were found between TKW and some seedling traits such as RW, RL, and SW/SW with values of 0.24*, 0.25*, and 0.21*, respectively.

Genetic control of seedling and kernel traits under AS conditions

Markers-traits association of seedling and kernel traits under controlled and AS stress conditions

The tested genotypes showed highly significant differences among the two studied conditions for seedling and kernel traits. Therefore, marker-trait association (MTA) analysis was done for each condition separately. Single marker analysis (SMA) was used for MTA analysis and Bonferroni correction *p*-value < 0.05 was applied to identify the highly significant MTAs. Under controlled conditions, a number of 292 markers were identified that could be classified into one and 291 markers were associated with seedling traits and kernel traits, respectively (Table S4). While under AS conditions, 52 significant markers were identified that could be classified into 13 and 39 markers associated with seedling and kernel traits, respectively (Table 3 and Table S5). Out of the significant markers associated with the studied traits under each condition, only 16 markers were common between the two conditions (Fig. S2). However, we will focus on the significant markers associated with AS conditions to understand more about the genetic control of AS tolerance. A summary of all the significant markers associated with AS tolerance is provided in Table 3, while the detailed results are presented in Table S5.

Table 3 Summary of significant markers associated with the studied seedling and kernel traits based on single marker analysis (SMA) using Bonferroni correction p-value < 0.05

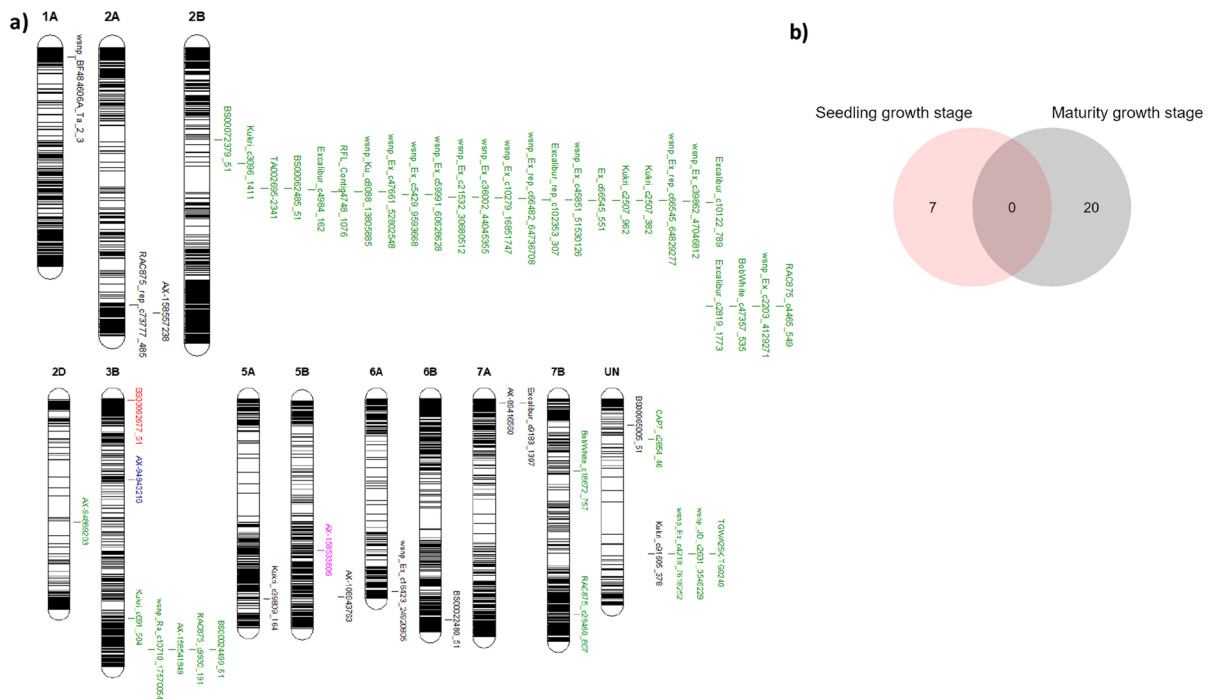| Growth stage	Total No. of sig markers	Studied trait	No. sig. markers	No. of chromosomes	R^2 (%)	P-value	Allele effect	Number of genes
Seedling	13	RW	1	3B	44.26	6.18E-07	0.05	1
		RW/SW	1	3B	43.80	7.41E-07	-0.26	0
		LA	11	1A, 2A,5A, 5B, 6A, 6B, 7A, and Un	40.67—48.42	7.94E-08 – 1.87E-06	150.64 – 379.59	6
Maturation	39	KL	38	2B, 2D, 3B, 7B, and Un	40.07 – 47.01	1.46E-07—2.35E-06	0.08 – 0.09	20
		TKW	1	5B	41.33	1.91E-06	15.094	0

Fig. 5 Single marker analysis for alkaline-saline tolerance in the 48 diverse wheat genotypes evaluated in seedling and mature growth stages. **a** number of significant SNP markers associated with leaf area (black), kernel length (green), root weight (blue), root weight/shoot weight (red), and TKW (pink). **b** number of identified gene models harboring the significant markers in each stage

Out of the 13 SNPs associated with the seedling traits, eleven markers were associated with LA. They are distributed as seven SNPs on the A genome (on chromosomes 1A, 2A, 5A, 6A, and 7A) two SNPs on the B genome (on chromosomes 5B and 6B), and two SNPs were signed as unknown. The remaining two SNPs associated with RW and RW/SW were both located on 3B chromosome (Table 3 and Fig. 5a). Of the 39 SNPs associated with the kernel traits, 38 SNPs were associated with KL and one SNP associated with TKW (Table 3). The majority of the 38 SNPs associated with KL were located in genome B with a number of 25 SNPs on chromosome 2B, five SNPs on chromosome 3B, and two SNPs on chromosome 7B (Fig. 5a and Table S5). The remaining five SNPs distributed as one SNP reside on the chromosome 2D and four SNPs were signed as unknown. The allele effects for all markers associated with all traits were positive except for RW/SW (Table 3, and Table S5). The phenotypic variation explained by these significant markers (R^2) ranged from 40.67—48.42% for the seedling traits and ranged from 40.07 – 47.01% for the kernel traits.

Gene models harboring the significant markers associated with seedling and kernel traits under AS stress conditions, their functional annotation, and gene network

To provide more understanding of the genetic control of AS tolerance in each growth stage, gene models harboring the identified significant markers were investigated. A total of seven and twenty gene models were found to harbor the significant markers associated with seedling and kernel traits, respectively (Table 3 and Fig. 5b). Out of the seven genes controlling the seedling traits, six are related to the LA, and one gene for the RW, meanwhile no candidate genes were identified for the RW/SW. All the 20 genes for the kernel traits are related to KL, and no genes were identified for the TKW (Table 3). No genes were found to tailor the variation of the measured traits at both stages at a time (Fig. 5b).

The functional annotation of the identified gene models and their network in relation to AS tolerance were investigated and presented in Table S5, and Fig. S3, S4. Out of the seven gene models identified in the seedling growth stage, three genes were directly associated with AS tolerance (Fig. S3). The remaining four genes were indirectly associated with AS tolerance as they were found to have a network associated with the effect of AS stress such as response to oxidative stress, response to hypoxia, regulation of DNA, and production of cellulose-synthase-like protein (Table S5).

The majority of the 20 genes identified in the mature growth stage were functionally annotated to control salt stress tolerance, abiotic stress tolerance, the response to oxidative stress, and the response to osmotic stress in wheat (Table S5 and Fig. S4). For example, *TraesCS2B02G276500* gene was annotated to produce F-box domain protein that controls the response to salt, response to osmotic stress, response to oxidative stress, and response to endoplasmic reticulum stress (Fig. S4b). Moreover, two genes, *TraesCS2B02G504900* and *TraesCS2B02G276300*, were functionally annotated to produce NBS-LRR disease resistance protein and tRNA dimethylallyl transferase (Table S5). Their networks are associated with stem rust and stripe rust resistance in wheat (Fig. S4c and S4m). Both of these genes harbored SNP markers associated with KL.

Gene enrichment analysis of the identified gene models

To understand more about the genetic control of AS tolerance in seedling and mature growth stages, gene enrichment of the identified seven and 20 genes in both stages was investigated. A cut-of-1% FDR was applied to detect the most important genes. A total of 41 and 57 biological process pathways were identified in the seedling growth and mature growth stage, respectively (Table S6). However, based on FDR 1%, this number was reduced to 18 and 19 significant pathways for seedling and mature growth stages, respectively (Fig. 6a). The 18 pathways identified in the seedling growth stage were found to be mainly controlled by two different genes that work in one network (Fig. 6b). This network mainly controls the production of beta-glucan, the cellular carbohydrate

process, and some important plant alcohols such as glycerophospholipids, and inositol phosphate. The 19 biological process pathways that control AS tolerance in the mature growth stage were found to form six different networks and were controlled mainly by eight gene models (Fig. 6c). Out of these networks, two were controlled by two different genes, while the remaining networks were controlled by only one gene. Network 1 was found to control the biosynthesizing of glycoproteins and carbohydrates. Network 2 was found to control the metabolic of tRNA, cytokinin biosynthetic, and hormone biosynthetic. Network 3 was found to mainly control the response of wheat plants to abiotic stresses such as drought recovery and protein autophosphorylation. Network 4 and network 5 were found to control only one pathway/network that is related to protein O-linked glycosylation and phenylpropanoid metabolism, respectively. The last network, network 6 was found to control the regulation of plant defense response.

Selection of superior genotypes for alkaline-saline stress tolerance

The stress tolerance index (STI) of TKW was used to select the best ten genotypes from the studied material (Table S7). Based on this STI, ten genotypes were selected to perform best (Table 4). Based on the origin of the selected genotypes, they could be classified into seven Egyptian genotypes, one Canadian genotype, one genotype from Afghanistan, and one with unknown.

To genetically confirm the superiority of the ten selected genotypes, the number of targeted alleles associated with improving AS tolerance in the seedling and mature growth stage was investigated and presented in Fig. 7a. Genotype "QADRY 003" has the highest number of alleles related to kernel traits with a number of 39 alleles, followed by "Giza-156", "Giza-36" and "Little club" with a number of 37, 33, and 25 alleles, respectively. Genotypes "93-11-14-2-2" and "SIDS12" have no target alleles associated with kernel traits. All the genotypes possess alleles for seedling-related traits, "Giza-156" has the highest number of target alleles with seven alleles out of 13 alleles. Based on the number of target alleles of the two growth stages, "Giza156", "Giza36", "Little club", and "QADRY 003" had the highest number of these alleles.
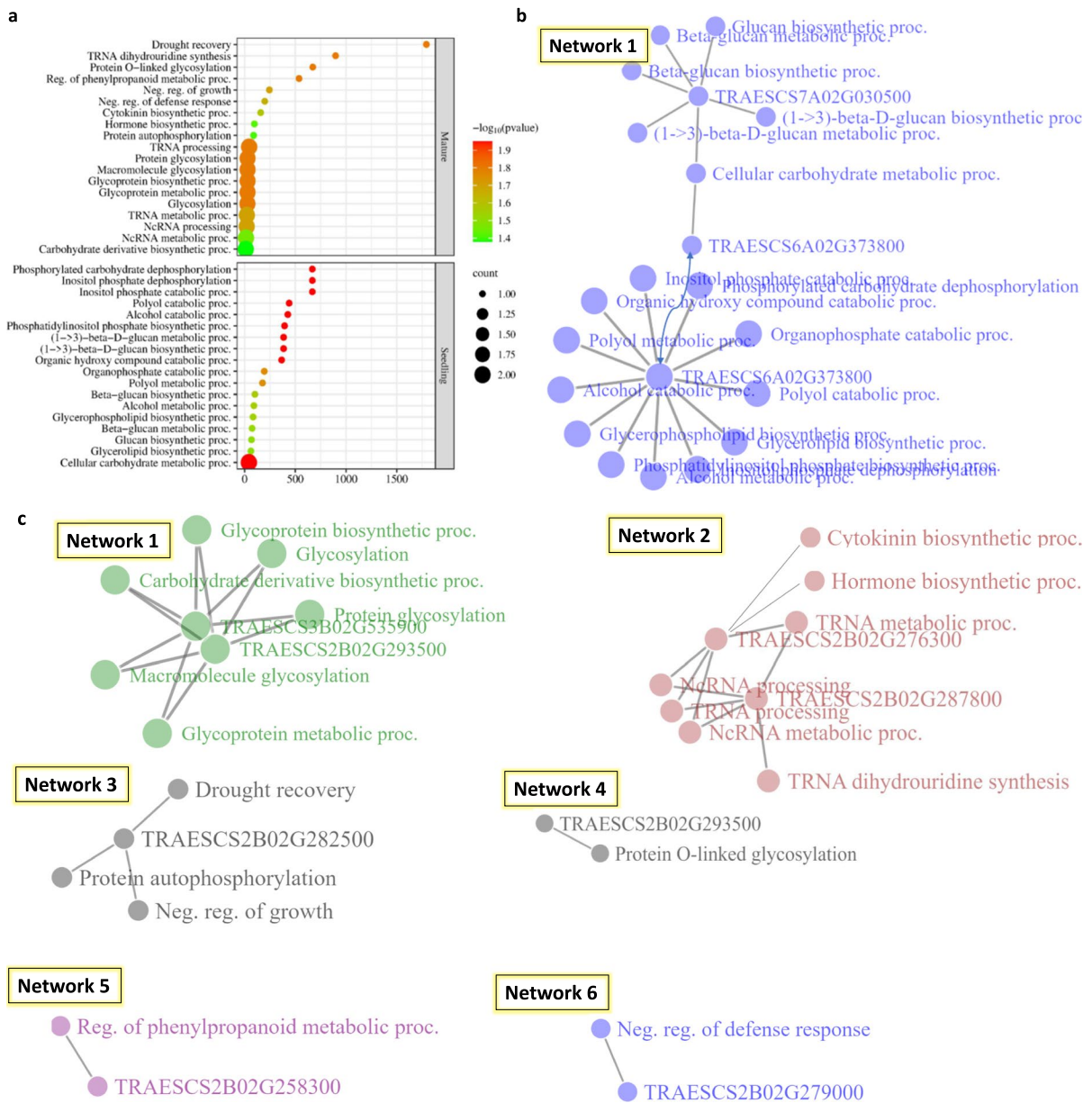
Fig. 6 Gene enrichment of the identified gene models associated with Alkaline-Saline tolerance. **a** significant biological process pathways identified in the seedling stage and mature growth stages, **b** network of the identified biological process pathways in the seedling growth stage, **c** network of the identified biological process pathways in the mature growth stage

To investigate the possibility of improving AS tolerance of wheat germplasm using the current studied materials, the genetic distance between each pair of the selected genotypes was calculated (Table S8). The phylogeny tree represented the genetic distance of the best ten genotypes is presented in Fig. 7b. These ten selected genotypes were found to be clustered in three different groups. Interestingly, the four genotypes

Table 4 List of the best tolerant ten genotypes based on the ranking of all stress tolerance indices for thousand kernel weight, and their country

Genotype	Country
Bahri Sirhosha	Afghanistan
Giza-152	Egypt
Giza-156	Egypt
93-11-14-2-2	Canada
Giza-36	Egypt
MISR2	Egypt
Giza-148	Egypt
Little Club	Unknown
SIDS12	Egypt
QADRY 003	Egypt

with the highest number of target alleles were distributed among the three clusters. Cluster 1 contains two superior genotypes, "Little club" and "Giza36". While the other two superior genotypes, "Qadry003" and "Giza156", were located in cluster 2 and cluster 3, respectively. High genetic distance was found between these four genotypes with values ranging from 0.3244 between "QADRY003" and "Giza 156" to 0.3844 between "QADRY003" and "Little club" (Table S8).

Discussion

Based on the soil analysis, the pH degree and soil content of the different cations and anions increased in the contaminated soils compared with the controlled ones. Based on Hayward and Wadleigh (1949), soils with pH < 8.5, EC > 4.0 dS/m, and high percentages of Ca_2^+, Mg_2^+, K^+, and Cl^- are defined as alkaline-saline soils. Therefore, we can conclude that the solution added to the tested soils was effective in converting them to alkaline-saline soils (Table 1).

Genetic variation and correlation of seedling and kernel traits under alkalinity-salinity stress

AS stress was found to reduce most of the seedling traits except RW and RW/SW (Fig. 3). Increasing RW/SW ratio under AS suggests that the reduction occurred in SW was greater than the reduction of RW. Similar increasing in root/shoot ratio was reported previously in wheat (Zhang et al. 2020), *Leymus chinensis* (Liu et al. 2015), and maize (Cao et al. 2020). However, some studies reported an inhibition in both shoot and root growth in wheat seedlings (Guo et al. 2010) and maize (Guo et al. 2017) under AS stress. This reduction in both roots and shoots was explained

Fig. 7 The best selected ten genotypes based on the highest TKW under AS stress conditions. **a** number of target alleles associated with the tolerance in the seedling and maturity growth stages. **b** phylogeny tree represents the genetic distance among the 10 selected genotypes

due to the negative effect of AS on cell division, and nutrient uptake, leading to less biomass accumulation and consequently shorter root and shoot. Furthermore, in the current study the highest reduction in seedling traits was found in LA (Fig. 3). Previous studies reported a great reduction of wheat LA due to the high pH, osmotic stress, and ion toxicity exerted by both the salinity and alkalinity stress (Yang et al. 2008a, b).

Moreover, all kernel traits were significantly reduced under AS stress. The highest reductions accounted for KD and KW (Figs. 2b, c and 3). Previous studies reported that the alkalinity-salinity stress greatly reduced yield-related traits which confirms our results (Sharma et al. 2010a). High pH environments were reported to inhibit ion absorption as a result of loss of the physiochemical gradient inside the root and reduce the solubility of some macro and micronutrients thus resulting in ion imbalance and impaired plant growth (Shi and Zhao 1997; Yang et al. 2008a, b). Therefore, the reduction of all kernel traits and some seedling traits in the current study can be attributed to ion imbalance under the effect of both alkalinity and salinity.

In the current study, either at the seedling stage or at the maturity stage we applied both alkalinity and salinity stress together, meaning that the plants experienced very severe stress. All of the aforementioned studies reported that alkalinity stress is distinctive from salinity stress. The effect of AS is more severe than salinity stress alone as well as alkalinity stress alone (Paz et al. 2012). This indicates that the plants had developed independent mechanisms to adapt and keep growing under both stresses. Based on the ANOVA, significant and highly significant differences were found between treatments and among the tested genotypes. This significant variation with the high heritability values for most traits, suggests that this collection is suitable for selection to improve these traits under AS conditions. Furthermore, the highly significant differences between the control and AS experiments allow us to discriminate between tolerant and susceptible genotypes under both conditions.

Significant and highly significant correlations were observed for the seedling traits under both conditions (Fig. 4a, b). Previous studies reported the presence of highly positive correlations among seedling traits under salinity-alkalinity stress in rice (Lv et al. 2015). In line with the aforementioned studies, our findings

suggested that the studied seedling traits could be valuable parameters for further assessing saline-alkaline stress tolerance in wheat. Out of the studied seedling traits, root weight had the highest correlation value with other seedling traits under both control and AS conditions confirming the role played by roots in improving AS tolerance. Moreover, highly positive correlations were detected among all kernel traits under both conditions (Fig. 4a, b). Previous studies reported a highly significant positive correlation between kernel traits (Li et al. 2007, 2019a; Mourad et al. 2021; Huang et al. 2006). These high correlations among the kernel traits may indicate that they are under common genetic control. Therefore, we can conclude that the studied kernel traits are indirectly correlated to grain yield since they are correlated with TKW. Moreover, negative or nonsignificant correlations were found between the seedling-related and kernel-related traits under AS. These results suggested that the traits of both stages are under independent genetic controls. Therefore, understanding the genetic control of AS tolerance in each growth stage is important and required to accelerate wheat breeding programs that target improving wheat tolerance to such severe stress.

Marker-trait associations and the putative candidate genes

The recently studied population is a highly diverse one that has been collected from 12 different countries (Table S1). Furthermore, it was reported as an effective population to detect marker-trait associations with different stresses (Mourad et al. 2020, 2021; Abou-Zeid and Mourad 2021; Ahmed et al. 2022; Amro et al. 2022; Mohamed et al. 2023). In the current study, highly significant variations and high degrees of heritability were found for almost all the studied traits. Therefore, testing marker-trait associations of the studied traits using this population would be highly informative. The number of studied genotypes (48-genotypes) was appropriate for single marker analysis (SMA). Due to the advances in sequencing methods, many marker data sets have been widely used in wheat genotyping. One of these marker sets is 25K-SNP array which was reported as an effective marker set (Aleksandrov et al. 2021). Moreover, this marker set was effective in detecting genomic regions controlling the resistance/tolerance

to different biotic/abiotic stresses in the same studied population (Esmail et al. 2023; Mourad et al. 2023a, b).

In the current study, SMA identified a total of 259 and 52 SNP markers significantly associated with the studied seedling and kernel traits under controlled and AS conditions, respectively (Fig. S2). Interestingly, only 16 markers commonly control the studied traits under both conditions. This low number of common markers suggests the presence of different genetic systems controlling the studied traits under both conditions. Due to the severe effect of AS on the studied traits and the urgent need to improve wheat tolerance to such stress conditions, more concern was drawn to the genetic control of the studied traits under AS in the current study.

The 52 significant markers identified under AS conditions had a major effect on the traits they are controlling as they explained more than 10% of the phenotypic variations (Table 3 and Table S5). The highest number of significant markers was identified for LA and KL in seedling and mature growth stages, respectively. These significant markers were distributed among eight and five chromosomes for LA and KL, respectively (Fig. 5a). Therefore, a wide genetic system was suggested to control each trait. Such a wide genetic system was reported previously to control LA and kernel traits in wheat which confirms our results (Wang et al. 2002; Ramya et al. 2010; Okamoto et al. 2013; Cui et al. 2014; Marzougui 2019; Mourad et al. 2023a). A total number of seven and 20 gene models was found to harbor the significant markers associated with seedling and kernel traits, respectively (Table 3). Interestingly, none of the identified gene models were found to commonly control seedling and kernel traits suggesting the presence of two different genetic systems controlling AS tolerance in each growth stage (Fig. 5b.). The absence of common gene models in the two growth stages supporting the nonsignificant or negative correlations found among the traits of both growth stages under AS conditions (Fig. 4b). Noteworthy to be mentioned that, in a previous study Hasseb et al. (2022) investigated the effect of salinity stress on seed germination and seedling development in a larger population ($n = 177$) encompassing the 48 genotypes harnessed in the current study. No common alleles/markers were identified in the current study and in the previous one, indicating that the alleles/markers are novel and suggesting that AS is under independent genetic control relative to salinity.

Based on the functional annotation and gene networks of the identified gene models in each growth stage, most of the identified gene models were associated with AS tolerance which confirmed our SMA results (Fig. S3, S4, and Table S5). For example, *TraesCS3B02G212900,* associated with RW in the seedling stage, was functionally annotated to encode respiratory burst oxidase homologs (RBOHs) (Fig. S2). The distribution of the RBOHs impaired root development, and reduced superoxide production in *Arabidopsis* (Esparza-Reynoso et al. 2023). RBOHs were reported to modulate plant development, especially root development under salinity and drought stresses by triggering the indigenous production of reactive oxygen species (ROS). They were found to be responsive to abiotic and biotic stresses including salinity stress in a range of crop and model plants (Wang et al. 2018). Moreover, *TraesCS2B02G276500* (associated with KL in the mature growth stage) was found to control the F-box domain. This gene family contains many genes that control plant response to different stresses in cereals. For example, the gene *TaFBA-2A* was reported to positively control salinity tolerance in transgenic plants of rice by increasing grain-related traits such as seed size and thousand kernel weight (Gao et al. 2022). Based on our results and the previous ones, we can conclude that our SMA results are accurate and could be used to provide more understanding about the genetic control of AS in wheat.

Gene enrichment analysis of the identified gene models

Due to the presence of two different genetic systems controlling AS tolerance in the two growth stages, it was worth detecting the biological processes that lead to the tolerance in each growth stage. Therefore, gene enrichment analysis was conducted for gene models identified in each growth stage (Fig. 6). Based on this analysis, a total of 18 and 19 highly significant pathways was found to control the tolerance in the seedling and mature growth stages, respectively.

The 18 pathways identified in the seedling growth stage were found to work in one network (Fig. 6b). This network controls the production of

some biological components such as β-glucan, inositol polyphosphate enzyme, cellular carbohydrates, and sugar alcohols. β-glucan metabolic biosynthesis includes some important proteins such as callose synthase-like proteins (GSLs). Callose is deposited primarily at the cell plates and cell walls of the newly proliferated cells (Zhong et al. 2023). It has been demonstrated that callose positively regulates plant tolerance to abiotic stress via prompt accumulation of callose under biotic and abiotic stressors (Wang et al. 2022). Inositol phosphate enzyme activates the sulphate transport to leave receptors. This enzyme is highly sensitive to high levels of sodium, thus characterized as a salt-tolerance enzyme (Gil-Mascarell et al. 1999). In this study, the production of this enzyme was controlled by *TraesCS6A02G373800* gene that was associated with LA and functionally annotated to encode 3(2),5-bisphosphate nucleosidase HAL$_2$ (Table S5). It was reported that the overexpression of HAL$_2$ conferred salinity tolerance via regulating osmolyte synthesis and ion transport (Murguía et al. 1995). The japonica rice cultivar HJ19 plants overexpressed the RiceHAL$_2$-like (RHL) gene and showed healthier leaves under salinity stress at the seedling stage (Li et al. 2002). In Arabidopsis, the Inositol phosphate enzyme is essential for leaf morphogenesis and venation (Robles et al. 2010). Furthermore, polyols and sugar alcohols were reported to play a vital function in the osmotic adjustment and stabilization of proteins and membranes (Hasanuzzaman et al. 2017). Therefore, enhanced salt stress tolerance is observed in plants overexpressing metabolic, polyols, and sugar alcohol genes. Moreover, cellular carbohydrates were reported to play an important role in improving wheat tolerance to salinity and abiotic stress (Naz et al. 2022). Based on our results and the previous studies, we can conclude that this network and its controlling genes are very important in improving wheat tolerance to AS at the seedling growth stage.

The 19 biological pathways identified in the mature growth stage were found to form six important networks (Fig. 6c). The first network was controlled by two different genes that are working together in improving the content of wheat kernels of different proteins such as glycoprotein (also known as gluten) and glycosylated proteins. Glycoproteins are essential in wheat flour. Glucosyltransferase enzymes and glycoproteins were reported to regulate kernel

size and improve plant tolerance to abiotic stresses in cereal crops (Dong et al. 2020; Chang et al. 2021). Therefore, this network and its genetic control are very important in improving wheat content of protein under AS stress conditions. The same role was found for Network 4 which controlled the Protein-o-linked glycosylation biological process pathway. Network 2 was found to control tRNA metabolism in wheat kernels. Some important enzymes are included in the tRNA metabolism such as tRNA dimethylallyltransferase (tRNA-IPT). The tRNA-IPT genes play a crucial role in plant-abiotic stress responses (Ghosh et al. 2018). In wheat, 24 IPT genes were identified including the gene *TraesCS2B02G276300* (encodes *TaIPT4-2B*) that upregulated under drought stress (Wang et al. 2023). In tomato, the tRNA dimethylallyltransferase coding genes were upregulated under salinity stress, and the high level of cytokinin enhanced salinity tolerance (Žižková et al. 2015). Accumulating cytokinin as a result of low expression of cytokinin oxidases enzymes positively affected grain yield in rice and wheat (Ashikari et al. 2005; Yeh et al. 2015; Zhang et al. 2012; Li et al. 2018). Network 3 controls the protein autophosphorylation pathway that leads to the recovery from abiotic stresses such as drought and salt. Moreover, *TraesCS2B02G282500* gene that controls this network was functionally annotated to produce a leucine-rich repeat kinase 2 (LRRK2) protein. Previous studies reported that knocking down the leucine-rich repeat in wheat resulted in a reduction in grain yield (Fang et al. 2020). Furthermore, in rice, a leucine-rich repeat protein is implemented in grain development and quality (Luan et al. 2022). Transgenic plants of rice showed drought and salt stress tolerance after overexpressing receptor-like kinase (*OsSIK1*) with extracellular leucine-rich repeat (Ouyang et al. 2010). This gene has an important role in controlling kernel quality and salt tolerance in wheat. Network 5 was found to mainly control phenylpropanoid metabolism. Phenylpropanoid metabolism produces flavonoids that are implemented in the scavenging of the excess ROS produced upon plant exposure to various abiotic stresses (Nakabayashi and Saito 2015). Overexpression of the phenylpropanoid regulating genes from wheat in tobacco increased osmotic stress tolerance as well as the chlorophyll content (Wei et al. 2017). The treatment of wheat with 50 mM NaCl enhanced the expression of the phenylpropanoid -related genes and increased the levels of

phenylpropanoid which in turn improved its nutritional value (Ma et al. 2019; Cuong et al. 2020). In rice, overexpression of the gene *Grain Size and Abiotic stress tolerance 1* (GSA1) increase the grain size under abiotic stresses, via increasing the biosynthesis of flavonoids after switching on the phenylpropanoid pathway (Dong et al. 2020; Zhang et al. 2021b). Network 6 was found to negatively control the plant defense system. *TraesCS2B02G279000* which controls this network was functionally annotated to encode tetratricopeptide repeat protein (TTL1). TTL1 was reported as a required protein for abscisic acid (ABA)-regulated responses (Rosado et al. 2006). The accumulation of ABA at later stages of grain growth was reported to prevent precocious germination and premature hydrolysis of starch (King 1976). Furthermore, ABA is known as a major phytohormone that plays an essential part in plant response toward varied range of abiotic stresses including high level of salinity. Previous studies reported the negative regulation of ABA in the plant immunity system pathways (Kim et al. 2011). Based on previous studies, the negative regulation of plant defense system results in improving wheat tolerance to abiotic stresses including AS stress.

The identified gene models seem to be highly important in improving AS tolerance in seedling and kernel growth stages. Furthermore, gene models identified in the mature growth stage control AS tolerance combined with high levels of wheat quality traits. Pyramiding of genes controlling the tolerance in both growth stages will accelerate wheat tolerance to such an expected severe stress by producing genotypes with high levels of tolerance in different growth stages.

Selection of the best candidate parents based on performance as well as genetic distance

Selection of the best genotypes that can be used as candidate parents in breeding programs is always challenging. One of the challenges that face plant breeders is the presence of many selection indices. It was reported that combining many selection indices provides more accurate results (Thiry et al. 2016; Pour-Aboughadareh et al. 2019). In the current study, the best ten genotypes were selected based on the average sum of ranks (ASR). ASR was reported

to provide more understanding of the response of the evaluated genotypes and has been used widely in different breeding programs to select superior genotypes (Pour-Aboughadareh et al. 2019; Belay et al. 2021). Furthermore, the selection was done based on TKW as it is the most important yield trait in wheat. Out of the ten selected genotypes, seven were Egyptian. Salt tolerance was reported previously in Egyptian wheat germplasm (El-Hendawy et al. 2005; Gadallah et al. 2017). However, no previous studies evaluated Egyptian wheat to combine alkalinity and salinity stresses. Combining phenotypic selection with extensive genetic analyses such as population structure, genetic distance, and association mapping results were found to be an effective approach to identifying the best parents that could improve target traits (Eltaher et al. 2021a, b, 2022). Therefore, the number of target alleles associated with seedling and kernel traits was detected in the ten-selected genotypes. Based on this number, four genotypes were detected. These four genotypes were found to be located in three different subpopulations and had a high genetic distance between them (Table S8). It was reported that the best parents to be used in breeding programs are those with high genetic distance (Bertan et al. 2007; Abou-Zeid and Mourad 2021; Mourad et al. 2022, 2023a, b; Esmail et al. 2023). Therefore, we can conclude that crossing the four genotypes in future breeding programs could accelerate wheat tolerance to AS and produce highly tolerant genotypes in different growth stages combined with highly qualified kernel yield.

Conclusion

Alkaline-salinity stress became a global problem that affects wheat yield. Based on the current study, a negative impact of AS on the seedling traits and the kernel traits, except RW and RW/SW, was observed. While the traits of each stage exhibited high positive correlations, the correlations among the traits of both stages were mostly negative or nonsignificant, indicating that they are under independent genetic controls. This is supported by the absence of common gene models controlling alkaline-saline tolerance in both growth stages. The recent study, for the first time, unlocked the genetic role of AS tolerance

in two different growth stages of the wheat life cycle and identified major genes controlling AS tolerance in each stage. Furthermore, tolerant and high-yielding genotypes were selected in this study based on the phenotypic and genotypic conceptions. These genotypes seem to be a good source of AS tolerance in spring wheat.

Declarations

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