Identification of Genomic Regions Associated with Agronomical Traits of Bread Wheat Under Two Levels of Salinity Using GWAS

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Abstract

Soil salinity is a major environmental stress that adversely affects the growth, development, productivity, and quality of crop species, in particular, in arid and semi-arid regions. Identification of chromosomal regions associated with agronomic traits under salinity stress is crucial for improving salinity tolerance in wheat. Genome-wide association study (GWAS) was employed to evaluate 289 elite lines of the Wheat Association Mapping Initiative (WAMI) population under low (LS) and high (HS) salinity conditions using 15,737 SNP markers for seven agronomical traits. The genotypes responded differently to the different environments for all traits, highlighting genetic diversity within the WAMI population in response to salt stress, where the heritability ranged from moderate (37%) to high (88%). GWAS identified 118 and 120 significant marker-trait associations (MTAs) under LS and HS conditions, respectively. Significant association of some markers with more than one phenotypic trait was observed, indicating possible pleiotropic or indirect effects. A high degree of significant linkage disequilibrium (>52%) was observed among SNPs on different chromosomes, indicating epistatic interaction. The salt stress index (STI) exhibited a positive significant correlation to grain yield per plant (GYP) under both LS and HS conditions ($R^2 = 0.851–0.856$). Linear regression analysis between STI and GYP under HS conditions indicated that STI is the best tolerance index for predicting high-yielding genotypes. The results present the WAMI population as a valuable source for improving yield potential for salt tolerance in wheat. Furthermore, our findings emphasize that GWAS is a powerful tool in promoting wheat breeding through accurate identification of molecular markers significantly associated with agronomic traits, which is essential for marker-assisted breeding.

Keywords Triticum · Salt stress · Salinity · Tolerance index · Association analysis · QTL

Introduction

Soil salinity is a major environmental stress that negatively affects the growth, development, productivity, and quality of crop species in arid and semi-arid regions, in particular. It inhibits the metabolic processes in the plants and reduces...
the photosynthetic capacity and biomass accumulation (Che-Othman et al. 2020; Jiang et al. 2021). An estimated 800 million hectares of irrigated soils worldwide are estimated to be salt-affected (Hernández 2019). The extent and severity of salinity-affected soils are expected to be increased because of deficit drainage of irrigated soil, rising water tables, and climate change (Aljabri et al. 2021; Oyiga et al. 2018; Peigné and Girardin 2004). Under salt stress conditions, plants exhibit a considerable reduction in growth rates, increasing leaf senescence, and reduced tillering, and during the long-term exposure to salinity, the reproductive development is adversely affected, resulting in a significant reduction in grain yield (Munns and Tester 2008). Therefore, understanding the mechanisms of salinity tolerance in crop plants is essential for the development of better salinity response varieties with improved tolerance to salt stress. These salt stress-tolerant varieties would improve the productivity of crop plants under the expected scenario of increasing salinity adverse effects to ensure sustainable food security for the growing population and avoid social perturbations resulting from starvation or food shortage (Abou-Elwafa and Shehzad 2020; Tanaka et al. 2015).

Wheat (Triticum aestivum L.), one of the earliest domesticated crops, is the major source of food and feed worldwide. The increasing demand for wheat consumption because of the rapid growth in the world population necessitates sustainable wheat production to ensure global food security (Garcia et al. 2019). Wheat is adapted to a wide spectrum of climatic regions including those subjected to salinity stress (Abou-Elwafa and Shehzad 2020; Monneveux et al. 2012). A representative core collection of wheat genotypes is essential for ensuring high genetic diversity, which is a crucial selection process in wheat breeding programs (Matus and Hayes 2002).

At the molecular level, plants have evolved several mechanisms to survive and grow under salt stress (Lv et al. 2020). Plants exhibit considerable plasticity at the morphophysiological, biochemical, and molecular levels to survive and resist or tolerate salt stress, including the expression of Na\(^+\) and K\(^+\) transporter genes. Rapid and efficient responses of plants to salt stress are essential for the survival, reproduction, and potential yields of cereal crops (Lohani et al. 2019). Plants would develop an adaptive salt stress tolerance mechanism to differentiate across growth stages as a result of long-term exposure to salinity (Oyiga et al. 2018). Dissecting the genetic factors underlying salt stress would facilitate breeding for improved abiotic stress-tolerant cultivars, which is the most feasible and cost-effective strategy for fighting against such a major abiotic stress (Abou-Elwafa 2016a; Abou-Elwafa and Shehzad 2018).

Genetic variations of salt-stress tolerance in bread wheat are limited and are mainly based on the ability of the plant to exclude Na\(^+\) from leaf blades and maintain an appropriate K\(^+\)/Na\(^+\) ratio (Oyiga et al. 2018). The limited application of marker-assisted breeding in improving crop plants to salt stress tolerance might be due to the complexity of salinity stress with respect to polygenic inheritance pattern, low heritability, and a high degree of genotype-by-environment interaction. However, recent advances in genetic and genomic strategies would facilitate unearthing the genetic control of important agronomic traits under abiotic stress conditions in different crop plants (Abou-Elwafa 2016b; Nezhadahmadi et al. 2013). The shared genomic regions and cross-compatibility between wild species and cultivated relatives provide wild species as a promising donor of valuable alleles to enhance the gene pool required for crop breeding and improvement (Abou-Elwafa 2016b).

Several studies employed genetic variations in agronomic traits evaluated under salt-stressed conditions to identify QTLs associated with salt tolerance at different growth stages in wheat (Ahmad et al. 2013; Munns et al. 1999; Rahnama et al. 2011; Schachtman et al. 1992). However, the main drawback of these studies is the reliance on bi-parental mapping populations that are characterized by poor resolution in detecting QTLs and considerable time and costs needed to develop the appropriate mapping population, besides the limited number of alleles that can be simultaneously studied at any locus (Mahmoud et al. 2018).

Genome-wide association study (GWAS) has emerged to overcome the drawbacks of conventional QTL analysis and improve and speed up breeding programs of crop plants. GWAS is a powerful approach that is used to enhance recent advances in genomic tools and statistical methods through efficient utilization of cumulative historical recombination and mutation events in a population to identify significant marker-trait associations (QTLs) with fine-mapping resolution and less research effort (Abou-Elwafa et al. 2019; Fleury et al. 2010). GWAS has been proven as a useful approach for uncovering the complex genetic mechanisms governing tolerance to abiotic stress in several crop plants (Long et al. 2013; Turki et al. 2015). However, although numerous research reports on GWAS for salt tolerance have been published in crop species, little research implementing GWAS for the identification of salt-stress tolerance loci has been carried out in wheat (Hu et al. 2021; Li et al. 2020; Quan et al. 2021; Yu et al. 2020). GWAS has identified 42 QTLs significantly associated with 10 salt tolerance-associated traits, from which 9, 16, and 17 QTLs are associated with physiological, shoot ionic, and biomass traits, respectively (Chaurasia et al. 2020). Using 395,675 SNP markers in a diversity panel of 323 wheat accessions, Li et al. (2020) identified 269 significant associations between phenotypic traits and SNP markers, from which 22 are overlapping with QTLs identified by bi-parental QTL mapping. Furthermore, GWAS in a panel of 191 wheat accessions genotyped by Wheat 660 K SNP array identified 389 significant SNPs.
representing 11 QTLs associated with several phenotypic traits including plant height, spike length, thousand kernels weight, and yield under different salt treatments, with an $R^2$ ranging from 9.14 to 50.45%. Repetitive and pleiotropic loci on different chromosomes were significantly identified to be linked to yield and yield-related traits such as thousand kernels weight, spike number, and spike length under low salinity conditions (Hu et al. 2021). Another GWAS in a wheat panel comprising 317 accessions genotyped with the wheat 90 K SNP chip revealed 37 SNPs located to 16 unique loci, each explaining 6.3–18.6% of the phenotypic variations. Ten loci were novel, whereas the remaining six were overlapped with previously reported genes or QTLs. Besides, nine loci are detected for two or more traits, indicating the complexity of the genetic architecture of salt tolerance in wheat (Quan et al. 2021). Employing both marker-based and pedigree-based kinship analyses in a panel of 307 wheat accessions including local landraces, exotic cultivars used in Chinese breeding programs and Chinese cultivars subjected to a GWAS revealed that favorable haplotypes were introduced in some exotic cultivars as well as a limited number of Chinese landraces (Yu et al. 2020).

The current study was carried out to evaluate a structured wheat population consisting of 289 elite spring bread wheat lines for salt stress tolerance. The genetic diversity in salt stress tolerance within the population was further estimated. We employed the GWAS approach using 15,737 SNP markers to identify significant associations between SNP markers and seven physiological and agronomical traits under two levels of salinity.

**Material and Methods**

**Plant Material and Field Experiments**

A genetically diverse structured population consisting of 289 elite spring bread wheat lines of the Wheat Association Mapping Initiative (WAMI) collected from several CIMMYT’s wheat international nurseries worldwide (Abou-Elwafa and Shehzad 2020); Suppl. Table 1) was used. Experiments were performed in 2017/2018 and 2018/2019 growing seasons at Sohag Governorate, Egypt (lat 26° 56′ N, long 31° 70′, and alt 61 m asl) at two experimental sites of low (control) and high salinity soils. Composite soil samples were collected from the upper 30 cm layer of each experimental site before the beginning of each growing season. The physical and chemical properties of the soil at the two experimental sites are shown in Suppl. Table 2. Seeds were sown in the field with a row space of 20 cm and a plant-to-plant space of 10 cm on November 25, 2017, and November 15, 2018, and harvested on April 13, 2018, and April 9, 2019, correspondingly. Irrigation was performed by drip irrigation with a 20-cm distance between drippers. Each treatment was irrigated twice a week using a total amount of 440 mm during the growing season. The chemical analysis of irrigation water is shown in Suppl. Table 3.

**Phenotyping**

The number of days to heading (HD) was estimated as the number of days from planting to the date when 50% of the plants have headed. The portable chlorophyll meter (SPAD502, Japan) was used to measure the leaf chlorophyll content (LCC) at the flowering stage. At harvest, five yield and yield-related traits were measured as average values of 10 individual plants from each plot. The measured traits include (i) plant height (PH), the main stem height at maturity, (ii) spike number/plant (SN), (iii) spike length (SL) excluding awns, (iv) thousand kernel weight (TKW), and (v) grain yield per plant (GYP). Salt-stress tolerance indices (STIs) for the measured traits were calculated according to the following formulas (Fernandez 1993), (Hossain et al. 1990), (Fischer and Maurer 1978), (Bouslama and Schapaugh 1984):

$$\text{Stress tolerance index (STI)} = \frac{y_p + y_s}{\bar{y}_p^2}$$

$$\text{Stress tolerance (TOL)} = y_p - y_s$$

$$\text{Stress susceptibility index (SSI)} = \frac{1 - (y_p/y_s)}{1 - (\bar{y}_p/\bar{y}_s)}$$

$$\text{Yield stability index (YSI)} = \frac{\bar{y}_s}{\bar{y}_p}$$

where $y_p$ is the average of a genotype under well-irrigated conditions, $y_s$ is the average of the same genotype under salt-stressed conditions, and $\bar{y}_p$ and $\bar{y}_s$ are the average yields of all genotypes in salt-stressed and well-irrigated environments, respectively.

**Genome-Wide Association, Population Structure, and Kinship Analyses**

The 90 K Illumina Infinium SNP array and SNP processing (https://data.cimmyt.org/file.xhtml?fileId=3822&datasetVersionId=332; Sukumaran et al. 2018) were employed to generate SNP markers by genotyping the WAMI population. A total of 15,737 SNP markers were used to perform the final genome-wide association analysis. The mixed linear model (MLM) with Structure and Kinship in the TASSEL 5 Version 20,160,901 software (Bradbury et al. 2007) was employed to
identify significant marker-trait associations between phenotypic traits and SNP markers. The significance of the tested molecular marker main effect at \( p \leq 0.0001 \) was considered an indicator of the significance of the marker-trait association (Chaurasia et al. 2020). Phenotypic data under either the control or stressed conditions were used to perform GWA analysis. Population structure of the 289 lines was carried out using the genotypic data of 15,737 SNP markers using a model-based (Bayesian) clustering algorithm in the software package STRUCTURE v. 2.2 (Falush et al. 2003; Pritchard et al. 2000). The analysis was performed with the linkage model on 15,737 SNP markers, allowing for correlated allele frequencies. The hypothetical subpopulations were considered \( K = 2 – 15 \), and the package was put on run with three independent runs for each value of \( K \). The iteration number for the Markov Chain Monte Carlo (MCMC) algorithm was set as 100,000, following a burn-in period of 100,000 iterations. The K-matrices, and the Q-matrix describing the assignment of each accession to specific clusters, were used in mixed linear model association mapping (Yu et al. 2006).

**Estimation of Linkage Disequilibrium**

The software Tassel 5 Version 20,160,901 was employed to calculate linkage disequilibrium (LD) between SNP markers using the entire set of wheat lines. LD was estimated separately for all loci pairs on different chromosomes. Significant \( p \)-values of LD for SNP pairs were estimated by permutation (10,000 simulations).

**Experimental Design and Statistical Analyses**

Experiments were carried out in a three-replicate split-plot design arranged in a randomized complete block design (RCBD). The main plots were assigned to salinity stress, and the sub-plots were assigned to the wheat genotypes. Plants from each of the wheat genotypes were sown in two lines, each of 2 m in length. Estimation of the additive and dominance effects was performed using the genetic model as essentially described in Gambel (1962). Broad sense heritability was estimated as a proportion of the genotypic variance to the phenotypic variance according to Allard (1960). The Proc Mixed of the SAS package version 9.2 (SAS 2008) was employed to perform the analysis of variance (ANOVA), correlation, and linear regression among stress indices of measured phenotypic traits.

**Results**

**Phenotypic Evaluation**

All measured agronomical traits were significantly affected across different environments. Salt stress significantly reduced HD, LCC, PH, SN, SL, TKW, and GYP (Fig. 1; Table 1; Suppl. Tables 4 and 5). All measured traits were significantly reduced in the first growing season under both salinity treatments. ANOVA revealed highly significant differences \( (p \leq 0.01) \) among evaluated genotypes in all measured traits. Genotype \( \times \) environment interaction was also significant for all traits (Table 1), indicating genetic diversity among the evaluated genotypes in their response to salt stress. Heritability degree ranged from moderate (37% for TKW under LS conditions in the second season) to high (88% for LCC under HS conditions in the first season; Table 1).

**Stress Tolerance Indices for Selection to Salt Stress**

Salt stress tolerance indices, i.e., STI, TOL, SSI, and YSI, and their correlations and regressions to GYP under HS and LS conditions (GYPP and GYPS, respectively) of all evaluated genotypes for all measured traits in both growing seasons were estimated. However, because of the high degree of similarities between the correlations and regressions among stress tolerance indices of all measured traits, only the correlations and regressions for GYP were presented and further studied (Table 2). Linear regression analysis revealed a strong positive relationship between STI and GYPPs with an \( R^2 \) value of 0.834. The higher the GYPPs, the greater the STI value and the higher the tolerance of a wheat genotype to salt stress observed (Fig. 2). TOL revealed a highly significant positive and negative correlation with GYPPs and GYPS, correspondingly (Table 2), suggesting that employing TOL as a selection index reduces grain yield per plant under salt-stressed conditions. STI exhibited highly significant positive correlations with either GYPPs or GYPS, indicating that STI could be efficiently employed as a selection index for GYPP and GYPS compared to TOL, SSI, and YSI. GYPPs was linearly correlated with GYPS (Fig. 2), indicating that the high-yielding elite genotype under low salinity conditions may produce a reasonable yield under salt-stressed conditions. In addition, the general linear regression revealed strong positive regressions between GYP under salt-stressed conditions and STI over the two growing seasons, with an \( R^2 \) of 0.834 (Fig. 2).

**Population Structure and Kinship**

STRUCTURE analysis indicated the presence of seven subpopulations in our germplasm (Fig. 3). The genetic makeup of the genotypes show obvious large proportions that had not been broken down by recombination. Cluster analysis also identified seven main clusters (Suppl. Figure 1) confirming the results of STRUCTURE. LD analysis revealed a low to medium level of linkage disequilibrium among the SNPs \( (r^2 \) ranging between 0.0 and 0.52) (Suppl. Figure 2).
Fig. 1 Boxplot for seven agronomical traits of 289 genotypes of the WAMI population evaluated under low (LS) and high (HS) salinity conditions during the 2017/2018 and 2018/2019 growing seasons.
Association Mapping

Genome-wide association analysis using 15,737 SNP markers and seven physiological, morphological, yield, and yield-related traits under low and high salinity conditions identified 118 and 120 significant marker-trait associations under LS and HS, respectively, with $R^2$ values ranging from 3.91 to 17.59 (Figs. 4 and 5; Suppl. Table 6).

Eleven SNP markers mainly clustered on chromosomes 1A and 1B exhibited significant association with the HD under LS with an $R^2$ value ranging between 3.91 and 4.87%. Meanwhile, under HS, HD exhibited significant associations with 28 SNPs with an $R^2$ value ranging from 4.13 to 5.40%. Out of those, 23 SNP markers were clustered on chromosome 5A (Fig. 4; Suppl. Table 6). Ten significant associations for LCC under either LS or HS were detected (mainly clustered on chromosomes 2B and 7A) with an $R^2$ value ranging between 3.41 and 4.67%. A total of 17 SNP markers clustered mainly on chromosomes 6B were significantly associated with plant height under low salinity conditions, with an $R^2$ ranging between 4.15 and 6.47%. Meanwhile, under high salinity conditions, 15 significant marker-trait associations clustered on chromosomes 2B and 6B were identified for plant height under salt-stressed conditions with an $R^2$ value ranging between 3.82 and 7.66% (Fig. 4; Suppl. Table 6). SN exhibited 12 and 19 significant associations with the SNP markers under LS and HS, respectively, with an $R^2$ value ranging between 3.98 and 7.46% (Fig. 4; Suppl. Table 6). Out of the 15 significant MTAs identified for SN under LS, 11 were located on chromosome 6A, and two markers are located on chromosome 7D. Under HS, two major clusters comprising seven and five significantly associated markers were identified for SN on chromosomes 2A and 4B, respectively. The analysis identified 31 significant marker-trait associations clustered on four genomic regions on chromosomes 2A, 2B, 3D, and 5B with SL under LS with an $R^2$ value ranging between 3.98 and 7.46% (Fig. 5; Suppl. Table 6).

### Table 1
Analysis of variance (ANOVA) for traits under individual environment

| Trait | HD | LCC | PH | SN | SL | GYP | TKW |
|-------|----|-----|----|----|----|-----|-----|
| Mean  | 75.8 | 89.0 | 98.4 | 8.4 | 10.0 | 31.2 | 0.3 |
| SD    | 41.6 | 40.2 | 52.8 | 2.1 | 0.8 | 18.6 | 0.1 |
| G  | 35.2 | 37.0 | 45.6 | 2.0 | 0.8 | 13.6 | 0.1 |
| h2   | 0.56 | 0.48 | 0.51 | 0.21 | 0.45 | 0.42 | 0.37 |

### Table 2
Pearson correlation coefficients (R) between salt stress indices and average plant grain yield over the two growing seasons

| GYPp | Gyps | STI | TOL | SSI |
|------|------|-----|-----|-----|
| 0.520* | 0.851** | 0.956** | 0.433* |
| STI | 0.856** | -0.244ns | -0.514* |
| TOL | 0.670** | 0.048 | -0.670** |
| SSI | -0.433* | 0.514** |

ns, *, and ** indicate non-significance, the significance levels at $P<0.05$ and $P<0.01$, respectively.
Twenty SNP markers clustered on three genomic regions on chromosome 2B, i.e., 88, 95–97, and 161 cM, were significantly associated with GYP under LS ($R^2 = 5.20$ and 13.63%). Meanwhile, under HS, 18 SNP markers located on chromosomes 2A, 4A, and 4B exhibited significant associations with an $R^2$ value ranging from 5.38 and 15.81%; Suppl. Table 6). GWAS revealed 17 significant marker-trait associations with TKW under LS/HS with an $R^2$ value between 3.91 and 13.25% (Fig. 5; Suppl. Table 6). Under normal conditions, the SNP markers significantly associated with TKW were mainly (14 SNPs) clustered on chromosome 1B, whereas under LS/HS, they were distributed to chromosomes 1B, 2A, 4B, 5A, 6A, 6B, and 7A, with a major cluster of markers on chromosome 6A (Fig. 5; Suppl. Table 6).

**Discussion**

GWAS which depends on the structure of linkage disequilibrium of alleles at different loci is a powerful strategy for accurate identification and fine mapping of genomic regions underlying quantitative traits (Abou-Elwafa and Shehzad 2018; Simko et al. 2006). This approach is more efficient when the purpose is to identify quantitative traits associated with a single marker (Abou-Elwafa 2016b; Doerge 2002). However, the choice of a population is a pivotal factor in determining the resolution of association analysis in plants. The selected population should exhibit a high degree of diversity with more extensive historical recombination to allow the detection of more alleles (Abou-Elwafa 2016a; Shehzad et al. 2009).
In the current study, the TASSEL software that implements a fixed-effects mixed linear model was employed to identify the association between SNP marker alleles and agronomic quantitative traits under LS and HS in the highly genetically diverse WAMI population (Abou-Elwafa and Shehzad 2020). The continuous variations observed in the evaluated agronomic traits phenotypic under both LS and HS conditions over the two growing seasons indicate polygenic inheritance of all evaluated traits. Besides, the genotypes responded differently to salinity conditions, emphasizing the adverse impacts of high salinity on wheat crops. The linear regression relationship observed between GYPp and GYPs indicates that indirect selection for salinity tolerance based on the high-yielding potential under low salinity conditions might be efficient (Talebi et al. 2009).

Fig. 3 Distribution of 289 wheat lines in seven groups. Each genotype is correspondent to a single vertical colored bar above the diagram defragmented into K differently colored fragments. The length of the colored fragment is equivalent to each one of the K inferred subgroups.

Fig. 4 Manhattan plots of the significant SNPs under low (LS) and high (HS) salinity conditions over the two growing seasons for the number of days to heading (HD-LS and HD-HS), leaf chlorophyll content (LCC-LS and LCC-HS), plant height (PH-LS and PH-HS), and spike number (SN-LS and SN-HS)
Of four stress tolerance indices (STI, TOL, SSI, and YSI), only STI proved its worth as it identified the high-yielding genotypes and revealed positive correlations with the mean GYP under LS and HS. Hence, the other indices (SSI, TOL, and YSI) may not be efficient under variable environmental conditions (Abou-Elwafa and Shehzad 2020) to identify the best lines. Further, the linear regression between STI and mean GYP under LS and HS emphasizes that STI is a powerful selection index for the prediction and selection of high-yielding genotypes under saline stress (Fig. 6).

The major challenge in GWAS is to separate true positive associations between molecular markers and phenotypic
Fig. 6 Relationship between grain yield per plant under high salinity conditions (GYPs) and salt tolerance indices (TOL, SSI, and YSI) over the two growing seasons.
traits from the false positives. To overcome this challenge, Q and K matrices were used in the MLM (Stich and Melchinger 2009). The QK method was essentially developed to abolish false associations caused by population structure and kinship (Stich and Melchinger 2009; Zhao et al. 2007). A major HD QTL (Qhd.4 W-5A.1) co-localized with the two major vernalization genes Vrn-A1 and Vrn-A2 on chromosome 5A has been identified (Guedira et al. 2016). Besides, a major cluster of molecular markers located on chromosome 5A has been reported to be significantly associated with heading date (Abou-Elwafa and Shehzad 2020). Additionally, a major HD QTL associated with the primary photoperiod gene Ppd-B3 on chromosome 7B has been identified and genetically mapped (Fowler et al. 2016). The significant associations between LCC and SNP markers were mainly clustered on chromosomes 2B and 7A. Furthermore, a major genomic region is associated with LCC under either low or high salinity conditions on the telomeric region of chromosome 7A, emphasizing that the LCC is mainly controlled by genetic factors (Dai et al. 2016) which is consistent with the high degree of heritability estimated for this trait (75–88%) under either at HS or LS (Table 1). Moreover, a major QTL associated with chlorophyll b designated qChla7A was identified on chromosome 7A (Zhang et al. 2009). SNP markers significantly associated with PH were located on specific genomic regions on chromosomes 2B, 3A, 4B, 5A, and 6B. Four genomic regions located on chromosomes 2B, 3A, and 6B were significantly associated with PH under either LS or/and HS conditions, indicating that plant height is mainly controlled by genetic effect. The high heritability degrees observed for plant height (77–83%) under all environmental conditions (Table 1) support this finding. Several PH-associated QTLs have been identified on chromosome 3A (Ali et al. 2011; El-Feki et al. 2018; Rustgi et al. 2013); however, major plant height QTLs were identified on chromosome 4B (Zhang et al. 2018). The SNP marker RAC875_c24550_1150 located on chromosome 4B and associated with PH under high salinity conditions (Suppl. Table 6) has been previously identified to be associated with plant height QTLs under different environmental conditions (Abou-Elwafa and Shehzad 2020; Zou et al. 2017). More recently, Arif et al. (2021) have located as many as nine PH QTLs on chromosome 4B. Besides, the identification of a cluster of markers on chromosome 4B that are significantly associated with PH is consistent with the identification of a cluster of the gibberellin acid-insensitive reduced height genes (RhtB1) that control plant height on chromosome 4B (Arif et al. 2021; Cabral et al. 2018; El-Feki et al. 2018). Furthermore, the identification of a cluster of markers co-localized with the genomic region of the Vrn-A1 gene that has a reducing effect on plant height (Chu et al. 2008) suggests that Vrn-A1 might be a promising candidate for this QTL. A major cluster of the markers significantly associated SN resides on chromosome 6A which is consistent with the identification of a genomic in close vicinity to the major tillering promoter Gli-A2 (a multigene protein family gliadin) on chromosome 6A (Li et al. 2002). Furthermore, a major SN-related QTL was mapped to chromosome 7B (Shah et al. 1999) at which three significant marker-trait associations were identified for SN in our study. SNP markers significantly associated with SL were mainly clustered to chromosomes 2A, 2B, 3B, 3D, 5B, and 7B, which is consistent with the results of El-Feki et al. (2018) where 10 SL QTLs were detected, three of which were mapped to chromosomes 2B, 3D, and 7B. A major SL-associated QTL designated (QEl. fcu-3D; Chu et al. 2008) was mapped to the short arm of chromosome 3D where a major cluster of 13 markers was identified to be significantly associated with SL under low salinity conditions in this study. Moreover, our results are in agreement with the identification of three SL-associated QTLs, i.e., QSL.cau-2B.2, QSL.cau-7B.1, and QSL.cau-7B.2, which are stable across different environmental conditions, on chromosomes 2B and 7B, respectively (Zhai et al. 2016). Additional verification findings for the importance of chromosomes 2B and 7B in the control of SL in wheat are the identification of two common loci associated with SL on chromosomes 2B and 7B, with the chromosome 7B locus being significant and stable across five environments (Li et al. 2019).

The SNPs associated with GYP were mainly clustered on the genomic region of 88–97 cM of chromosome 2B under LS, two of which, i.e., wsnp_Ex_c20786_29875033 and BS00003404_51, were associated with SL, whereas under HS, five of the SL significantly associated markers located on chromosomes 2A were also significantly associated with SN. The significant association of markers with more than one phenotypic trait indicates possible pleiotropic or indirect effects of the QTLs harboring those markers. In a similar GWAS, 12 significant associations between SNP markers and grain yield were identified, of which two were located on chromosomes 2A and 2B (Li et al. 2019). The SNPs significantly associated with TKW under low and high salinity conditions were clustered mainly on chromosomes 1B, 2A, and 6A. Two markers, i.e., RFL_Cotig785_535 and RFL_Cotig785_1700, are located on chromosome 1B and explain that 8.22–13.25% of the phenotypic variance in this trait was identified under both the normal and the stressed environments. Significant associations between those two markers and TKW were previously identified in a GWAS using the same WAMI population (Sukumaran et al. 2018). A major QTL significantly associated with marker BS00023092_51 under the salt-stressed conditions that is closely linked to the marker BS00036878_51 on chromosome 6A identified previously to be associated with TKW-QTL (Zou et al. 2017). In addition, the genomic region of chromosome 6A (78–85 cM) that harbors seven
SNP markers (BS00023092_51, Tdurum_contig76709_195, Excalibur_c56264_188, BobWhite_c17086_197, IAAV1263, BobWhite_c1082_134, and Tdurum_contig47663_321) has been earlier identified to harbor a major QTL for TGW (Sukumaran et al. 2018). In conclusion, this study revealed 238 significant MTAs associated with seven phenotypic traits. Certain SNPs were associated with multiple traits, indicating possible pleiotropic or indirect effects of the QTLs associated with those markers. The high degree of significant linkage disequilibrium (> 52%) observed among SNP markers on different chromosomes indicates epistatic interaction. The results present the WAMI population as a valuable source for improving yield potential for salt tolerance in wheat. Furthermore, our findings emphasize that GWAS is a powerful tool in promoting wheat breeding through accurate identification of molecular markers significantly associated with agronomic traits, which is essential for marker-assisted breeding.

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Author Contribution SFA conceived the study, analyzed the data, performed the experiment, collected the data, and wrote the manuscript. TS performed GWAS and structure analyses. FA, RA, MAJ, and MAB helped with data analysis and presentation. All authors read and approved the final version of the manuscript.

Data Availability All data are included within the manuscript and its supplementary material.

Code Availability Not applicable.

Declarations

Ethics Approval and Consent to Participate Not applicable.

Consent for Publication Not applicable.

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References

Abou-Elwafa SF, Shehzad T (2018) Genetic identification and expression profiling of drought responsive genes in sorghum. Environ Exp Bot 155:12–20

Abou-Elwafa SF, Shehzad T (2020) Genetic diversity, GWAS and prediction for drought and terminal heat stress tolerance in bread wheat (Triticum aestivum L.), Genet Resour Crop Evol

Ahmad M, Shahzad A, Iqbal M, Asif M, Hirani AH (2013) Morphological and molecular genetic variation in wheat for salinity tolerance at germination and early seedling stage. Aust J Crop Sci 7

Ali ML, Baenziger PS, Ajlouni ZA, Campbell BT, Gill KS, Eskridge KM, Mueeb-Kazi A, Dwiekait I (2011) Mapping QTL for agronomic traits on wheat chromosome 3A and a comparison of recombinant inbred chromosome line populations. Crop Sci 51:553–566

Aljabri M, Alharbi S, Al-Qthainan RN, Ismaeil FM, Chen J, Abou-Elwafa SF (2021) Recycling of beet sugar byproducts and wastes enhances sugar beet productivity and salt redistribution in saline soils. Environ Sci Pollut Res

Allard RW (1960) Principles of Plant Breeding. John Wiley and Sons, New York, NY

Arif MAR, Shokat S, Plieske J, Ganal M, Lohwasser U, Chesnokov YY, Kocherina NV, Kulwai P, Kumar N, McGuire PE (2021) A SNP-based genetic dissection of versatile traits in bread wheat (Triticum aestivum L.). Plant J 160:960–976

Bouslama M, Schapaugh WT (1984) Stress tolerance in soybeans. I. Evaluation of three screening techniques for heat and drought tolerance. Crop Sci 24:933–937

Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES (2007) TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics (oxford, England) 23:2633–2635

Cabral AL, Jordan MC, Larson G, Somers DJ, Humphreys DG, McCartney CA, Zhang A (2018) Relationship between QTL for grain shape grain weight test weight milling yield and plant height in the spring wheat cross RL4452/AC Domain'. PLOS ONE 13(1) e0190681

Chaurasia S, Singh AK, Songachan LS, Sharma AD, Bhardwaj R, Singh K (2020) Multi-locus genome-wide association studies reveal novel genomic regions associated with vegetative stage salt tolerance in bread wheat (Triticum aestivum L.). Genomics 112:4608–4621

Che-Othman MH, Jacoby RP, Millar AH, Taylor NL (2020) Wheat mitochondrial respiration shifts from the tricarboxylic acid cycle to the GABA shunt under salt stress. New Phytl 225:1166–1180

Chu C-G, Xu SS, Friesen TL, Faris JD (2008) Whole genome mapping in a wheat doubled haploid population using SSRs and TRAPs and the identification of QTL for agronomic traits. Mol Breeding 22:251–266

Dai W, Girdtai T, Huang Z, Ketudat-Cairns M, Tang R, Wang S (2016) Genetic analysis for anthocyanin and chlorophyll contents in rapeseed. Ciência Rural 46:790–795

Doerge RW (2002) Mapping and analysis of quantitative trait loci in experimental populations. Nat Rev Genet 3:43–52

El-Feki WM, Byrne PF, Reid SD, Haley SD (2018) Mapping quantitative trait loci for agronomic traits in winter wheat under different soil moisture levels. Agronomy 8

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164:1567–1587

Fernandez GCJ (1993) Effective selection criteria for assessing plant stress tolerance. In ‘Adaptation of food crops to temperature and soil moisture levels. Agronomy 8

Fleury D, Jefferies S, Kuchel H, Langridge P (2010) Genetic and genomic tools to improve drought tolerance in wheat. J Exp Bot 61:3211–3222
Nezhadahmadi A, Prodhan ZH, Faruq G (2013) Drought tolerance in
Munns R, Hare RA, James RA, Rebetzke GJ (1999) Genetic variation
Monneveux P, Jing R, Misra SC (2012) Phenotyping for drought adap-
Lv B, Wu Q, Wang A, Li Q, Dong Q, Yang J, Zhao H, Wang X, Chen H, Li C (2020) A WRKY transcription factor, FWRKY46, from Tartary buckwheat improves salt tolerance in transgenic Arabidopsis thaliana. Plant Physiology and Biochemistry : PPB 147:43–53
Mahmoud AF, Abou-Elnawa SF, Shehzad T (2018) Identification of charcoal rot resistance QTLs in sorghum using association and in silico analyses. J Appl Genet
Matus IA, Hayes PM (2002) Genetic diversity in three species of barley germplasm assessed by simple sequence repeats. Genome 45:1095–1106
Monnevoux P, Jing R, Misra SC (2012) Phenotyping for drought adap-
tation in wheat using physiological traits. Front Physiol 3:429
Munns R, Hare RA, James RA, Rebetzke GJ (1999) Genetic variation for improving the salt tolerance of durum wheat. Aust J Agric Res 51:69–74
Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–681
Nezhadahmadi A, Prodhan ZH, Faruq G (2013) Drought tolerance in wheat. Scientific World Journal 610721
Oyiga BC, Sharma RC, Baum M, Ogbonnaya FC, Léon J, Ballvora A (2018) Allelic variations and differential expressions detected at quantitative trait loci for salt stress tolerance in wheat. Plant, Cell Environ 41:919–935
Peigné J, Girardin P (2004) Environmental impacts of farm-scale compo-
ting practices. Water Air Soil Pollut 153:45–68
Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959
Quan X, Liu J, Zhang N, Xie C, Li H, Xia X, He W, Qin Y (2021) Genome-wide association study uncover the genetic architecture of salt tolerance-related traits in common wheat (Triticum aestivum L.). Front Genet 12:66:3941–663941
Rahnama M, Munns R, Poustini K, Watt M (2011) A screening method to identify genetic variation in root growth response to a salinity gradient. J Exp Bot 62:69–77
Rustgi S, Shaqfhat MN, Kumar N, Baenziger PS, Ali ML, Dweikat I, Campbell BT, Gill KS (2013) Genetic dissection of yield and its component traits using high-density composite map of wheat chromosome 3A: bridging gaps between QTLs and underlying genes. PLoS ONE 8:e70526–e70526
SAS (2008) SAS/STAT® 9.2 user’s guide. SAS Institute Inc., Cary, North Carolina, USA
Schachtman DP, Lagudah ES, Munns R (1992) The expression of salt tolerance from Triticum tauschii in hexaploid wheat. Theor Appl Genet 84:714–719
Shah MM, Gill KS, Baengiser PS, Yen PS, Kaeppler SM, HM HMA (1999) Molecular mapping of loci for agronomic traits on chromosome 3A of bread wheat. Crop Sci 39:5
Shehzad T, Iwata H, Okuno K (2009) Genome-wide association mapping of quantitative traits in sorghum (<i>Sorghum bicolor</i> (<i>L.</i> Moench)) by using multiple models. Breed Sci 59:217–227
Simko I, Haynes KG, Jones RW (2006) Assessment of linkage disequi-
librium in potato genome with single nucleotide polymorphism markers. Genetics 173:2227–2245
Stich B, Melchinger AE (2009) Comparison of mixed-model approaches for association mapping in rapeseed, potato, sugar beet, maize, and Arabidopsis. BMC Genomics 10:94
Sukumaran S, Reynolds MP, Sansaloni C (2018) Genome-wide associa-
tion analyses identify QTL hotspots for yield and component traits in durum wheat grown under yield potential, drought, and heat stress environments. Front Plant Sci 9:81
Talebi R, Fayaz F, Naji AM (2009) Effective selection criteria for assessing drought stress tolerance in durum wheat (<i>Triticum durum Desf.</i>). Gen Appl Plant Physiol 35:64–74
Tanaka A, Takahashi K, Masutomi Y, Hanasaki N, Hiijoka Y, Shiogama H, Yamanaka Y (2015) Adaptation pathways of global wheat production: importance of strategic adaptation to climate change. Sci Rep 5:14312
Turki N, Shehzad T, Harrabi M, Okuno K (2015) Detection of QTLs associating with salt tolerance in durum wheat based on association analysis. Euphytica 201:29–41
Yi J, Pressoir G, Briggs WH, Vroh Bi I, Yamazaki M, Doebley JF, McMullen MD, Gaut BS, Nielsen DM, Holland JB, Kresovich S, Buckler ES (2006) A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. Nat Genet 38:203–208
Yu S, Wu J, Wang M, Shi W, Xia G, Jia J, Kang Z, Han D (2020) Haploype variations in QTL for salt tolerance in Chinese wheat accessions identified by marker-based and pedigree-based kinship analyses. The Crop Journal 8:1011–1024
Zhai H, Feng Z, Li J, Liu X, Xiao S, Ni Z, Sun Q (2016) QTL analysis of spike morphological traits and plant height in winter wheat (Triticum aestivum L.) using a high-density SNP and SSR-based linkage map. Front Plant Sci 7:1617–1617

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Zhang J, Gizaw SA, Bossolini E, Hegarty J, Howell T, Carter AH, Akhunov E, Dubcovsky J (2018) Identification and validation of QTL for grain yield and plant water status under contrasting water treatments in fall-sown spring wheats. Theor Appl Genet 131(8) 1741-1759

Zhang K, Zhang Y, Chen G, Tian J (2009) Genetic analysis of grain yield and leaf chlorophyll content in common wheat. Cereal Research Communications 37:499–511

Zhao K, Aranzana MJ, Kim S, Lister C, Shindo C, Tang C, Toomajian C, Zheng H, Dean C, Marjoram P, Nordborg M (2007) An arabidopsis example of association mapping in structured samples. PLoS Genetics 3:e4

Zou J, Semagn K, Iqbal M, Chen H, Asif M, N’Diaye A, Navabi A, Perez-Lara E, Pozniak C, Yang R-C, Randhawa H, Spaner D (2017) QTLs associated with agronomic traits in the Attila × CDC Go spring wheat population evaluated under conventional management. PLoS One 12:e0171528

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