Pak. J. Bot., 55(5): 1739-1746, 2023. DOI: http://dx.doi.org/10.30848/PJB2023-5(32)

0GENOME-WIDE ASSOCIATION MAPPING OF SEED RESERVE UTILIZATION DURING EARLY SEEDLING GROWTH OF SWEET CORN UNDER SALT STRESS

JING-YI HUANG, WANG-FEI HE, ZI-QIANG HONG, YOU-XIN Li, BAIYU ZHOU AND XIN-XIN CHENG*

College of Agriculture, Anhui Science and Technology University, Fengyang, Anhui, 233100, PR China
**Corresponding author's email: chengxin0901@163.com*

Abstract

Sweet corn is a special type of cultivated maize only used in food production. It results from a naturally occurring recessive mutation in the genes controlling sugar conversion to starch inside the corn kernel's endosperm. In this study, we have found that the seed germination rate, seedling height, root length, weight of the mobilized seed reserve, and seed reserve utilization efficiency of 100 sweet corn seeds were significantly different. They also showed a downward trend with increasing salt concentration. A total of 37297 SNP markers were screened to identify valuable quantitative trait loci associated with the seed germination rate, seedling height, root length, weight of the mobilized seed reserve, and seed reserve utilization efficiency by genome-wide association study. Significant SNPs were successfully identified, and 28 quantitative trait loci were identified for all salt-related traits in hydroponic systems. Three major loci of Affx-91146, Affx-91055, and Affx-90658 were common. Their contributions were 15.06～19.56%, and positions 162789062, 2136131, and 1230444, respectively.

Key words: Sweet corn; Seed reserve utilization; Seed germination; Salt stress.

Introduction

Sweet corn (*Zea mays* L. var. saccharata Bailey) is widely planted in China because of its pleasant taste. Sweet corn kernels are consumed in the green ear/milky stage, generally at 21 days after pollination, and sold as highly prized fresh or canned vegetables (Brijesh *et al*., 2017). However, the seed of the sweet corn can be shriveled, and the germination can be lower from mutation of the gene-controlling endosperm. As a result, the decreasing germination rate (GR) and poor emergence of sweet corn seeds have become the bottleneck factors restricting the development of its production.

Seed germination is a complex trait that the heterotrophic seedling growth (mg per seedling) comprises the Weight of Mobilized Seed Reserve (WMSR) in mg per seed, and the conversion efficiency of mobilized seed reserve to seedling tissue ($mg.mg^{-1}$) (Cheng *et al*., 2013). Seed vigor forms in the process of seed development, and the accumulation of seeds is the basis for the formation of seed vitality. In the germinating seed, the emergence of seeds with high vigor is more uniform and therefore forms more vigorous seedlings, which in turn, provide better stand establishment (Cui *et al*., 2020). Viable seeds can produce new plants under favorable climatic conditions.

Sweet corn is subjected to various stresses during its lifecycle, including abiotic stress factors such as salinity and biotic stress factors such as pathogens. Salinity is a major environmental factor that restricts plant growth and yield during all developmental stages. So, developing salinized or marine agriculture (growing salt-tolerant crops on land using seawater for irrigation) has become one of the world's research hotspots.

Soil salinity is the primary abiotic stress affecting crop growth and productivity worldwide (Ali *et al*., 2013). It is estimated that 6% of the earth's landmass and 20% of irrigated land are affected by salinity (An *et al*., 2020). Salt tolerance is a polygenic trait, which is highly influenced by the environment. So, it is crucial to breed new varieties of sweet corn tolerant of salinity. That necessitates the identification of genetic determinants conferring salinity tolerance (Chen *et al*., 2020). Over the past several years, traditional methods (such as seed preparation) have improved the salt tolerance of plants but are short of what is needed. In recent years, genetic engineering has significantly improved the salt tolerance gene of the plant. Recently, Genome-Wide Association Studies (GWAS) have been used as powerful tools to dissect the genetic basis of many phenotypic traits by using genetically diverse populations (Le *et al*., 2021; Maulana, *et al*., 2018). Until now, previous studies have reported many quantitative trait loci (QTLs) for rice, wheat, maize, soybean, cotton and tomato under salt stress (Wang *et al*., 2014; Shi *et al.*, 2017; Maulana *et al*., 2018; Ma *et al*., 2019; Jin *et al*., 2019; Huang *et al*., 2021; Li *et al*., 2021; Luo *et al*., 2021). Zeng (2001) mapped 13 QTLs for the seed GR and Germination Index (GI) underwater and conditions of 300 mM NaCl. Wang (2012) detected 16 QTLs for rice seed's germination ability at 100 mM NaCl for the japonica variety, *Jiucaiqing*. However, no reports have been based on the salt tolerance of sweet corn associated with a genome-wide location.

In this study, the salt tolerance of sweet corn at the seed germination stage was screened for identifying the tolerant cultivars and detecting the novel QTLs or genes associated with germination traits such as the GR, root length (RL), seedling height (SH), WMSR, and Seed Reserve Utilization Efficiency (SRUE) using genome-wide association mapping. Dynamic analysis of salt tolerance genotype/s could provide a breakthrough in breeding for salinity tolerance in sweet corn. These results should contribute to understanding the genetic control of seed vigor in sweetcorn, and the identified QTLs could be used to improve sweet corn seed vigor by MAS (Molecular assisted breeding).

Materials and Methods

Plant materials: One hundred super sweet (sh_2) sweet corn cultivars from Anhui Science and Technology University were randomly selected and used in this study. All cultivars had single nucleotide polymorphisms (SNP) data for GWAS. Fifteen plants of each cultivar were selected to self-cross with the same performance before silking. All seeds were harvested at the mature stage when seeds were dried at the university's Experimental Station.

Evaluation of salt tolerance at the seedling stage: The experiment was designed with randomized groups and used three replications. The seeds were surface sterilized in 1% sodium hypochlorite, washed with deionized water, and then germinated. Seeds were placed in boxes (19×13×12 cm) with three sheets of filter paper, and each box contained 20 mL of salt solution for the germination test. Each cultivar's run consisted of twelve boxes, three for control and another nine for salt treatment (30 mmol/L, 60 mmol/L, and 90 mmol/L). Fifty seeds per replication of each cultivar were weighed and incubated in an incubator for seven days in the dark at 28±1ºC.

Evaluation of seed reserve utilization: To determine each cultivar's Initial Seed Dry Weight (ISDW) in mg per seed, three replications of 300 seeds were weighed (W_1), dried at 104 ºC for 24 h, and then reweighed (W_2). The seed Water Content (WC) was calculated as $[(W_1-W_2)/W_1]$ then the ISDW in each replication was calculated as $[W_1\times(1\text{-WC})]$. The dry weight of seedlings and remnant cotyledons was obtained after oven drying at 104ºC for 24 h. The WMSR in mg per seed was calculated as the ISDW minus the dry weight of the seed remnant. SRUE (in mg per seed) was estimated by dividing the seedling dry weight (ISDW) by the WMSR (Cheng *et al*., 2016). The shoot length and RL were measured, and their samples were collected separately and dried in an oven at 85°C for four days, after which the shoot biomass and root biomass were weighed. Three independent experiments were conducted for the evaluation of SRUE after seven days of germination.

Data analysis: The experimental data were analyzed using the Statistical Analysis System (SAS) software, and the traits were compared using Student's t-test at the 5% and 1% levels of probability. The correlations of the traits were computed using PROC CORR by SAS software.

Genome-wide association mapping: The General Linear model (GLM) in TASSEL 2.1 was used to identify the association between each SNP and phenotype across all cultivars. SNPs with minor allele frequency <0.05 were filtered out, and the maximum missing per SNP was set at 5% for GWAS. After GWAS, the significance threshold for the association between SNPs and traits was set at $p<0.0001$, a value previously used for this population.

Results

Structure analysis of sweet corn: 56,000 SNP markers were filtered by using the Plink software to reduce the influence of LD on population structure with high linkage imbalance intensity ($r^2>0.3$), and 37297 SNP markers were obtained, covering each sh_2 sweet corn chromosome evenly (Table 1). The density of SNP on each chromosome was 7.65–21.08 per Mb; the polymorphic information content was 0.194–0.195, and the variation amplitude of gene diversity on different chromosomes was 0.361–0.377, with an average of 0.371.

The filtered markers were selected for population structure analysis (Fig. 1), and it was found that the corresponding ΔK value was the maximum, and then ΔK decreased rapidly and tended to be gentle when K was 2. Therefore, the 100 sh_2 sweet corn samples could be divided into four subgroups, in which Subgroup 1 contained 12 germplasms, Subgroup 2 contained 29 germplasms, Subgroup 3 contained 31 germplasms, and Subgroup 4 contained 28 germplasms.

Seed reserve utilization phenotypes: The GR, SH, RL, WMSR, and SRUE of the 100 sh_2 sweet corn seeds were significantly different (Table 2). With increased salt stress concentration, GR, SH, RL, WMSR, and SRUE trended downward. When the salt concentration was 60 mmol/L, GR varied from 5% to 59%, the mean was 29%, and the standard deviation was 0.15. The SH ranged from 5.52 to 17.6 cm, and the mean was 11.02 cm. The RL ranged from 5.52 to 17.6 cm, the mean was 11.02 cm, and the standard deviation was 3.13. While the WMSR ranged from 0.01 to 0.08 g/g, and SRUE varied from 0.21 to 0.50 g/g. Fig. 2 showed that germination traits were normally distributed and were representative to a certain extent, which met the requirements of genome-wide association analysis.

Table 1. PIC values and gene diversity in 10 chromosome of the sweet corn.

Chromosome	SNP No.	Length (bp)	SNP density (SNP/Mb)	PIC	Gene diversity
01	4930	23267756	16.44	0.194	0.376
02	1795	24793637	7.65	0.195	0.361
03	4702	29767390	20.44	0.194	0.369
04	4749	19705878	19.24	0.194	0.374
05	4394	23067498	20.27	0.194	0.372
06	3446	24396286	20.36	0.195	0.368
07	3400	24006421	19.95	0.194	0.372
08	3676	18961841	21.08	0.194	0.377
09	3102	33865240	20.38	0.194	0.373
10	3103	17398127	20.75	0.195	0.373
Total	37297	645950889	186.57	0.194	0.371

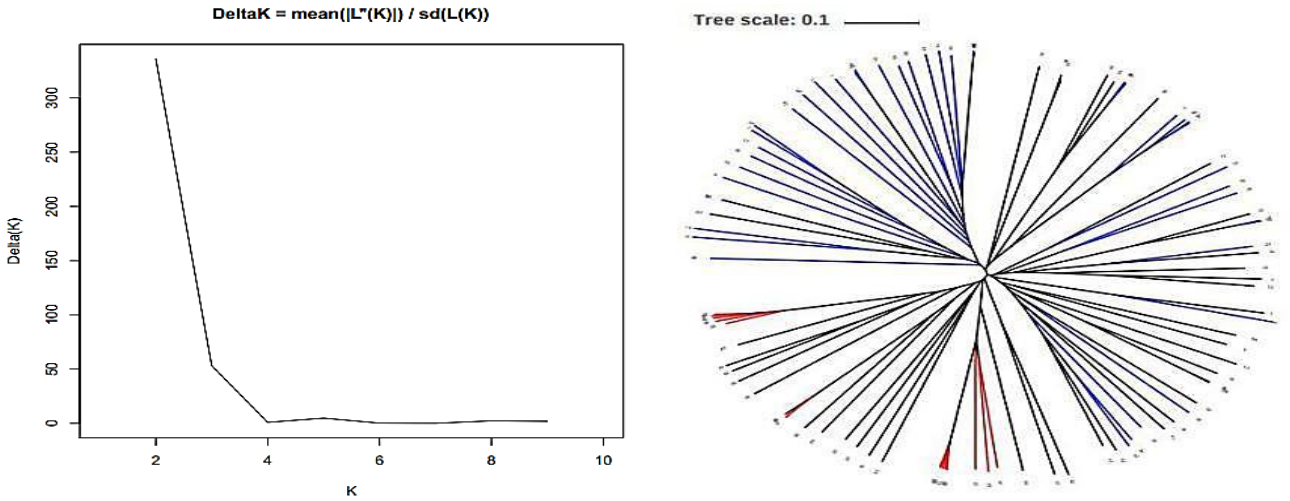

Fig. 1. Plot of posterior probabilities (y axis) to two subgroups on each cultivar (x axis) using STRUCTURE software.

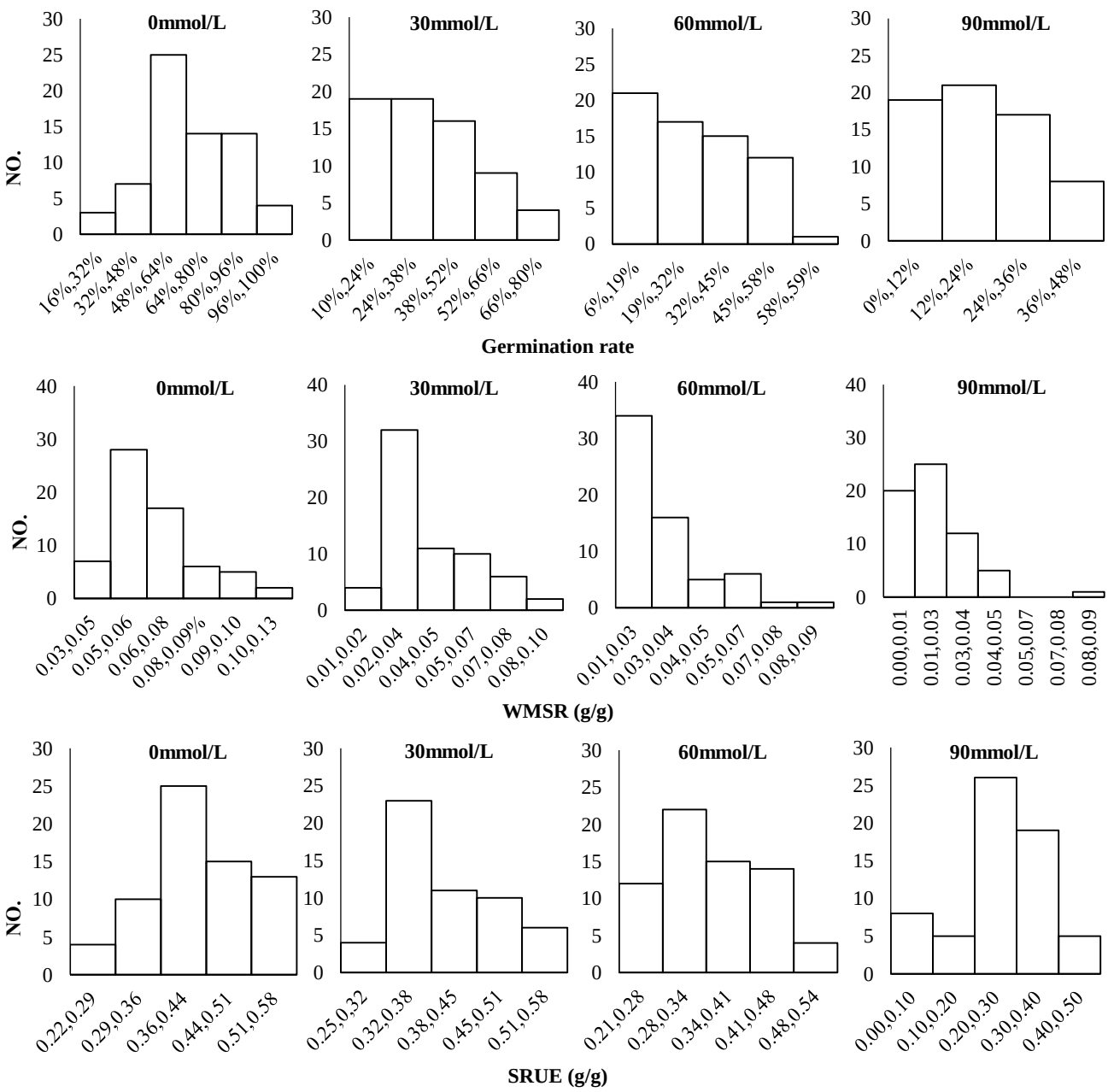

Fig. 2. Frequency distribution of GR、WMSR and SRUE of Sweet Corn Inbred Lines.

Table 2. Phenotypic values of seed reserve utilization among population at different salt solutions.

Traits	GR (%)					Seedling height (cm)					Root length (cm)				
	Mean	Rangeability	SD	Skewness	Kurtosis	Mean	Rangeability	SD	Skewness	Kurtosis	Mean	Rangeability	SD	Skewness	Kurtosis
0 (CK)	66%	16～100%	0.18	-0.09	-0.18	13.39	7.1～23.41	3.41	0.33	-0.36	15.59	6.90～23.60	3.91	-0.09	-0.11
30mmol/L	36%	10～71%	0.15	0.40	-0.78	11.02	5.52～17.60	3.13	0.17	-1.09	10.83	4.90～19.0	3.14	0.49	-0.02
60mmol/L	29%	5～59%	0.14	0.19	-1.16	9.13	3.92～17.20	3.21	0.26	-0.79	9.60	3.14～14.6	2.57	0.06	-0.61
90mmol/L	20%	2～46%	0.13	0.09	-1.04	4.08	0.00～9.50	2.50	0.1	-0.6	3.78	0.00～9.40	2.65	0.37	-0.88

Table 2. (Cont'd.).

Traits	WMSR (g/g)					SRUE (g/g)				
	Mean	Rangeability	SD	Skewness	Kurtosis	Mean	Rangeability	SD	Skewness	Kurtosis
0 (CK)	0.06	0.03～0.12	0.02	0.83	0.28	0.42	0.22～0.57	0.08	-0.27	-0.44
30mmol/L	0.04	0.02～0.09	0.02	0.76	0.48	0.39	0.25～0.54	0.08	0.21	-0.97
60mmol/L	0.03	0.01～0.08	0.01	1.31	1.01	0.35	0.21～0.50	0.08	0.23	-0.94
90mmol/L	0.02	0.00～0.08	0.01	0.97	2.48	0.26	0.00～0.45	0.12	-0.92	0.51

Table 3 Correlation analysis of SRUE in germination of 100 sweet corn inbred lines in salt stress.

Correlation	0					30mmol/L				
	GR	Seedling height	Root length	WMSR	SRUE	GR	Seedling height	Root length	WMSR	SRUE
GR	1					1				
Seedling height	0.15	1				0.23	1			
Root length	0.40**	0.57**	1			0.35**	0.56**	1		
WMSR	0.33**	0.28*	0.41**	1		0.13	0.29*	0.26*	1	
SRUE	0.55**	0.22	0.37**	0.02	1	0.45**	0.34**	0.38**	-0.11	1

Table 3. (Cont'd.).

Correlation	60mmol/L					90mmol/L				
	GR	Seedling height	Root length	WMSR	SRUE	GR	Seedling height	Root length	WMSR	SRUE
GR	1					1				
Seedling height	0.19	1				0.45**	1			
Root length	0.15**	0.65**	1			0.41**	0.71**	1		
WMSR	0.11	0.24	0.26*	1		0.34**	0.39**	0.45**	1	
SRUE	0.39**	0.24*	0.25*	-0.08	1	0.64**	0.59**	0.56**	0.36**	1

Note: * and ** indicate significant differences at 0.05 and 0.01 levels respectively

Relationship of seed reserve utilization traits: In the seed germination stage, correlation analysis showed that there was a positive correlation between seed GR, SH, RL, WMSR, and SRUE (Table 3). With the increase of salt stress, WMSR was significantly correlated with SH and RL, and the correlation coefficient was 0.26–0.45. SRUE was significantly positively correlated with GR, RL, and WMSR, and the correlation coefficient was 0.34–0.64.

GWAS mapping for salt tolerance: In this study, a total of 46 significant loci were shown to be related to three seed traits, namely GR, WMSR, and SRUE, among which 16 loci were significantly associated with GR. There were 13 significantly associated loci for controlling WMSR and 17 for controlling SRUE.

Seven SNP loci on chromosomes 1, 2, 5 and 9 of sh_2 sweet corn were significantly associated with GR traits under CK at $-\log_{10}P>4.30$, while there were nine SNP loci on chromosomes 1, 2, 5, 6, 7 and 9, and 10 under 90 mmol/L salt stress at $-\log_{10}P>3.25$ (Table 4 and Fig. 3).

Nine SNP loci on chromosomes 1, 6, 7, 8 and 9 of sh_2 sweet corn were detected on WMSR under CK at $-\log_{10}P>4.50$, while there were 4 SNP loci on chromosomes 5 and 9 under salt stress at $-\log_{10}P>4.5$ (Table 5 and Fig. 4).

SRUE is a complex trait that is susceptible to the influence of the seed production environment. In the germination stage, there were nine SNP loci on chromosomes 1, 2, 4, 6, 7 and 10 of sweet corn at $-\log_{10}P$ ＞3.25 under CK, while there were eight SNP loci on chromosomes 1, 3, 6, 7 and 10 of sh_2 sweet corn at $-\log_{10}P$ ＞4.0 under salt stress. Three major loci of Affx-91146, Affx-91055, and Affx-90658 were common, and their contributions were 15.06～19.56%. Their positions were 162789062, 2136131, and 1230444, respectively (Table 6 and Fig. 5).

Table 4. The SNP loci significantly correlated with the GR traits of sweet maize under the GLM-Q method.

Treatments	No.	QTL ID	SNP	Chr	Position	$-\log_{10}P$	R^2(%)
CK	1	qGR-1-1-CK	Affx-90096	1	56673003	2.5866E-6	23.97
	2	qGR-2-1-CK	Affx-11532	2	193636902	2.5907E-5	20.32
	3	qGR-5-1-CK	Affx-90319	5	181111119	2.9754E-5	20.08
	4	qGR-5-2-CK	Affx-90362	5	182949016	6.0972E-5	18.48
	5	qGR-9-1-CK	Affx-91040	9	124304177	4.0037E-6	23.02
	6	qGR-9-2-CK	Affx-90600	9	26434264	1.0513E-5	21.44
	7	qGR-9-3-CK	Affx-90784	9	141460572	4.2904E-5	19.45
Salt stress	1	qGR-1-1-ST	Affx-90473.	1	145740201	4.2418E-4	21.85
	2	qGR-2-1-ST	Affx-90183	2	1763050	4.4769E-4	17.64
	3	qGR-5-1-ST	Affx-90518	5	179626240	2.6401E-4	23.02
	4	qGR-6-1-ST	Affx-91012	6	122404413	2.8197E-4	22.86
	5	qGR-7-1-ST	Affx-91142	7	161993995	3.3575E-4	22.43
	6	qGR-7-2-ST	Affx-90901	7	149736612	5.0388E-4	21.73
	7	qGR-9-1-ST	Aftx-90252	9	122950390	3.3177E-4	18.36
	8	qGR-9-2-ST	Affx-91163	9	123303194	4.0309E-4	17.89
	9	qGR-10-1-ST	Affx-91008	10	141820337	2.8413E-4	20.16

Table 5. The SNP loci significantly correlated with the WMSR traits of sweet maize under the GLM-Q method.

Treatments	No.	QTL ID	SNP	Chr	Position	$-\log_{10}P$	R^2(%)
CK	4	qWMSR-1-1-CK	Affx-90286	1	170917949	7.6337E-6	22.38
	1	qWMSR-6-1-CK	Affx-90961	6	157026970	3.7069E-7	27.27
	2	qWMSR-7-1-CK	Affx-90564	7	14505509	3.4983E-6	23.46
	3	qWMSR-7-2-CK	Affx-90924	7	154582104	8.9078E-6	21.92
	5	qWMSR-8-1-CK	Affx-91341	8	157278269	2.4247E-5	20.43
	6	qWMSR-8-2-CK	Affx-91377	8	164574099	3.1073E-5	19.82
	7	qWMSR-9-1-CK	Affx-90786	9	2419863	2.5457E-5	20.35
	8	qWMSR-9-2-CK	Affx-90994	9	2420471	3.785E-5	19.86
	9	qWMSR-9-3-CK	Affx-90266	9	32373390	6.3115E-5	18.60
Salt stress	1	qWMSR-5-1-ST	Affx-91242	5	3406289	8.1193E-6	31.07
	2	qWMSR-5-2-ST	Affx-90361	5	213256843	2.0221E-5	24.88
	3	qWMSR-9-1-ST	Affx-90780	9	74813183	9.5763E-7	36.99
	4	qWMSR-9-2-ST	Affx-11533	9	748880725	2.6288E-6	30.15

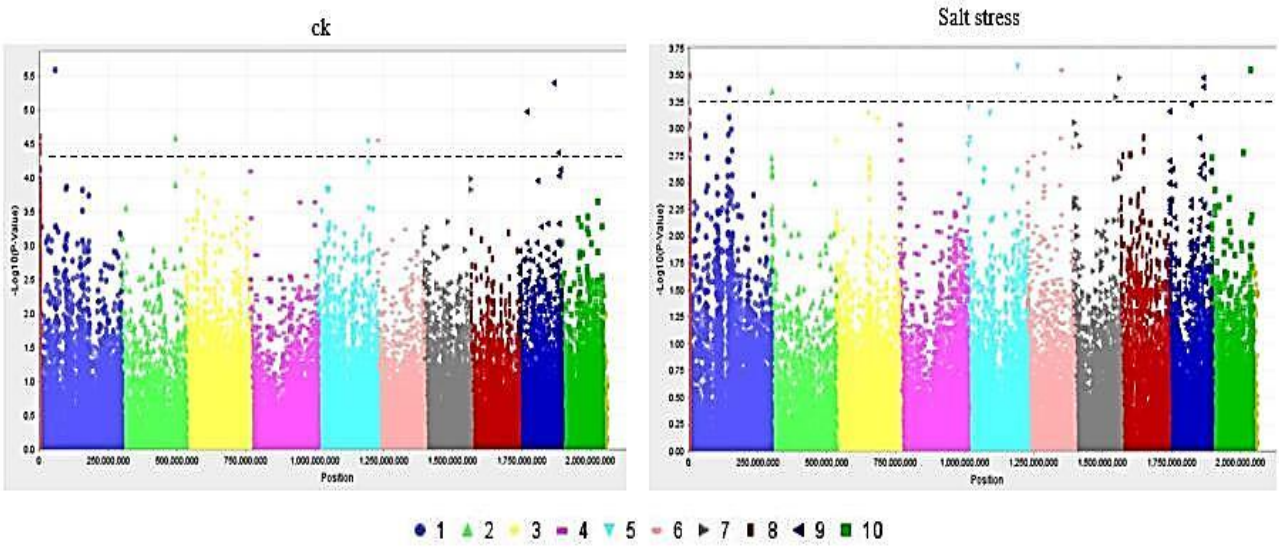

Fig. 3. Manhattan diagram of the GLM-GWAS method on GR.

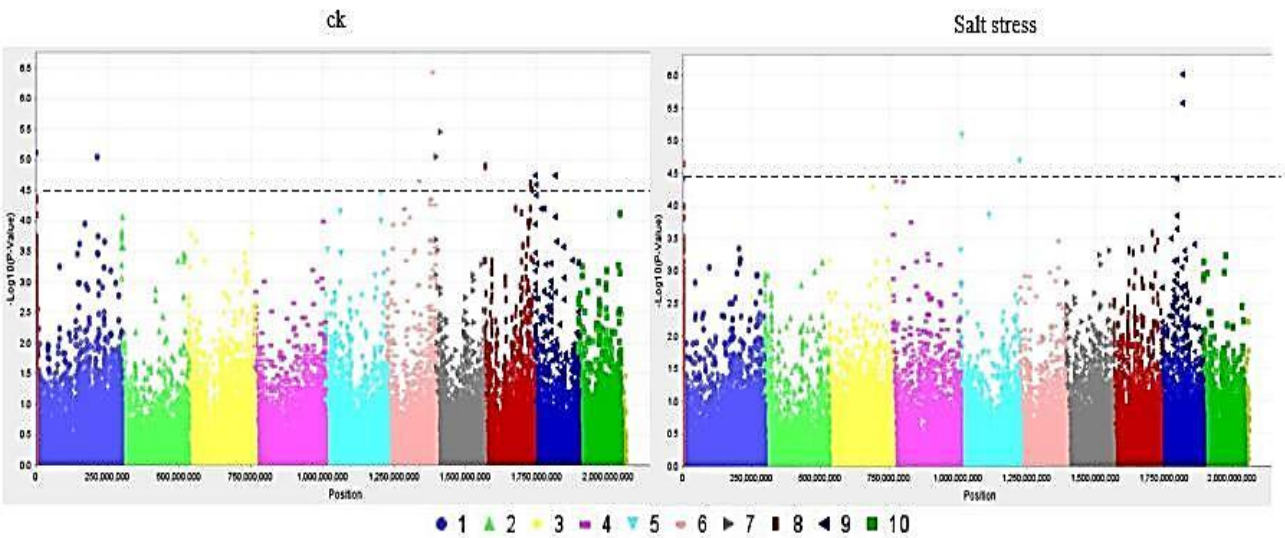

Fig. 4. Manhattan diagram of the GLM-GWAS method on WMSR.

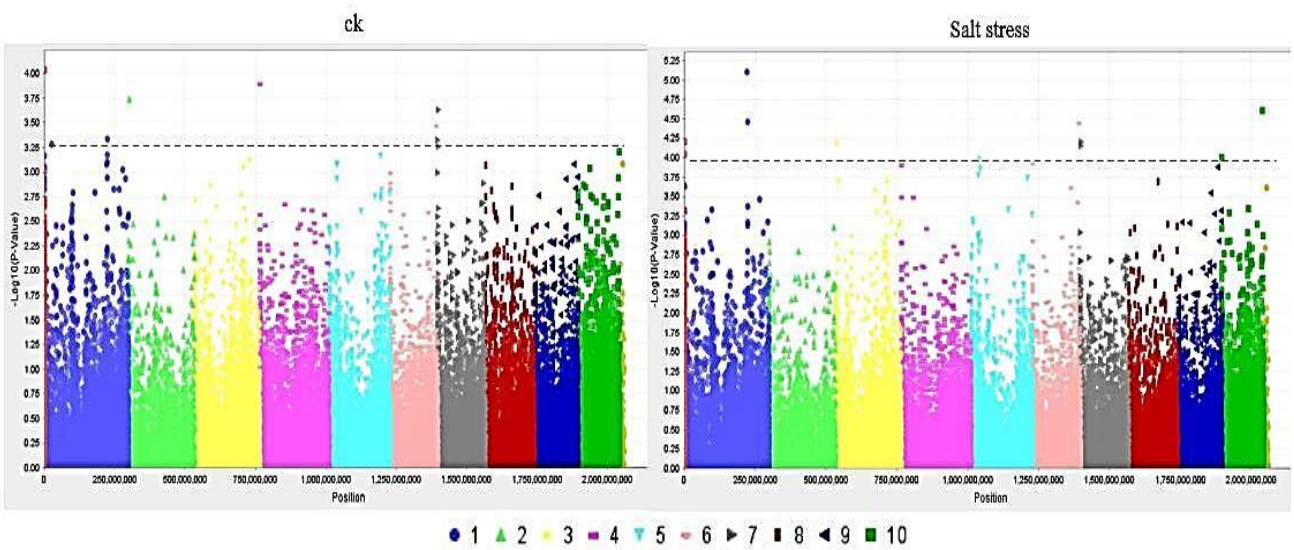

Fig. 5. Manhattan diagram of the GLM-GWAS method on SRUE.

Table 6. The SNP loci significantly correlated with the SRUE traits of sweet maize under the GLM-Q method.

Treatments	No.	QTL ID	SNP	Chr	Position	$-\log_{10}P$	R^2(%)
CK	1	qSRUE-1-1-CK	Affx-91381	1	223466976	4.6232E-4	15.22
	2	qSRUE-1-2-CK	Affx-90581	1	25494471	5.1788E-4	14.87
	3	qSRUE-2-1-CK	Affx-90647	2	1640973	1.8232E-4	16.74
	4	qSRUE-4-1-CK	Affx-91262	4	42099597	1.2954E-4	17.51
	5	qSRUE-6-1-CK	Affx-91146	6	162789062	3.4206E-4	15.62
	6	qSRUE-7-1-CK	Affx-91055	7	2136131	5.4976E-4	15.06
	7	qSRUE-7-2-CK	Affx-90658	7	1230444	2.3442E-4	16.29
	8	qSRUE-7-3-CK	Affx-91142	7	161993995	4.7079E-4	18.93
	9	qSRUE-10-1-CK	Affx-90465	10	146850891	6.2463E-4	14.53
Salt stress	1	qSRUE-1-1-ST	Affx-90453	1	220797171	7.8656E-6	19.61
	2	qSRUE-1-2-ST	Affx-91381	1	223466976	3.4577E-5	19.82
	3	qSRUE-3-1-ST	Affx-91263	3	180730724	1.9925E-4	17.08
	4	qSRUE-6-1-ST	Affx-91146	6	162789062	3.6108E-5	19.56
	5	qSRUE-7-1-ST	Affx-91055	7	2136131	6.2723E-5	18.97
	6	qSRUE-7-2-ST	Affx-90658	7	1230444	6.9637E-5	18.43
	7	qSRUE-10-1-ST	Affx-91075	10	143964640	2.4968E-5	20.78
	8	qSRUE-10-2-ST	Affx-90444	10	94804176	4.5396E-4	18.55

Discussion

Field-grown crops are subject to a variety of stresses during their life cycle, and salt stress negatively impacts their growth. Sweet corn is considered very sensitive to salinity. Previous studies have shown salinity stress causes rapid osmotic stress to plant roots resulting in a reduction in leaf and shoot growth. In this study, we have found that GR, SH, RL, WMSR, and SRUE of 100 sh_2 sweet corn seeds were significantly different and showed a downward trend with increasing salt stress concentration.

Salt tolerance is a genetically and physiologically complex trait. Molecular evidence indicates that genes related to reserve mobilization and endosperm weakening could possibly affect seed germination (Choe *et al*., 2021; Horvath *et al*., 2015; Li *et al*., 2000). In this study, we already screened 37297 SNP markers to identify valuable QTLs associated with GR, SH, RL, WMSR, and SRUE by GWAS. The density of SNP on each chromosome was 7.65–21.08 per Mb; the polymorphic information content was 0.194–0.195, and the variation amplitude of gene diversity on different chromosomes was 0.361–0.377, with an average of 0.371. Therefore, the 100 sweet corn samples could be divided into four subgroups when K was 2.

The GWAS approach has been used to discover genes controlling both polygenic and monogenic traits. Until now, some scholars have extensively studied the GI, vigor index (VI), GR, mean germination time (MGT), and imbibition rate (IR) using high-density genome-wide SNPs. A previous study detected only 16 QTLs associated with seed germination traits under salt stress and control conditions in a recombinant, inbred rice population (Wang *et al*., 2012). It found that the strongest association region for the SSI of VI on chromosome 2 harboring two nitrate transporter family genes (OsNRT2.1 and OsNRT2.2), which affect gene expression under salt stress in rice (Shi *et al*., 2017). Nine salinity tolerance-related traits in leaves and 24 QTLs were measured by using GWAS analysis (Le *et al*., 2021). Protein folding and photosynthetic processes were revealed to be common response mechanisms for DMC21-84 and GSS2259P hybrids in sweet corn (Choe *et al*., 2021). In this study, we succeeded in identifying 49 significant SNPs assigned to 46 QTLs, and a total of 28 QTLs were identified for all salt-related traits in hydroponic systems. The three major loci of Affx-91146, Affx-91055, and Affx-90658 were common, and their contributions were 15.06～19.56% (Fig. 6). Their positions were 162789062, 2136131, and 1230444, respectively. The position of Affx-91146 is consistent with qSRUE6 (Cheng *et al*., 2016). In this region, we have detected a total of 96 candidate genes and future research will aim to find the specific genes.

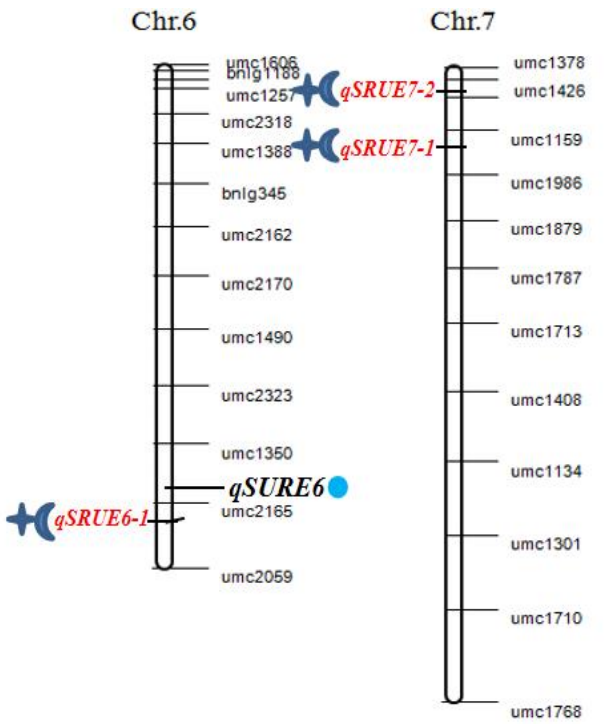

Fig. 6. Three major loci of the SRUE under CK and salt stress conditions in sweet corn. The mapped QTLs in this study are shown on the left of the bar and previously mapped QTLs shown on the right of the bar.

Conclusion

This study analyzed the structure of 100 sh_2 sweet corn cultivars using GWAS. The density of SNPs on each chromosome was 7.65–21.08 per Mb, and the 100 sweet corn samples could be divided into four subgroups when K was 2. The three major loci are Affx-91146, Affx-91055 and Affx-90658, and their contributions were 15.06～19.56% under salt stress.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant No. 31440066; 31101598), the Fundamental Research Funds in Anhui Province (Grant No. KJ2019ZD57), and the Stable Fund for the Anhui Science and Technology University.

References

An, H.Z., K. Liu, B.X.Wang, Y.L. Tian, Y.W. Ge, Y.Y. Zhang, W.J. Tang, G.M. Chen, J. Yu, W. Wu, X.L. Liu, Y.Q. Zhao, J. Li, H.Z. Zhang, B. Yang, X.Z. Jiang, C. Peng, C. Zhou, C.S. Zhu, C.M. Wang and J.M. Wan. 2020. Genome-wide association study identifies QTLs conferring salt tolerance in rice. *Plant Breed*., 139:73-82. https://doi.org/10.1111/pbr.12750

Ali, S., R.K. Gautam, R. Mahajan, S.L. Krishnamurthy, S.K. Sharma, R.K. Singh and A.M. Ismail. 2013. Stress indices and selectable traits in SALTOL QTL introgressed rice genotypes for reproductive stage tolerance to sodicity and salinity stresses. *Field Crops Res.*, 154: 65-73.

Brijesh, M.H., Firoz, M. Vignesh, B. Aanchal, Z. Rajkumar, K. Shailendra and S. Hari. 2017. Microsatellite-based genetic diversity analyses of sugary1-, shrunken2- and double mutant- sweet corn inbreds for their utilization in breeding programme. *Physiol. Mol. Biol. Plants*, 23(2): 411-420.

Chen, C., G.J. Norton and A.H. Price. 2020. Genome-wide association mapping for salt tolerance of rice seedlings grown in hydroponic and soil systems using the Bengal and Assam Aus Panel. *Front. Plant Sci.*, 11: 1-18. https://doi:10.3389/fpls.2020.576479

Cheng, X.X., J.P. Cheng, X. Huang, Y.Y. Lai, L. Wang, W.L. Du, Z.F. Wang and H.S. Zhang. 2013. Dynamic quantitative trait loci analysis of seed reserve utilisation during three germination stages in rice. *PLoS One*, 8.11, e80002.

Cheng, X.X., S. He and G.H. Geng. 2016. Dynamic QTL analysis of seed reserve utilization in sh2 sweet corngermination stages. *Gen. Mol. Res.*, 3:1-10.

Choe, E., Y. Ko and M.M. Williams. 2021. Transcriptional analysis of sweet corn hybrids in response to crowding stress. *PLoS One*, 16.6: e0253190. doi: 10.1371/journal.pone.0253190.

Cui, H., Z.S. Cheng, P. Li and A.M. Miao. 2020. Prediction of sweet corn seed germination based on hyperspectral image technology and multivariate data regression. *Sensors*, 20(17): 4744. doi:10.3390/s20174744

Horvath, D., S. Hansen, J. Moriles-Miller, R. Pierik, C. Yan, D. Clay, B. Scheffler and S. Clay. 2015. RNA-seq reveals weed induced PIF3-like as a candidate target to manipulate weed stress response in soybean. *New Phytol.*, 207: 196-210.

Huang, Z.B., J.F. Ying, L.L. Peng, S. Sun, C.W. Huang, C. Li, Z.F. Wang, Y.Q. He. 2021. A genome-wide association study reveals that the cytochrome b5 involved in seed reserve mobilization during seed germination in rice. *Theor. Appl. Genet.* 134(12):4067-4076. doi: 10.1007/s00122-021-03948-2

Jin, T., Y.Y. Sun, R.R. Zhao Z. Shan, J.Y. Gai and Y. Li. 2019. Over-expression of Per-oxidase gene GsPRX9 confers salt tolerance in soybean. *Int. J. Mol. Sci.*, 20(15): 3745.

Le, T.D., F. Gathignol, H.T. Vu, K.L. Nguyen, L.H. Tran, H. Vu, T.X. Dinh, F. Lazennec, X.H. Pham, A. Véry, P. Gantet, and G.T. Hoang. 2021. Genome-Wide association mapping of salinity tolerance at the seedling stage in a panel of vietnamese landraces reveals new valuable QTLs for salinity stress tolerance breeding in rice. *Plants*, 10(6): 1088.

Li, P.S., W.L. Kong and X.Q. Wu. 2021. Salt tolerance mechanism of the rhizosphere bacterium JZ-GX1 and its effects on tomato seed germination and seedling growth. *Front. Microbiol*., 12: 657238. doi: 10.3389/fmicb.2021.657238.

Li, X., O. Bjo, E. Rkman, C. Shih, A. Grossman, M. Rosenquist, S. Jansson and K. Niyogi. 2000. A pigment-binding protein essential for regulation of photosynthetic light harvesting. *Nature*, 403: 391-5.

Luo, M.J., Y.X. Zhang, J.N. Li, P.P. Zhang, K. Chen, W. Song, X.Q. Wang, J.X. Yang, X.D. Lu, B. S. Lu, Y. Zhao and J.R. Zhao. 2021. Molecular dissection of maize seedling salt tolerance using a genome-wide association analysis method. *Plant Biotech.*, 10: 1937-1951.doi: 10.1111/pbi.13607.

Ma, J.J., W.F. Pei, Q.F. Ma, Y.H. Geng, G.Y. Liu, J. Liu, Y.P. Cui, X.Q. Zhang, M. Wu, X.L. Li, D. Li, X.S. Zang, J.K. Song, S.R. Tang, J.F. Zhang, S.X. Yu, and J.W. Yu. 2019. QTL analysis and candidate gene identification for plant height in cotton based on an interspecific backcross inbred line population of *Gossypium hirsutum* × *Gossypium barbadense*. *Theor. Appl. Genet*., 132(9): 2663-2676.

Maulana, F., H. Ayalew, J. Anderson, T. Kumssa, W. Huang and X. Ma. 2018. Genome-wide association mapping of seedling heat tolerance in winter wheat. Front. *Plant Sci*., 9: 1272.

Shi, Y., Y. Gao, Z. Wu, X. Zhang, M. Wang, C. Zhang, Y. Zhou and Z. Li. 2017. Genome-wide association study of salt tolerance at the seed germination stage in rice. *BMC Plant Biol.*, 17: 92.

Wang, S., D. Wong, K. Forrest, A. Allen, S. Chao, B. Huang, M. Maccaferri, S. Salvi, S. Milner, L. Cattivelli, A. Mastrangelo, A. Whan, S. Stephen, G. Barker, R. Wieseke, J. Plieske, M. Lillemo, D. Mather, R. Apples, R. Dolferus, G. Guedira, A. Korol, A. Akhunova, C. Feuilet, J. Salse, M. Morgante, C. Pozniak, M. Luo, J. Dvorak, M. Morell, J. Dubcovsky, M. Ganal, R. Tuberosa, C. Lawley, I. Mikoulitch, C. Cavanagh, K. Edwards, M. Hayden, and E. Akhunov. 2014. Characterization of polyploid wheat genomic diversity using a high-density 90000 single nucleotide polymorphism array. *Plant Biotech. J*., 12: 787-96.

Wang, Z., J. Cheng, Z. Chen, J. Huang, Y. Bao, J. Wang and H. Zhang. 2012. Identification of QTLs with main, epistatic and QTL × environment interaction effects for salt tolerance in rice seedlings under different salinity conditions. *Theor. Appl. Genet*., 125: 807-815.

Zeng, L., M. Shannon and S. Lesch. 2001. Timing of salinity stress affects rice growth and yield components. *Agric Water Manag.*, 48(3):191-206.

(Received for publication 17 April 2022)