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Identification of Salinity Tolerant Gene in Six Wheat Genotypes (*Triticum aestivum*) Via SSR and Sequencing Technique

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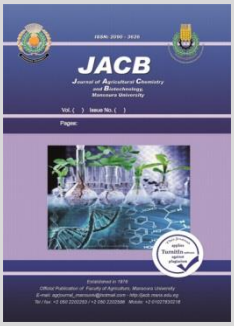
Cross Mark



ABSTRACT

Six wheat genotypes which included two lines named by Line-1 and Line-2 in addition to four cultivars (Masr-1, Shandaweel-1, Giza-171 and Sakha-94)) were used in this study.To evaluate the salt tolerant genes in the studied wheat genotypes, 13 SSR markers were applied. Three (10%) were monomorphic, 26 (86%) were polymorphic, and one (3.34%) produced no results. A total of 37 alleles were detected using 30 polymorphic markers. The polymorphic information content (PIC), which found in a range of 0.00 to 0.38 on average, was applied to assess each SSR locus's capacity to differentiate between different wheat cultivars. The significant genetic heterogeneity of wheat genotypes was revealed by cluster analysis. Using salinity primers, amplification of antiporter gene, and sequencing, the six genotypes were specified. Applying the BLAST bioinformatics programe, the sequences were compared with those from the NCBI online database. According to the results, the base size of the salinity-tolerant gene is 200 bp, compared to 600 bp for the antiporter gene(OM200013). The phylogenetic analysis showed that the sequence of the antiporter gene was nearly typical to four gene bank accession numbers. The antiporter gene-based phylogeny tree grouping four clades, and phylogenetic analysis revealed 0.01 of genetic deviation at the root of each clade. As the bootstrap value between 6 and 8 %, the support for the clades grouping was low. Moreover, as can be shown from the phylogenetic tree, the detected salinity-tolerant gene sequences were aligned with four different NCBI website accession codes.

Keywords: *Triticum aestivum*, antiporter gene, salinity tolerant gene, bioinformatics analysis, SSR.

INTRODUCTION

Increasing bread wheat's salinity tolerance is important as it is a main food crop in the most of nations having saline soils (Tuna *et al.*, 2008). Salinity and drought remain an important concerns for the plant breeders. After two to three years, it is expected that 1.8 billion of people would pass through water deficit, and 65% of people will live in regions suffered from salinity and water deficiency. The crop performance can be influenced by many factors when it needs to be salt tolerance and or to tolerate water stress. Breeders of a large number of species are today applying many SSR markers (Song *et al.*, 2010). as a result of improvements in breeding methods for genotyping and DNA sequencing, the data of genome sets have been expected, which move to the development of SNP markers and sequence-based simple sequence repeats (SSRs), which have recently hurried the development of MAS for wheat (Filiz *et al*., 2009). SSRs have been the most widely used markers for plant breeding for genotyping during the last four decades, this is related to their traits as co-dominant, informative, replicable multi-allele genetic markers, and convertible among linked species, (Mason, 2015). SSRs found to be especially beneficial for integrating physical, genetic, and sequence-based maps (Temnykh *et al*., 2001) and for giving a tool to correlate diversity (genotypic and phenotypic diversity) for scientists of agriculture especially genetics and plant breeding when linkage maps are created applying full-sib families (Grohme *et al*., 2013, Pereira *et al*., 2013). The rice plant, *Oryza sativa*, has had numerous salt tolerant gene and water deficient gene cDNAs cloned and characterized in recent years (Dubouzet *et al*., 2003; Tian *et al*., 2005). As a result, it was employed the RACE technique to detect the cDNA sequences for these genes. Then, applying cDNA pools which formed from start codons and stop codons, then these coding areas will amplified and cloned. All four homologous pairs, including the DNA-binding domain, showed strong homology with subtype 1 DREB2s of Arabidopsis. They all shared the conserved motif CMIV-3, although the GmDREB2A homologs were the most similar to DREB2A (Nakano *et al*., 2006). It was found that the major frequent ions are Na and H, which have key functions in cell physiology. Both are important for the bioenergetics of the cell, and the concentration of protons inside the cell is important for the suitable operation of the cell and its proteins. In fact, these ions represent powerful stressors for all cells when their concentrations are either too high or too low (Padan *et al*., 2000). therefore, there is a very obvious homeostatic mechanism for these ions in each cell. The Na./H. antiporters are proteins that are important for this homeostatic mechanism. These proteins are found in the membranes, and they convert Na (or Li) to H. According to Stockinger *et al*. (1997), DREBs have a conserved apetala2/ethylene response factor (AP2/ERF) domain that is around 60 amino acids long. The six minor groups A-1 to A-6 consisted of the large multigene family which is named as the DREB genes (Sakuma *et al*., 2002). Utilizing a collection of six wheat genotypes, consisted of four Egyptian cultivars and two lines kindly provided by the Agriculture Research

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Centre, field crops research institute, department of wheat research, Giza. The aim of the current investigation was to assess the wheat germplasm's tolerance or resistance to saline conditions. The salt responsive SSR markers used in this study were generated in 2019 and were unvalidated at the time. The study suggests confirming the associations between markers and phenotypic features. This study's objectives is to cluster analysis, similarity between the six genotypes and phylogenetic tree beside to do functional genomic analysis to decide whether local cultivars of bread wheat (*Triticum aestivum* l.) are suitable for breeding or direct cultivation in the area suffered from salinity.

MATERIALS AND METHODS

Genetic materials

The six local genotypes of wheat used in this study were kindly provided by the Ministry of Agriculture, Cairo, Egypt's Wheat Research Department, Field Crops Research Institute, Agricultural Research Centre (ARC), (Table 1).

Table 1. The names and pedigree of the six wheat genotypes used in the study.

Genotype name	Pedigree and selection history	Origin
Line1	ATTILA50Y//ATTILA/BCN/3/STAR*3/MUSK-3. AISBW05-0043-9AP-0AP-0AP-9AP-0AP-0SD	Egypt
Line 2	SAKHA94/MISR1 GZ2008-06DH2	Egypt
Misr 1	OASIS/SKAUZ//4*BCN/3/2*PASTOR CMss00Y01881T-050M-030Y-030M-030WGY-33M-0Y-0S	Egypt
Shandweel 1	Site/Mo/4/Nac/Th.Ac//3*Pvn/3/mirlo/Buc CMSS93 B00S 67S-72Y-010M-010Y-010M-3Y-0M-0THY-0SH	Egypt
GIZA-171	SAKHA93/GEMMEIZA9 AND ITS SELECTION history is "GZ 2003-101-1GZ- IGZ-2GZ-OGZ"	Egypt
Sakha94	OPATA/RAYON//KAUZ.CMBW 90Y3180 –OTOPM-3Y-010M-010M-010Y-10M-015Y-OY-OAP-0S.	Egypt

DNA extraction and PCR amplification

DNA was extracted following (Plaschke et al. 1995) with some modifications. Total genomic DNA was extracted from tissues of all the studied genotype plants according to the manufactures of QIAGEN DNeasy Plant DNA extraction Mini Kit. The primers were tested by *in silico* PCR tool (http://insilico.ehu.eus/PCR/). The expected PCR amplicons resulted from the used SSR primers as well as primers name and sequence are listed in Table 2. PCR reaction was performed in a 25 µl mixture containing 0.2 µM of each primer with concentration of 10 pmol, 400 µM of dNTPs mix, 2.5 µl of 10x PCR reaction buffer, 2 µM MgCl2, 1.25 units of TAKARA Taq DNA polymerase, 1 µl of template DNA and the final volume was adjusted with sterilized doubled distilled water (d.dH2O). A PCR thermocycler was used to amplify the reactions consisting of 94 °C for 5 min followed by 35 cycles at 94 °C for 30 sec, 56°C as annealing temperature for 1 min with an extension of 72^0 C for 1 min followed by final extension temperature at 72°C for 10 min. Amplified PCR products were stored at -20°C for further purification and downstream application. About 5 µl of PCR amplified product was loaded on 0.9% agarose gel electrophoresis stained with Ethidium bromide, then visualized under UV Transilluminator (Bio RAD).

Table 2. Name of SSR primers employed in this study with the forward and reverse sequences, expected size.

SSR marker	F	R	Expected size
OM200013	CTACCTATTCTTCACCAGCAC	AGCAGCATTGACAGCATATAC	580 bp
Gwm484-2D	AGTTCCGGTCATGGCTAGG	ACATCGCTCTTCACAAACCC	150
Xwmc273-7D	AGTTATGTATTCTCTCGAGCCTG	GGTAACCACTAGAGTATGTCCTT	179
Xwmc407-2A	CATATTTCCAAATCCCCAACTC	GGTAATTCTAGGCTGACATATGCTC	120-135
Gwm11-1B	GTGAATTGTGTCTTGTATGCTTCC	GGATAGTCAGACAATTCTTGTG	196
Xbarc121-7A	ACTGATCAGCAATGTCAACTGAA	CCGGTGTCTTTCTAACGCTATG	170-197
Xgwm294-2A	GGATTGGAGTTAAGAGAGAACCG	GCAGAGTGATCAATGCCAGA	112
Xcfa2129-1A	ATCGCTCACTCACTATCGGG	GTTGCACGACCTACAAAGCA	100
Wms136	GACAGCACCTTGCCCTTTG	CATCGGAACATGCTCATC	296
Xgwm637-4A	CGGTAGTTTTTAGCAAAGAG	CCTTACAGTTCTTGGCAGAA	137-167
Xgwm160	TTCAATTCAGTCTTGGCTTGG	CTGCAGGAAAAAAAGTACACCC	165-205
Xgwm219-B	GATGAGCGACACCTAGCCTC	GGGGTCCGAGTCCACAAC	194
Xgwm369-3A	CTGCAGGCCATGATGATG	ACCGTGGGTGTTGTGAGC	160-320
XWMC313-4A	GCAGTCTAATTATCTGCTGGCG	GGGTCCTTGTCTACTCATGTCT	197

Cloning and Sequencing

The resulted DNA amplicons were eluted from agarose gel and purified using QIAquick Gel Extraction Kit. The purified PCR fragments were ligated into pGEMR-T Easy Vector Systems according to its manufacturer. The competent cells of E. coli top 10 strain were prepared and transformed as described by (Inoue et al., 1990). From LB/Amp/Xgal plates, white colonies were selected and inoculated on LB/Amp broth media. Then it was incubated overnight at 33 oC with shaking for stabilizing the plasmid inside the transformed cells. The alkaline method of Birnboim and Doly (Bimboim and Doly, 1979) was used to isolate the plasmid. To confirm the recombinant plasmids the purified plasmids were examined by electrophoresis on 1.5% agarose gel. Macrogen Company (South Korea) sequenced the obtained recombinant plasmids. The obtained sequence for ……..genes were examined for vector contamination using the VecScreen tool (http:// www.ncbi.nlm.nih.gov/tools/vecscreen). Construction of the phylogenetic tree was done using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) and MEGA7 software (Kumar, et al., 2016). The obtained sequences were deposited at NCBI database (http://www.ncbi.nlm.nih.gov) under accession numbers……..., respectively.

Sequencing methodology and Data analysis:

Data scoring and analysis

The gels were scored as presence or absence alleles or bands. Because of the studied genotypes of bread wheat was described as hexaploid species. So, the SSR markers can amplify more than two alleles per locus, where it is able to amplify alleles from the origins genome. Hence, these alleles in the studied genotypes of *Triticum aestivum* can be used to

determine the origin of these genotypes depending on the size of alleles in the six studied hexaploid species. The analyses were repeated at least twice to assure the reproducibility of the products. The PCR products of the microsatellite markers were detected by agarose gel (1.5%) to separate PCR products with different sizes, and then stained with ethidium bromide solution and visualized under gel documentation, for gel analysis. Recorded data as a presence/absence (1/0) were used to calculate the similarity degree according to Dice coefficient (Sneath and Sokal 1973), using the SPSS software ver. 16. The dendrogram was produced by clustering the data with the average Linkage (Between Groups) utilizing all detected alleles to estimate the genetic relationship and similarity among all the studied genotypes.

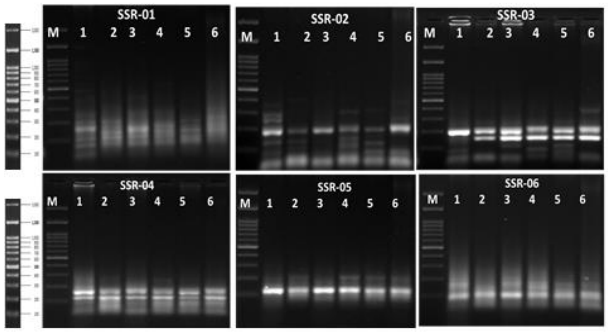
RESULTS AND DISCUSSION

13 SSR markers were used in this experiment, and there were an average of 4.769 alleles per marker, ranging from one to nine. The results of Almanza-Pinzon et al. (2003), who stated that two to four alleles per marker using 37 SSR markers in 70 wheat genotypes, were supported by the data. Two to six alleles per marker and an average of 2.9 alleles per SSR locus were reported by Sing et al. (2006). PIC varied from 0.00 to 100.00, with an average of 0.5439 (Table 6).

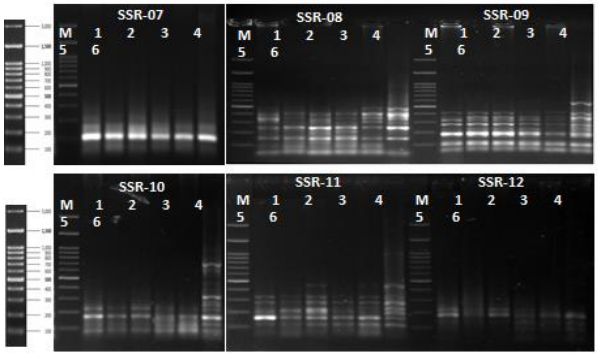
Table 5 shows that the similarity matrix has a range of 0.43 to 0.77 and an average of 0.41. This demonstrated that wheat genotypes exhibit high levels of variation. Sing et al. (2006) reported 0.47 average PIC value in 21 SSR markers, compared to 0.45 reported by Almanza-Pinzon et al. (2003) with 37 SSR primers. It is evident from the dendrogram in Figure 2 that some genotypes from the same wheat region were clustered together, while others displayed greater divergence. One explanation for this could be that, unlike Line-1 and Line-2, the genetic diversity of wheat genotypes was not entirely correlated with geographic dispersion. According to Huang et al. (2002), this may be because identical genetic variation occurred independently in various geographic locations.

The results of the hydroponic evaluation at the vegetative stage were confirmed by the SSR markers utilized in the current study, which likewise demonstrated significant genetic variation in wheat genotypes. Indicating that modern plant breeding has significantly eroded the genetic makeup of the current wheat varieties, the level of polymorphism identified in the landraces was considerably higher than in the cultivars. The landraces are a good source of undiscovered genes, which can be used to increase the genetic diversity of wheat cultivars. The fact that the salt-tolerant genotypes grouped together shows how effectively SSR markers may distinguish between salt-tolerant and susceptible genotypes. In comparison to the SSR markers from the B and D genomes, those from the A genome displayed more polymorphism (Table 5). This was most likely caused by the fact that genotypes were chosen for the current study based on salt tolerance, which is predominantly regulated by genes from the A genome. Nine SSR markers were found to be linked with genotypes' ability to tolerate salt. These markers can be utilized in salt tolerance marker-assisted selection. Therefore, the salt tolerance of wheat genotypes can be assessed using these SSR markers.

According to SSR markers, the acquired data demonstrated that the tested wheat genotypes had significant genetic variation. Landraces showed significantly higher genetic variation than cultivars, demonstrating that plant breeding has reduced genetic variation in cultivars of commercial wheat. Genotypes that are typically salt tolerant grouped together, showing greater genetic similarity and less genetic diversity. This suggested that new cultivars are less genetically related to landraces than previously thought. The genetic makeup of the salt-tolerant cultivar 'Masr-1' from Egypt was discovered to be unique from all other commercial cultivars examined (Figures. 1, 2, and 3). According to Roder et al. (2002), varieties with the same paternal line gave identical allele patterns. Our findings demonstrated that Masr-1 differed genetically from other commercial cultivars.



1: Masr-1 2: Shandaweel-1 3: Line-1 4: Line-2 5: Giza-171 6: Sakha-94.



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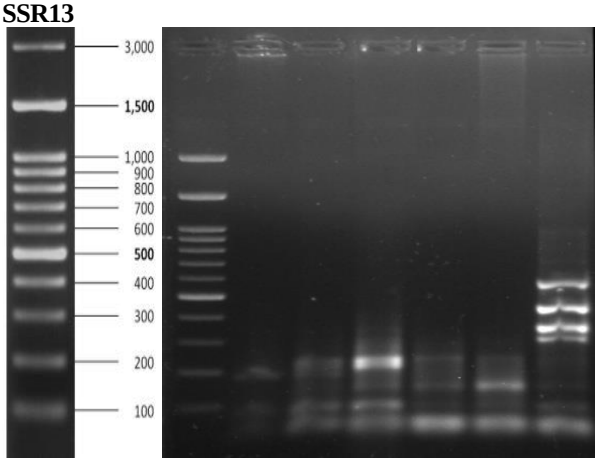


Figure 1. 13 SSR primers employed for detection of salinity tolerance in 6 wheat genotypes,

1: Masr-1 2: Shandaweel-1 3: Line-1 4: Line-2 5: Giza-171 6: Sakha-94.

For the past decade years, SSRs have been widely utilized to test the genetic variety (Almanza-Pinzon et al., 2003; Sing et al., 2006). The employment of SSR markers for research on salt tolerance, however, is restricted. In the experiment, 6 wheat genotypes chosen based on salt tolerance indices had 101 polymorphic alleles amplified by 13 SSR markers. According to SSR data, genetic similarity ranged from 0.43 to 0.77 (Table 5), which revealed that the 6 wheat genotypes had a substantial amount of genetic diversity. The three salt-tolerant genotypes—Shandaweel-1, Line-2, and Sakha-94—were discovered to be genetically the most dissimilar (52% similarity). The genotype Giza-171 was discovered to differ from the other genotypes the most genetically (Fig. 2). Genetic similarity between cultivars Shandaweel-1 and Line-1 was found to be 77%. PIC values for these markers' results ranged from 0.00 (Gwm11-1B and Xgwm294-2A) to 0.819 (Xgwm369-3A), showing significant genetic variation. Only showing that these markers were related to salinity tolerance in wheat were the eleven SSR markers that amplified particular alleles in salt-tolerant genotypes. The six wheat genotypes' significant genetic diversity was found using SSR markers, with PIC values ranging from 0.141 (Xgwm 219-B) to 0.819 and 0.810 (Xgwm369-3A and wmc 136).

Sakha-94 and Masr-1 had the lowest genetic similarity (43%) and Line-1 and Shandaweel-1 had the highest (77%) (Table 5), showing that there were enough genetic diversity among the six wheat genotypes.

The salt-tolerant and sensitive genotypes were successfully separated into four clusters using cluster analysis (Fig. 2). All of the tolerable genotypes were found in the four sub-clusters, but Sakha-94 remained the sole susceptible genotype. These findings suggested that, with appropriate screening, cluster analysis might be employed to differentiate between salt-tolerant genotypes and vulnerable genotypes.

According to Zhang et al. (2002) and You et al. (2004), about 70 SSR markers with high polymorphism levels are needed to identify genetic connection in wheat genotypes. The wheat genotypes in the current study's SSR markers demonstrated enough genetic variation for salt tolerance, which might be utilized in subsequent breeding operations.

Simple Sequence Repeat (SSR) or Microsatellite Markers

Table 3. 13 SSR primers used for detection of salinity tolerance in 6 wheat genotypes and their products of amplicons.

Genotypes	Masr-1	Shandaweel-1	Line-1	Line-2	Giza171	Sakha-94
Amplicons	32	30	34	32	34	44

Table 4. The list of SSR primers, Total Number of Bands (TB), Monomorphic Bands (MB), Polymorphic Bands (PB), Percentage of Polymorphism (%P) and Frequency (F) as revealed from the experimental results.

Sr Nu	Primer Name	Total bands	Monomorphic bands	Polymorphic bands	% of Polymor bands	Unique bands
1	Gwm484-2D	8	0	6	75.00 %	2
2	Xwmc273-7D	17	6	8	47.00 %	3
3	Xwmc407-2A	10	0	10	100.00 %	0
4	Gwm11-1B	12	12	0	00.00 %	0
5	Xbarc121-7A	9	6	3	33.33 %	0
6	Xgwm294-2A	9	0	6	66.66 %	3
7	Xcfa2129-1A	6	6	0	00.00 %	0
8	Wms136	30	18	8	27.00 %	4
9	Xgwm637-4A	35	6	23	66.00 %	6
10	Xgwm160	18	6	8	44.00 %	4
11	Xgwm219-B	25	6	18	72.00 %	1
12	Xgwm369-3A	6	6	0	00.00 %	0
13	XWMC313-4A	17	0	11	65.00 %	6
Total	Total	202	72	101		29
Mean	Mean	15.	5.538	7.769	40.07 %	2.2307

Six different genotypes of wheat were found to have 101 polymorphism alleles of different sizes from the investigations on wheat salinity tolerance. One to nine alleles were amplified (Table 4,5, 6). There were 4.714 alleles on average detected per locus. From the Table 3, a salt-tolerant Masr-1 amplified no more than (32) amplicons, whereas a salt-vulnerable Sakha-94 amplified (45) amplicons. All the genotypes that were examined, the SSR marker Xgwm637-4A generated a 183 bp and a 241 bp DNA fragment.These genotypes either salt-tolerant or sensitive showed amplified a 59 bp DNA amplicon in a similar manner using Gwm484-2D. Both a 151 bp and a 110 bp DNA segment were produced by Xwmc407-2A in all salt-tolerant and salt-susceptible genotypes. All of the genotypes under study had a 110 bp DNA amplicon generated by Gwn11-1B. Xbarc121-7A produced a single distinct band. All of the genotypes under investigation yielded a 102 bp DNA fragment from Xgwm294-2A. Xcfa2129-4A generated DNA fragments of 241 bp, 151 bp, and 74 bp in each genotype that was examined. An 183 bp DNA fragment was produced by Wms 136 in each genotype that was being studied. A 151 bp DNA fragment from Xgwm160 was produced by every genotype that was looked at. An 183 bp DNA fragment from Xgwm219-1B was produced by each of the investigated genotypes. Sakha-94 was affected by Xgwm369-3A, which produced four different bands. The XwmC313-4A gene produced a 151 bp DNA fragment in each genotype analyzed.

In every genotype under investigation, Gwn11-1B generated a 110 bp DNA amplicon. One distinct band was produced by Xbarc121-7A. In each of the genotypes that were examined, Xgwm294-2A generated a 102 bp DNA fragment. In each of the genotypes that were looked at, Xcfa2129-4A produced a 241 bp, 151 bp, and 74 bp DNA fragment. In each of the genotypes examined, Wms 136 generated an 183 bp DNA fragment. In every genotype under investigation, Xgwm160 generated a 151 bp DNA fragment. In each of the genotypes that were examined, Xgwm219-1B generated an 183 bp DNA fragment. In the Sakha-94 example, Xgwm369-3A generated four distinct bands. In all the genotypes that were investigated, XwmC313-4A generated a 151 bp DNA fragment.

Table 5. Coefficients of similarity based on 13 SSR markers data between pairs of 6 wheat genotypes in the current experiment.

0	Masr -1	Shandaweel -1	Line -1	Line -2	Giza-171	Sakha-94
Masr-1	1	0.61	0.58	0.50	0.65	0.43
Shandaweel-1		1	0.77	0.63	0.71	0.52
Line-1			1	0.57	0.71	0.64
Line-2				1	0.64	0.54
Giza-171					1	0.57
Sakha-94						1

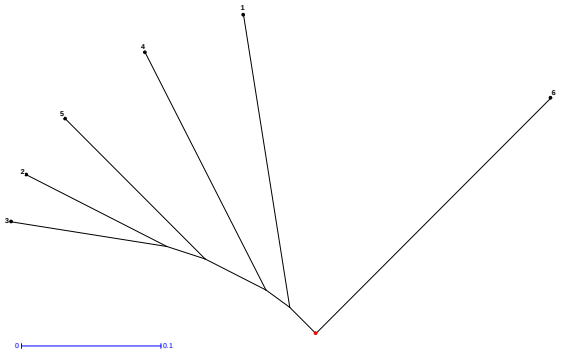


Fig. 2. UPGMA cluster analysis based on Dice Similarity Coefficient showed the genetic relationship created by SSR data between the six wheat genotypes.
1: Masr-1 2: Shandaweel-1= 3: Line-1 4: Line-2 5: Giza-171 6: Sakha-94.

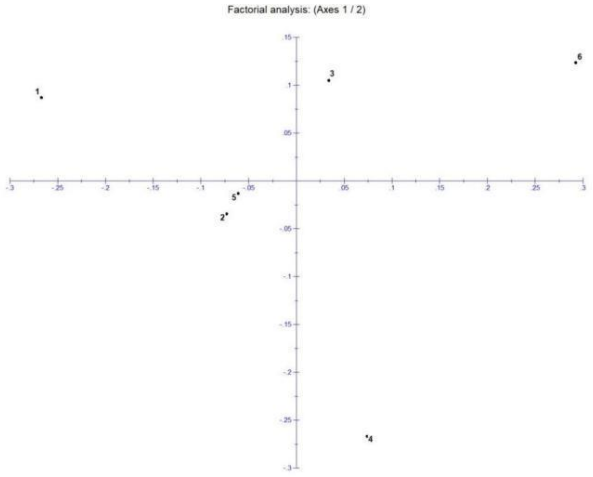


Figure 3. Factorial analysis for the studied wheat genotypes.
1: Masr-1 2: Shandaweel-1 3: Line-1 4: Line-2 5: Giza-171 6: Sakha-94.

Information on genotype diversity and allele frequency is provided by polymorphism information content. For the 13 SSR markers examined in 6 wheat genotypes, PIC varied significantly. With an average of 40.07%, the PIC value ranged from 0.00 (Gwm11-1B), (Xcfa2129-1A), and (Xgwm369-3A) to 100% (Xwmc407-2A). Gwm11-1B and Xgwm294-2A, two primers that were discovered to be monomorphic, have PIC values of 0. 9 primers had PIC values above 0.5 (50%) indicating a high degree of genetic variability in wheat genotypes for salinity tolerance. In terms of genetic polymorphism, chromosomes 1A, 3A, and 4A showed the highest levels (Table 6). SSR marker data also distinguished between genotypes that are salt resistant and susceptible. The best SSR markers for assessing salt tolerance were Gwm484-2D, Xwmc407-2A, Gwm11-1B, Xgwm294-2A, Xcfa2129-4A, Wmc 136, and Xwmc 313-4A.

UPGMA cluster analysis of similarity matrix in the form of a dendrogram was used to determine the genetic relationships between the 6 wheat genotypes (Fig. 2). The six wheat genotypes were divided into four subclusters using cluster analysis. Due to its genetic dissimilarity, Sakha-94 was discovered on its own and was not a part of any cluster. Masr-1 made up the first sub cluster. Line-2 and two further sub clusters were broken up into the sub sub cluster. Line-1 and Shandaweel-1 made up Giza-171's subcluster. Giza-171 was located at the base of the dendrogram and was not a part of any cluster, indicating that it is genetically distinct from other genotypes.

Table 6. Using of 13 SSR markers for the six wheat genotypes to obtain the allele size, observed heterozygosity, expected heterozygosity, number of detected polymorphic alleles (P. Allele), and (PIC) polymorphism information content.

Sr No.	Primer	Size	H_{ob}	H_{ex}	Allele	PIC value
1	Gwm484-2D	59-241	0.000	0.770	6	0.708
2	Xwmc273-7D	151-213	0.000	0.674	3	0.563
3	Xwmc407-2A	110-151	0.000	0.522	2	0.375
4	Gwm11-1B	110	0.000	0.000	1	0.000
5	Xbarc121-7A	59-151	0.000	0.784	5	0.699
6	Xgwm294-2A	102	0.000	0.000	1	0.000
7	Xcfa2129-1A	74-459	0.000	0.869	9	0.834
8	Wms136	183-548	0.000	0.850	8	0.810
9	Xgwm637-4A	241-352	0.000	0.790	7	0.735
10	Xgwm160	110-435	0.000	0.836	6	0.790
11	Xgwm219-B	163-183	0.167	0.167	2	0.141
12	Xgwm369-3A	74-459	0.000	0.863	9	0.819
13	XWMC313-4A	151-459	0.000	0.638	3	0.535
	Total					
	Mean			0.6045	4.769	0.5439

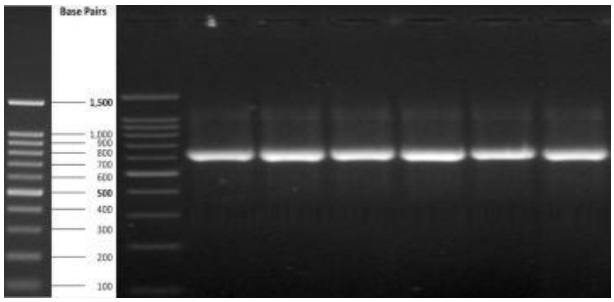
OM200013.1 *Triticum aestivum* Na+/H+ antiporter (NHX1) mRNA, partial cds

CTATCTTGCAATTGGGGCTATCTTCTCAGCA
ACAGATTCTGTTTGCACCTTACAGGTGCTTAACCA
GGATGAAGCACCCCTACTGTATAGTCTAGTTTTTG
GTGAAGGTGTTGTTAATGATGCTACATCAGTTGTG
CTCTTCAATGCAATTCAAAACATTGATATTAATCA
TTTTGATGTCTTCGTTCTACTACAATTCATCGGAA
AATTCCTCTACCTATTCTTCACCAGCACCGTTCTT
GGAGTAGCTGCTGGGTTGCTTAGTGCATACATTAT
TAAGAAACTTTGTTTTGCAAGACACTCAACTGAC
AGAGAAGTTGCTATCATGATACTCATGGCATACC
TTTCGTATATGCTGTCAATGCTGCTGGATCTGAGT
GGCATTCTAACCGTGTTCTTCTGTGGAATAGTAAT
GTCACATTACACTTGGCATAATGTCACAGAAAGC
TCAAGGGTTACTACCAAGCATACTTTTGCAACTTT
ATCATTCATTGCTGAGATTTTTCTTTTTCTCTATGT
CGGGATGGATGCATTGGACATTGATAAATGGAAA
TTAGCTAGTAGCAGTCCTAAGAAACCA

According to the study's findings, the wheat genotypes Shandaweel-1 and Masr-1 can be chosen to thrive in irrigation water with high salinity levels (Maha et al., 2017).

Phylogenetic tree and gene sequencing of the antiporter, the base size of the antiporter gene was 600 bp in the isolate's sequences. The antiporter gene was 100% identical to the DNA sequences of this strain. The present antiporter gene sequences were sequenced and entered into the DNA database under the accession number OM200013.

The results were compared in the NCBI gene bank. In order to create a phylogenetic tree, the sequences of accession number OM200013 and four other accession numbers from a gene bank were matched. Antiporter sequence phylogenetic analyses revealed a connection between the sequences from accession number OM200013 and four other accession numbers from the NCBI. According to the findings, the current specimens belonged to the Phylum antiporter gene (Figure. 5). The antiporter gene-based phylogenetic tree included four clades, and the length of the branch representing an amount of genetic change of 0.01 was shown in the phylogenetic tree derived from the sequence comparison of the antiporter gene area. The given specimen was assigned to the antiporter gene ID: OM200013 by the clades grouping, which had minimal support (bootstrap value between 6-8%). One number in the phylogenetic tree is next to two numbers in the same tree. And demonstrated how number (1 in the evolutionary tree) was distant from numbers (3, 4, and 5).



1: Masr-1 2: Shandaweel-1 3: Line-1 4: Line-2 5: Giza-171 6: Sakha-94.

Figure. 4. a PCR product for OM200013.1 gene. b PCR product for Na+/H+ antiporter gene

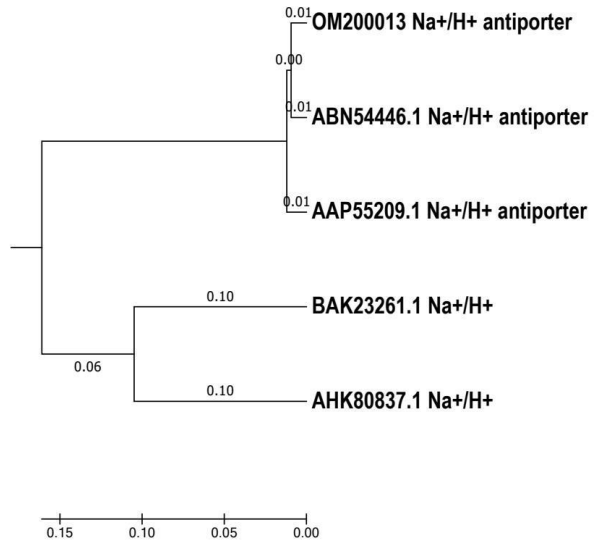


Figure. 5. The Sequences of the salt tolerant gene with the accession number OM200013 and four genes with the accession numbers indicating the relationship between them from gene bank. T

This isolate's DNA sequences revealed complete identity to the OM200013 gene.The present OM200013 gene sequences were sequenced and registered in a DNA database with the accession number OM200013. The results were compared in the NCBI gene bank. The OM200013 entry number's sequences and four other gene bank accession numbers were aligned, and a phylogenetic tree was created using the MEGA 7 programme. Salinity tolerance gene sequences were the subject of phylogenetic analyses, which revealed a connection between the sequences from accession number OM200013 and four other numbers from the NCBI. The outcome revealed that the current specimens belonged to a gene family that was tolerant to salinity (Figure. 5). The salinity tolerance gene-based phylogenetic tree had four clades and revealed that the length of the branch reflects an amount of genetic change of 0.01, according to the sequence comparison of the salinity tolerance gene area. The present specimen was placed in the salinity tolerance gene ID: OM200013 number (1) in the phylogenetic tree and was compared to the other accession number because of the low support for the clades grouping (bootstrap value between 8 and 12%).

CONCLUSION

This study aimed to determine a genotype (Line or cultivar) suitable for growing in salt soil and had the ability to tolerate the salinity. Therefore, cluster analysis, similarity between the six genotypes and phylogenetic tree beside to do functional genomic analysis to decide whether local cultivars of bread wheat (*Triticum aestivum* l.) are suitable for breeding or direct cultivation in the area suffered from salinity.

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تحديد جين تحمل الملوحة فى ستة تراكيب وراثية من قمح الخبز من خلال استخدام تكنيك ال اس اس ار و التتابعات.

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الملخص

تم تطبيق 13 بادىء لتقييم الجينات المقاومة للملوحة في التراكيب الوراثية للقمح. كانت 3 بادئات (10 %) احادية الحزم، و 26 (86 %) كانت عديدة التباين فى الحزم و كانت 1 فقط (3.34 %) لم تعطى نتائج. تم الحصول على ما مجموعه 37 اليل باستخدام 50 بادىء متعدد التباين. تم حساب محتوى المعلومات المتعددة الاشكال او التباين والتى تراوحت قيمها من الصفر الى 0.38 فى المتوسط ، التى استخدمت لمساعدة سعة كل موقع اس اس ار للتمييز بين اصناف القمح المختلفة. تم عرض عدم التجانس الوراثي المعنوى للتراكيب الوراثية للقمح من خلال تحليل الشجرة الوراثية. بإستخدام بادئات الملوحة تضخيم جين الانتبورتر الصوديوم-الهيدروجين و التتابع، تم تحديد التراكيب الوراثية الستة. بإستخدام برنامج المعلوماتية الحيوية بلاست، تمت مقارنة التتابعات الناتجة مع التتابعات الموجودة فى قاعدة البيانات أون لاين ان سى بى اى. و طبقا للنتائج كان حجم القاعدة لجين تحمل الملوحة 200 قاعدة مقارنة مقارنة بال 400 قاعدة لجين الانتى بورتر. تحليل الشجرة الوراثية اوضح ان التتابع لجين الانتى بورتر الصوديوم - الهيدروجين كان بيرتبط بشفرات اربعة جينات ببنك الجينات. و ان تحليل الشجرة الوراثية بين ان التغيير الوراثى يكون فى حدود 0.01 عند جذر اى جين. أن الدعم لمجموعات الجينات كان قليلا (بقيمة 6-8 %) . بالاضافة الى ذلك كما هو واضح فى الشجرة الوراثية، ان تتابعات جين تحمل الملوحة التى تم جمعها تكون فى نفس اتجاه اربعة شفرات مختلفة فى موقع ال ان سى بى اى .