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POPULATION STRUCTURE AND GENOME-WIDE ASSOCIATION ANALYSIS FOR SALINITY TOLERANCE IN BREAD WHEAT USING SNP, SSR AND SCOT MARKER ASSAYS

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ABSTRACT

Wheat is an essential staple food in the developing world, where demand is projected to grow exponentially in the future; simultaneously, climate changes are projected to reduce supply in the near future. One of the main consequences of climate change is salinity, which negatively impacts the world's cultivated area and therefore affects the global wheat production. Our objectives are to study the population structure of several Egyptian and international wheat accessions and to identify the genetic factors controlling the salinity stress response of bread wheat. In addition, we have attempt to identify genes that control some important agronomic parameters of wheat under salinity stress were identified. The wheat germplasm panel consisted of 70 accessions obtained from Egypt, Syria and Iran. The assessment of salinity tolerance was conducted over the years of 2018 and 2019 in the field and in the greenhouse. The genome association analysis (GWAS) and population structure analysis was conducted using six SCoT, five SSR and 93 SNP markers. Analysis of the population structure using allele frequency and phylogenetic analysis indicated that the studied wheat accessions were belong to four population groups. Where, for the most portion, Egyptian, Syrian and Iranian accessions are clustered depending on their country of origin. The GWAS analysis revealed 13 SNP markers that were significantly associated with morpho-agronomic wheat traits during salinity stress. These markers were closely related to genes that are known to have a direct link to wheat response to salinity stress such as *CYP709B2*, *MDIS2*, *STAY-GREEN*, *PIP5K9*, and *MSSP2 genes*. This study revealed the genetic structure of adapted and imported wheat accessions, which could be used to select potential wheat accessions for local breeding programs. In addition, the SNP genotyping assay is a very potential technology that could be efficiently applied to detect genes that control bread wheat response to salinity stress.

Keywords: Wheat, Salinity, SNP genotyping, SSR, SCoT, GWAS

INTRODUCTION

The domestication of wheat (*Triticum* sp.) began in the Fertile Crescent around 10,000 years ago **(Faris, 2014)** . It is the most essential staple food of about 36% of humans, where 55% of the world's population relies on wheat for about 20% intake of food calories **(Aryan et al 2018)**. In the developing world, wheat demand is projected to grow by 60% by 2050; at the same time, climate change-induced temperature rises are predicted to decrease wheat supply **(Alexandratos and Bruinsma, 2012)**. In addition, global wheat production will be exponentially affected by major biotic and abiotic stress, including drought, salinity and plant diseases. Salinity will have a negative impact on 6.5% of the total land, which translates into 8 million km^2 of cultivable land **("FAOSTAT," 2018).**

As a response to environmental changes, the rapid growth of genotypic and phenotypic analysis technologies has enabled the examining of the genomic content of many economic crops **(Aliyu et al 2011; Awan, 2019; Girish and Dubey, 2018)**. Such methods would provide efficient information that could be used to improve the response of these crops to dramatic changes in the environment and therefore to maintain global cereal production. The yield of wheat grains is a dynamic trait based on multiple genes interacting with each other and the environment **(Wu et al 2012).** In this regard, molecular marker technologies have proved their value in the detection and tagging of several genetic loci associated with crop tolerance to biotic and abiotic stresses **(Hassan et al 2020; Nahas et al 2020)**. For instance, simple sequence repeat (SSR) marker technology was successfully used to evaluate the diversity and genetic structure of wheat cultivars, corresponding to their origin, productivity and ability to perform effectively in different environments **(Abbasabad et al 2017; Würschum et al 2013)**. Aditionally, SSR markers, which chracterized bymulti-allelism, high reproducibility, co-dominance, and genomic abundance and transferability have support its usefulness in identifying genetic loci associated with the ability of wheat to tolerate drought, salinity and several diseases **(Qadir et al 2014; Turki et al 2015)**. Other marker assays such as Start Codon-Targeted (SCoT) were developed on the basis of a short standard area flanking the start codon of ATG in plant genome. SCoT markers could be more effective than other random marker technologies, particularly due to high annealing temperatures and longer PCR primers **(Collard and Mackill, 2009)**. Various molecular studies have used the SCoT marker to study different plant species including wheat **(Etminan et al 2016)**, olive **(Alsamman et al 2017)**, maize **(Vivod'ík et al 2017)**, and tomato **(Abdein et al 2018)**.

Owing to its evolutionary relationship, genome abundance, applicability for population structure assessment, and agronomic traits association, single nucleotide polymorphism (SNP) markers have acquired remarkable value in crop genetics **(Rafalski, 2002)**. Genome-wide association (GWAS) analysis through SNP genotyping technology has a major influence on the detection of genetic loci correlated with quantitative and complex features **(Zaimah, 2019)**. This methods have been used to study and analyze the genetic architecture for crop resilience and grain production in wheat under salinity **(Hussain et al 2017)**, drought **(Ballesta et al 2020)** and disease stresses **(Perez-Lara et al 2017)**. Moreover, recent bioinformatics techniques provide a golden opportunity to filter trait-associated SNPs, depending on their impact on gene activity.

The study of the response of economic crops, such as wheat, to environmental changes, is therefore vital for future genetic improvement **(Nassar et al 2018)**. Unfortunately, the large genome of wheat limits such studies, requiring advanced biological data analysis techniques. Our objective is to study the population structure of several Egyptian, Syrain and Iranian wheat accessions and to identify the genetic factors controlling the salinity stress response of bread wheat. In addition, we have attempt to identify genes that control some important agronomic parameters of wheat under salinity stress.

METHODOLOGY

Plant Material

The studied germplasm panel consisted of 70 bread wheat accessions obtained from Egypt, Syria and Iran. This subset was chosen from the International Center for Agricultural Research in the Dry Areas (ICARDA) and Agricultural Research Center gene banks, Giza, Egypt.

Salinity tolerance phenotyping

Forty-four foreign international accessions (Syrian and Iranian genotypes) were chosen using Focused Identification of Germplasm Strategy (FIGS) method for the assessment of salinity tolerance over the years 2018 and 2019. Evaluations were performed in the field (Arish province, Sinai, Egypt) and in a greenhouse (Agricultural Research Center – ARC, Giza, Egypt) using a hydroponic system. The evaluation was done in three replications using the Alpha Lattice design. In the field, an irrigation system of dripping water was installed and the field was irrigated one time every two weeks. A sample of soil was air-dried and used as a soil solution for pH and salt concentration analysis to evaluate the salt content in the field soil **(Sparks et al 2020)**. The concentration of salt in the field at depths of 30 and 60 cm was 344, 904 ppm, respectively, and 848 ppm at depths of more than 60 cm. The morpho-agronomic traits of plant hight (PH), number of tillers (NT), Days to 50% of flowering (DF), number of spikes (NS), spike height (SH), and number of spikelets (NSL) were measured. In the greenhouse, three seeds of each accession were germinated in

small pots containing a mixture of perlite (60%) and peat moss (40%). The plantlets were transferred after two weeks to hydroponic tanks. Electrical conductivity (EC) meter (Hanna HI8733) was used to measure the salt concentration in the greenhouse, which was calibrated at 5,844 ppm (pH 8). The plant salinity tolerance rate (STR) was measured in the range of 1 (normal) to 5 (dead). Throughout the trial, plant performances were scored three times, at an interval of two weeks.

DNA extraction and analysis of molecular markers

DNA was extracted from young leaves of four to six week old seedlings using the cetyl trim ethyl ammonium bromide (CTAB) method (Rogers and Bendich, 1989). Through PCR-based assays, five SSR and six SCoT primers were applied (**Table 1**). The reactions of SSR and SCoT PCR analyses were performed in a 15 µl volume of reaction contained a 5 ng of DNA, a 10 pmol of each primer, a 2 mM of dNTPs, a 25 mM of $MgCl_2$, a 0.1 U of Taq DNA polymerase, and 10X PCR buffer. For 35 cycles, the SSR PCR program included 95 °C (5 min), 95 °C (15 sec), 55 °C (15 sec) and 72 °C (30 sec). Thereafter a final extension step was applied at 72 ° C for 7 min. The SCoT PCR program and reaction content were conducted as reported by **Ibrahim et al (2016)**. Ethidium bromide stained agarose gel (8%) was used to distinguish among PCR fragments. Gel images were documented using the Gel Doc XR system (Bio-Rad, Hercules, CA, USA). Fragments of PCR products were counted as present (1) or absent (0) for all tested wheat accessions.

The SNP panel of DarT® company (Triticarte Pty. Ltd. Australia) was applied to the forty four 44 wheat accessions. The DNA was sent to marker genotyping as a supplier for profit-oriented service. A 93 SNP marker loci (**Table 2**) were used for GWAS analysis. The BLAST software **(Altschul et al 1997)** was used to locate SNP markers on wheat chromosomes **(Wheat Consortium, 2014)**.

Statistical and genetic analyses

The phylogenetic and diversity analysis was conducted using Dice's similarity matrix coefficients using Dendro-UPGMA online tool (http:// genomes.urv.es/UPGMA/). The online iTOL software was used to construct phylogenetic trees that show evolutionary relationships among tested wheat accessions. The GWAS analysis of the SNP markers and morpho-agronomic traits of wheat was conducted using GAPIT software (R Package) **(Lipka et al 2012)**. The population genetic structure of the tested wheat accessions was studied by STRUCTURE (https://web.stanford.edu) and strplot2 software (http://omicsspeaks.com/strplot2/) using 5000 burn-in and MCMC iterations. The Circos software package was used to illustrate the different results of GWAS on the wheat genome **(Krzywinski et al 2009)**.

RESULTS AND DISCUSSION

Genetic polymorphism of the PCR markers

Studying the genetic diversity of local and international accessions of wheat could benefit local breeding programs by enriching their genetic resources with more adaptive and stable accessions. PCR-based techniques such as SCoT and SSR could provide different but complementary information regarding wheatevolutionary adaptation to environment. In this regard, a total number of 61 PCR-bands were revealed using SSR and SCoT primers, where SCoT assay provided a higher number of bands (46 bands) compared to SSR assay (15 bands) (**Table 3 and Fig. 1**). The maximum number of bands was obtained from SCoT-05 were 10 bands. Additionally, the total number of polymorphic bands was 48 bands, where SCoT-10, and SCoT-01 revealed the maximum number of polymorphic bands (8 bands). The PCR primers of SCoT-02, and SSR-01 revealed the maximum percentage of polymorphism (100%) (**Table 3 and Fig. 1**). On the other hand, among the 91 SNP primers used for SNP genotyping of wheat, only 17 makers were monomorphic.

Etminan et al (2016) used six SCoT primer to study the genetic diversity of several durum genotypes, where they obtained 54 PCR bands with polymorphism percentage of a 100%. SCoT assay was used to assess the genetic variability of some Egyptian wheat cultivars, where 32 bands with a 59% of polymorphism using six SCoT primers were detected **(Abdel-Lateif et al 2018)**. In addition, 14 ScoT primers were used to study the population structure of 17 durum wheat genotypes, generating a total of 118 bands with a polymorphism of 83.24% **(Heidari et al 2017)**. SCoT was used to identify the allelic variation among multiple olive genotypes, moderate ability of SCoT markers to detect genetic variation compared to other molecular assays was recorded **(Alsamman et al 2017)**. The genetic diversity of 480 bread wheat accessions, chosen from

Table 1. Sequences and code names of SCoT and SSR primers used for the PCR-based genotyping of wheat genotypes.

Name	Forward primer	Reverse primer
SSR-1	GACAGCACCTTGCCCTTTG	CATCGGCAACATGCTCATC
SSR-2	AAAGAGGTCTGCCGCTAACA	TATACGGTTTTGTGAGGGGG
SSR-3	TTCAATTCAGTCTTGGCTTGG	CTGCAGGAAAAAAAGTACACCC
SSR-4	GATGAGCGACACCTAGCCTC	GGGGTCCGAGTCCACAAC
SSR-5	CTGCAGGCCATGATGATG	ACCGTGGGTGTTGTGAGC
SCoT-1	CGACATGGCGACCACGC	-
SCoT-2	ACCATGGCTACCACCGGC	-
SCoT-3	CGACATGGCGACCCACA	-
SCoT-4	ACCATGGCTACCACCGCA	-
SCoT-5	CAATGGCTACCACTAGCG	-
SCoT-10	ACAATGGCTACCACCAGC	-

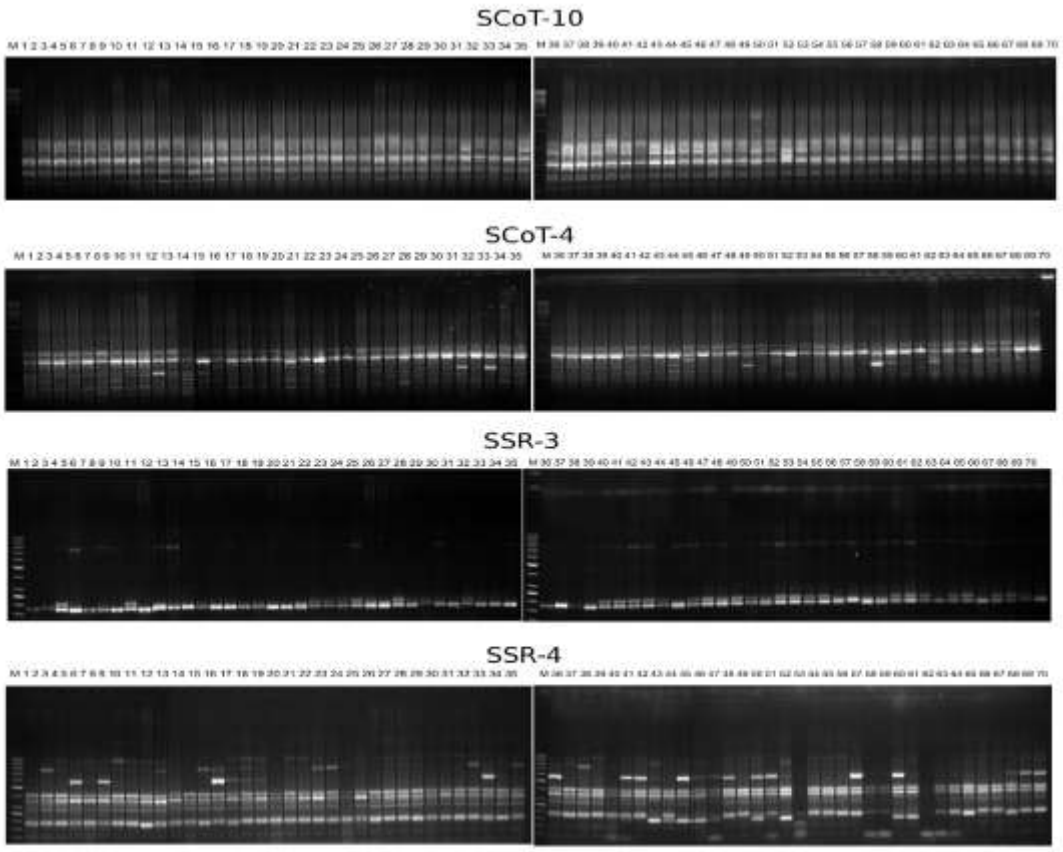

Fig. 1. Selected gel electrophoresis profiles of 70 wheat genotypes studied using SCoT and SSR PCR primers.

Table 2. Sequences and primer name (PN) of DNA primers used for the SNP genotyping of wheat genotypes where targeted SNPs are surrounded by square brackets.

PN	Sequence	PN	Sequence
AX-86163814	TTCTGTTAGGCATGG[R]AACTCTTCTCTGTTT	BS00044237	GGGTGTTCCTTTGTT[Y]TTTGGTGGTATATTC
AX-86167869	TCACTTGTCACTGCC[K]GTGCTCAAAGTCATC	BS00044720	CTTAGTAGTTCACGT[A/G]CTCAGTAGCCCTGCA
AX-94382081	TTTGCGAAAGGGGCT[S]AAGCTAGTAGTTCGG	BS00046264	CCATCGATCACCGGT[Y]GGCGCTACCAAAGGG
AX-94392216	TCCATTTGTACCTAA[K]CTGTGTAGTTGGTAA	BS00046963	CCCGTCGCCGCCCGC[R]AGTCGCGGCTGAACC
AX-94401211	CACCTACAAGCTAAT[R]ATAAGGAAGCAGTTA	BS00049370	GTATTTGTCCTTTTC[A/G]TCATTTGGGTCATTT
AX-94406983	ATCTTTACCACCTGG[S]CTTCTTGCTTTCTAT	BS00049818	TGTATAGATACACTC[C/T]ATTACCTTCTGGTCT
AX-94415898	TAACACACACAGTTG[R]TGCTTAAACTGATTC	BS00050057	GAACATCATCAACCT[K]GCAACCATTAATTCT
AX-94431524	GATAAGGTGCATGAA[R]GTCGTCTGATCTACT	BS00050109	CAAAGAGAACTCTGC[Y]GGTCGATGTGTGATA
AX-94442305	GTCATCTAGTAAGGA[R]ACCAAATCACTCATC	BS00050993	ACATTGTTGTGGGCT[M]TGTCCGAACTGATCA
AX-94446956	GGAATTGTGGGTCGA[K]CAAGAGATGGTTCGT	BS00057851	CAATATCGTTGAGGA[Y]TTCCAAGTTATGACA
AX-94454241	GTTCTTGTGCTTGAG[Y]GTCTTAAGCAACCGC	BS00058591	TGTACGTTCCCGCCT[Y]CCATGGACTACGGCG
AX-94457966	ATACACTGAGATTTC[Y]TGGAGATGTTGGTCG	BS00060686	GCTCGGTGAGCAATT[K]ATGTTGGTTGCGCTT
AX-94486277	TACAATGCATAGAAC[K]AGTGGTTATGTTGTG	BS00063425	ACTGATAGGTAGATA[Y]GGTTGCCTCTTCAGT
AX-94488939	AGACTTAAATGGACT[M]CCAGAGGCACTTCTT	BS00064146	ACGGCCATTGCAGGT[R]CGGTTGGTGATTGTC
AX-94527869	CCTATAGGTACACTG[Y]AGGATGCGAAACTTA	BS00064691	ATCTGCTTCTCATCC[T/G]TGCCGATGCAATCGT
AX-94529943	GCGATACACATGCCC[Y]GCCATCCGCGGATGA	BS00066143	AGCCACAGCAAATTT[Y]GCAGATAATATTGAA
AX-94540417	TGTATTCTGTTCTGA[S]ATCGTTTACACAGGA	BS00070791	TGACAAGGATTTATT[Y]AGTATTAAATTTTGA
AX-94544363	GAAGGAGGTGACCAG[R]AAGAAGAACGAGACG	BS00070903	ACGATCTCGTGCGGG[R]TAGCCAAATCAACAT
AX-94545917	CTGAACCCTTCCTGT[Y]AATTGTTTCCGAGTA	BS00071183	AGCAGAGCAGACATG[R]TAGACTTGCCGCATT
AX-94558874	CCAGCAGCTTCATTC[S]TCACCGGCCAGGTCA	BS00071558	GACACTGTATAGATG[Y]GAATGGTCCCCTTCA
AX-94559367	CCCTGAGGGAGTGCT[Y]CAAGGGAGGGGGTTT	BS00073116	TGATAAGGATTCTGG[K]CAGTGCTGTGCTCCA
BS00049977	AAAGGAATTTCCTGG[Y]GTAGTACATTAGGAT	BS00074083	GTTCATATACTTCAG[R]CATACTATATATTTC
BS00000006	TCCCGCAGTGGGTGC[K]GAATGTCGGTGCGAG	BS00075815	CGCAAAAAGGTGTGC[Y]GAAACCACGGCCAAA
BS00018707	AAGTCCAAAATCCGC[R]ATTCTTGGGTTCATG	BS00076033	TGAGCAGTGTTGGAA[R]CTGATTAAAACATTT
BS00021704	TCACTTTTCAGTGCC[Y]GCTTTACCGTTGCAG	BS00076192	ACCAACAAGAGAGCC[R]GATCCACGAGGAATC
BS00021745	CACGACAGAAGCAAC[R]CGTTTGCGAGGTTTG	BS00076248	GACGGTAGGAATACG[R]CAGTTTCAGTCTTTT
BS00022411	ATCTATGACTATCTA[K]GAATTTGTATCTCCT	BS00076622	TCTGCCGCATTCCGA[Y]CGATCACAAATAGAA
BS00022625	TTTCTTTTTGTTGTG[R]GCTTGTTTCGTATGC	BS00077716	GTTCATTCAGCACTA[Y]GAAAAAGCTCTCGAC
BS00022653	TGTAGTTTATGCTTA[M]TCACTTTGGCTGAAA	BS00077891	TCTCGATTATGCCTG[K]GTTTTCGAGTAGATC
BS00023673	TTGCCGGCTGATGGA[Y]CTTAAAAGCGGCACT	BS00078124	AGAGGATGCCGCTGA[Y]GCCGGAGAATTGTTG
BS00024548	TTGCCATCCATATTT[R]CATGCCCCATGAATA	BS00080749	AGAAAACACACCCCC[A/C]GCTCAATATTCCATA
BS00024786	CTCCCCATTCAGTCC[Y]GACAAATGTAAATAT	BS00082503	CAAGGGTATTACCCA[R]GCGTAACTTCTCCTC
BS00024921	TCACAACAAGCGCAC[R]CAAAATTAGCAGCAC	BS00083630	CGACCTGACTGCCAA[K]ATCAACGGCCGCGTC
BS00025017	GAGCAGACTGTAGAG[C/T]TTTTACAATGGCAAG	BS00084133	GAACATAGTTTTTGA[T/C]CTACGGGATGACAAG
BS00030651	TGACCGGACCCTGTA[Y]GCCGACGAGATTTTG	BS00089403	ATCATGTGTATCATA[Y]GATTAATTGCAATAA
BS00031140	ACATACAGACCACTA[Y]TAAAACCAAAAATAC	BS00089597	AGTTGAGAGAACAGC[T/C]ACGCATAATTCTCCG
BS00031178	TATGTTGTCTCCTTT[Y]CATTCATTTGTCATG	BS00089954	CAACTCCCAGCTATC[R]TCGCCGGCTGCTGCG
BS00032039	CCCGGTGATTTCACT[K]TAACATGAGTAAGGA	BS00097126	GGAGCCTCCTTCGAA[T/C]GAACGTATTGTTACC
BS00033795	CAGCGCCGTCGCTTC[Y]AGGAGATCCAGCCCG	BS00100939	TTACTCAAGGCATGG[Y]GGTTCGCGTCAAGGG
BS00035234	TAGTGCAAACTGAGT[R]TACTGGGTTCAAAAG	BS00101408	GATGGAGAACCGTCC[M]GCTGCCAATACTTGT
BS00037020	ACAACCCCCATTGGA[K]AGGGATTTCTAAAGA	BS00104432	TTTTGTAGCTCCTGA[M]TCGATGCAGCTTTAG
BS00038820	GATAGCATACTGCCT[Y]GAGCAAATGCACAAG	BS00105878	CTGATGCGTGTTGGG[Y]GGAAGAATAATCTCA
BS00039211	GAGCTAGTAGTGATG[T/C]ATTGGTCAGATCGAT	BS00106043	AGGCCGAACCATCAG[Y]GGCTAATTAATTATT
BS00040283	CTGCTCCATCATCTC[R]TGGTCCAGGTGAAGA	BS00107766	GCTAGTCTGTGTCCA[Y]CATGTGTAATCTCGT
BS00040798	TGGATCGATGCGCTG[R]TGTTTACTGCATTTT	BS00107837	ATGCATACAATACAT[R]CTTGCTGCAAATGAT
BS00042105	CAACAACTTCATTCG[Y]CCGCTCGCTAGGGGT	BS00109036	CTGAAGGAGGTGTAC[Y]GAATCCTTGCCCATG
BS00043169	CCCTATATGTGCGAC[A/C]GTTGATTTCTTTTGT		

Table 3. The primer name (PN),total number of bands (TB), monomorphic bands count (MB), polymorphic bands count (PB) and polymorphism percentage as revealed by SCoT and SSR marker assays

PN	TB	MB	PB	PP
SCoT-01	9	1	8	89%
SCoT-02	6	0	6	100%
SCoT-03	5	1	4	80%
SCoT-04	7	1	6	86%
SCoT-05	10	3	7	7%
SCoT-10	9	1	8	89%
SSR-01	3	0	3	100%
SSR-02	2	2	0	0
SSR-03	2	1	1	50%
SSR-04	6	2	4	67%
SSR-05	2	1	1	5%
Total	**61**	**13**	**48**	**7.8%**

15 European countries or geo-graphic groups, were genotyped using 39 polymorphic SSR primers which, generated 635 PCR bands with a 72% of polymorphism, with a number of bands was ranged from 4 to 40 **(Roussel et al 2005).**

Genetic diversity and population structure

Analysis of population structure involves allocating each individual to a group in a population, and reporting the number of clusters. It has many applications in diversity studies including clustering of individuals, inferring demographic history of the population and identifying immigrants. There are many methods for inferencing population structure, such as the allele frequencies based analyses, Kinship analysis, and principal component analysis (PCA) **(Lee et al 2009)**.

In this study, our aim is to identify the structure of the population of several accessions collected from different regional areas. We used the SSR, SCoT and SNP markers were used to compare between genetic structure and geographic origin (**Figs. 2 and 3**). Population structure analysis through STRUCTURE is widely conducted using Markov Chain Monte Carlo (MCMC), which uses genetic allele frequencies to allocate individuals of different groups (Pritchard et al., 2000). Analysis of the genetic structure using allele frequency of the Egyptian, Syrian and Iranian wheat accessions indicate that these accessions belong to a four different population groups. Where, for the most portion, Egyptian, Syrian and Iranian accessions were clustered depending on their country of origin. On the other hand, some accessions showed a type of genetic migration, which could be caused by varietal adaptation. Similar results were retrieved using the phylogenetic analysis (**Fig. 3**). Most accessions were almost clustered, depending on their geographical origin, although some Egyptian accessions were clustered with Iranian and Syrian accessions, which could indicate their source of origin. Such analysis could indicate a number of potential foreign accessions that could be successfully adapted in the Egyptian environment through local breeding programmes.

GWAS analysis of wheat morpho-agronomic traits

GWAS was emerged as a promising method for detecting important marker-trait associations based on the correlation between genotyping and agronomic data of several wheat accessions with low genetic relationships. GWAS has proven to be a useful tool for dissecting complex genetic mechanisms which regulate biotic stress in wheat **(Liu et al 2018; Oyiga et al 2018).**

SNP genotyping was used to detect genes that were related for wheat response to salinity stress. The statistical correlation between the 93 SNP markers and the seven agronomic traits of wheat was calculated (**Table 4 and Fig. 4**). GWAS analysis revealed 13 SNP markers. Which were distributed across the chromosomes of 7B (3), 6A (3), 5A (2), 2B (1), 2A (1), 5B (1), 3B (1), and 1B (1) (**Table 4 and Fig. 4**). Five different markers are correlated with STR trait (BS00064146, BS00101408, BS00089954, BS00000006, and BS00076622) (**Table 4**). The effect of these markers on STR trait was ranged from -0.56 (BS00076622) to 0.469 (BS00064146 and BS00101408). DF trait is correlated with four SNP markers (BS00024921, BS00083630, BS00078124, and BS00038820), where its effect was ranged from -2.544 (BS00078124) to 2.526 (BS00038820, and BS00024921). Some SNP markersshowed a correlation with multiple traits such as BS00038820 (DF and PH), BS00107837 (NS and NT), and BS00089954 (NS and STR).

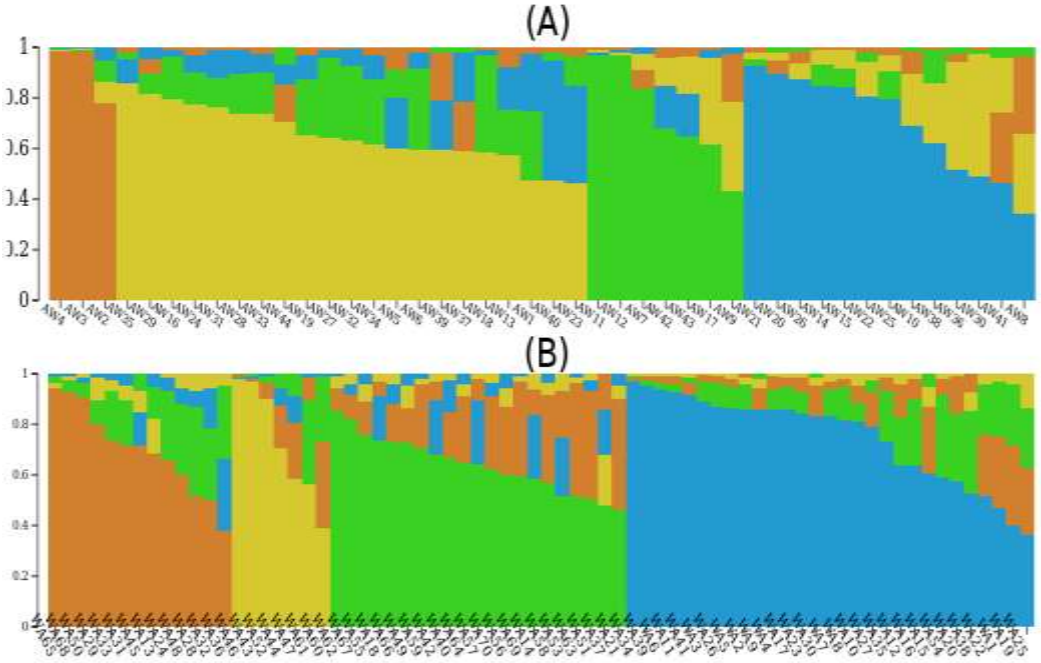

Fig. 2. Analysis of the population structure based on the allele frequencies using STRUCTURE software for the tested 70 wheat genotypes used in this study. (A) The population structure of the forty four accessions using SNP assay. (B) The seventy local and foreign genotypes structure using SCoT and SSR assays.

Table 4. The statistical effect and correlation significance (p.value) of the SNPs markers showing correlation with the agronomic traits of wheat under salinity stress

SNP			DF		NS		NSL		NT		PH		SH		STR	
Name	Chr	Pos	p. value	effect	p. value	effect	p. value	effect	p. value	effect	p. value	ef-fect	p. value	ef-fect	p. value	ef-fect
BS00064146	7B	655818377	0.83	-0.298	0.146	0.383	0.459	0.289	0.267	0.296	0.823	0.51	0.217	0.364	0.015*	0.469
BS00101408	7B	657662487	0.83	-0.298	0.146	0.383	0.459	0.289	0.267	0.296	0.823	0.51	0.217	0.364	0.015*	0.469
BS00066143	5A	533072163	0.078	-1.903	0.131	0.326	0.057	-0.578	0.122	0.339	0.03*	-3.853	0.461	-0.179	0.987	-0.002
BS00024921	2A	733091124	0.021*	2.525	0.068	-0.375	0.093	0.508	0.056	-0.4	0.054	3.417	0.938	-0.018	0.885	-0.021
BS00089954	3B	543718628	0.583	-0.578	0.045*	0.43	0.994	0.002	0.104	0.351	0.451	1.299	0.66	0.105	0.037*	0.319
BS00107837	1B	674821632	0.403	1.071	0.025*	-0.564	0.363	0.328	0.014*	-0.638	0.229	2.533	0.69	0.109	0.6	0.091
BS00083630	6A	5604416	0.02*	-2.372	0.852	0.035	0.075	-0.503	0.997	-0.001	0.145	-2.384	0.352	-0.196	0.804	0.033
BS00000006	5A	706240365	0.931	-0.083	0.913	0.02	0.799	-0.068	0.81	0.045	0.544	-0.951	0.363	-0.189	0.044*	-0.269
BS00078124	6A	617182750	0.036*	-2.544	0.375	0.197	0.714	0.122	0.34	0.217	0.126	-3.001	0.368	0.223	0.738	-0.053
BS00021704	6A	611851563	0.089	2.055	0.271	-0.261	0.557	-0.196	0.267	-0.267	0.102	3.229	0.032*	-0.593	0.102	-0.278
BS00050057	5B	658370171	0.568	0.7	0.843	-0.053	0.057	0.67	0.808	-0.064	0.377	1.768	0.022*	0.733	0.964	-0.008
BS00076622	7B	717202678	0.639	-0.733	0.781	0.095	0.594	-0.234	0.783	0.094	0.941	0.188	0.26	-0.448	0.024*	-0.561
BS00038820	2B	64988240	0.047*	2.526	0.598	0.139	0.379	0.308	0.532	0.166	0.045*	4.161	0.297	0.317	0.531	0.117

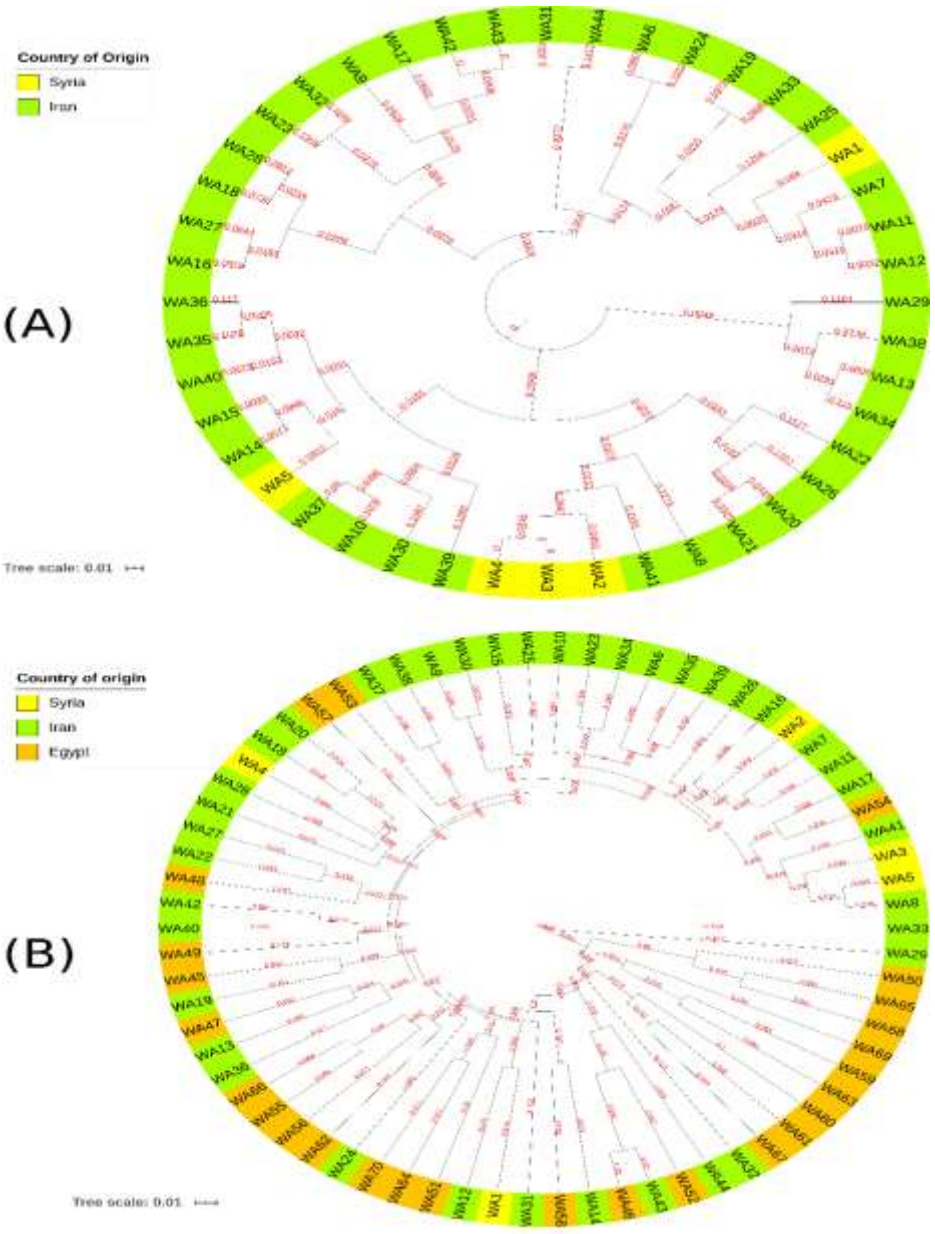

Fig. 3. The phylogenetic diversity for the tested wheat genotypes used in this study. (A) The phylogenetic tree using SNP genotyping assay for the forty four foreign accessions. (B) The phylogenetic tree of the severity local and foreign genotypes using SCoT and SSR assays

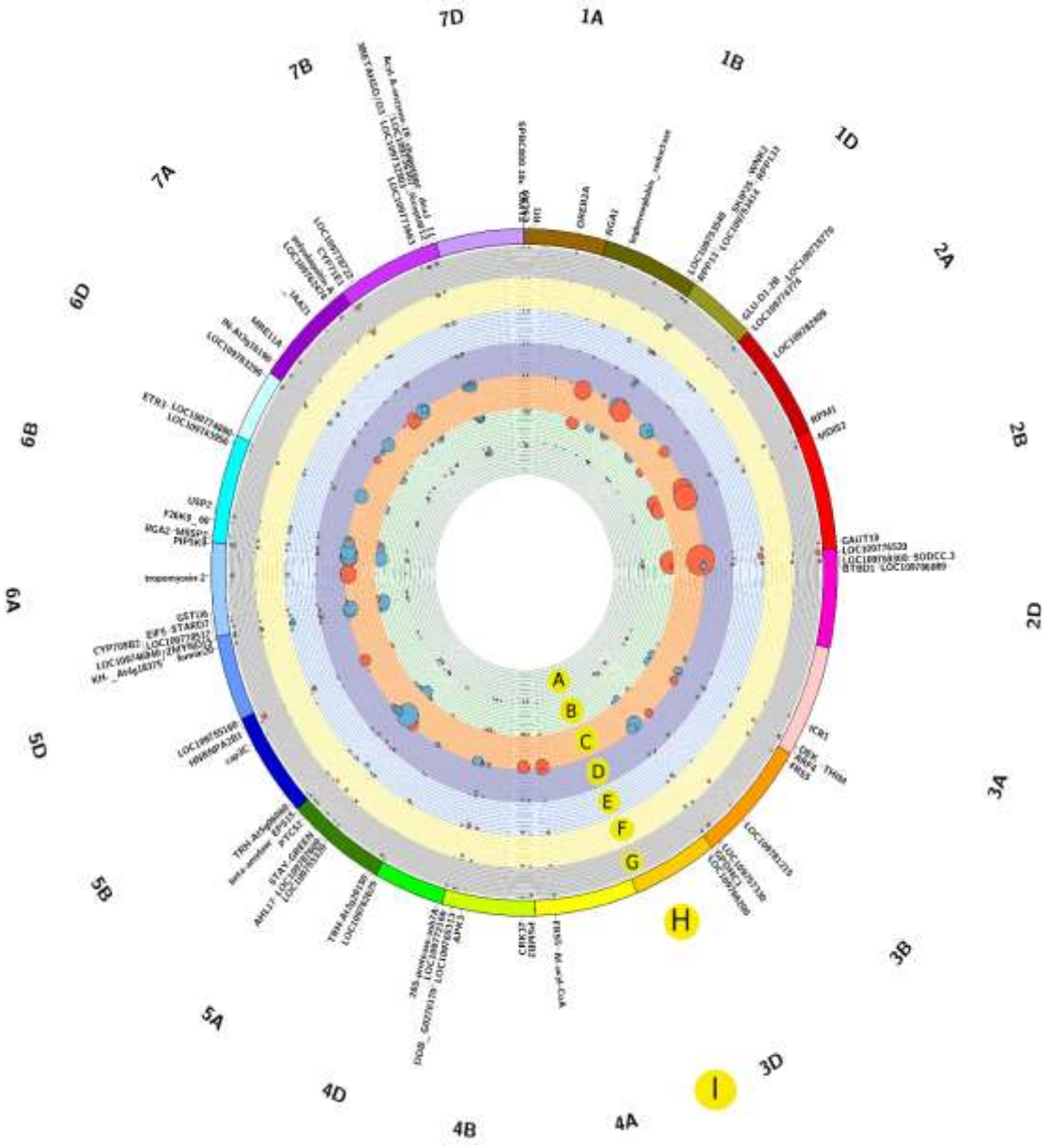

Fig. 4. The genomic distribution and GWAS analysis of the studied SNP markers. The significance value and statistical effect regarding SNP marker and their correlation with STR (A), DF (B), PH (C), NT (D), NSL (E), NS (F), SH (G) traits. The size and color of the circles indicate the p.value and effect markers. (H) Genes near these markers are shown by their chromosome location on the wheat genome (I).

By studying genes located near the SNP markers associated with wheat agronomic traits under salinity, 13 different genes were identified (**Table 5**). These genes included *CYP709B2,* which has been reported to be highly associated with salinity tolerance in *Arabidopsis thaliana*. It has been concluded that plants with mutant *CYP*709b3 may be sensitive to ABA and salt stress during germination **(Mao et al. 2013)**. Additionally, an association with BS00038820 marker was detected, which is located near *MDIS2* gene and associated with DF and PH traits. The *MDIS2* gene is highly correlated with root hair morphogenesis, which is extremely important process in the response of plants to salinity. Such associations have been detected in chickpea **(Kaashyap et al 2018)** and soybean **(Duzan et al 2004)**. The significant association between *STAY-GREEN* gene and PH trait under salinity stress is not surprising (**Table 5**). Recently, it has been reported that the STAY-GREEN gene that encodes chlorophyll-degrading Mg++-dechelatase is essential for the regulation of lifespan and yield in rice cultivars **(Shin et al 2020)**. The *PIP5K9* gene has showed a significant association with SH trait (**Table 5**). This indicated a relationship between PIP5K9 gene and wheat salinity tolerance which could be due to the importance of polyamines in stress reactions, such as drought, salinity and heat stresses, where *PIP5K9* gene is required for polyamine-triggered K+ efflux in plant roots **(Zarza et al 2020)**. As shown in **Table 5**, *MSSP2* gene is genetically near to BS00078124, which is correlated with DF trait (**Table 4**). A significant expression of *MSSP2* transport protein was reported in the phosphoproteome analysis during the study of defense mechanisms for wheat against drought stress **(Zhang et al 2014)**. A beta-amylase-related wheat gene was detected that correlated with STR trait during salinity stress (**Table 5**). It has been reported that beta-amylases are stress-induced proteins that is related to light- and stress-dependent enhancement of amylolytic activities in barley **(Dreier et al 1995)**. The promotion of wheat seed germination under salt stress could be increased by beta-amylase activity **(Duan et al 2007)**. The association between disease resistance genes and plant response to salinity stress has been recognized in different plant species **(Zhang et al 2018)**. GWAS analysis revealed a significant association with BS00024921, which is associated with DF trait and is located near the *RPM1* gene, which is a disease resistance gene that regulates a sustained increase in cytosolic calcium that is essential for oxidative bursting and hypersensitive cell death **(Grant et al 2000)**.

Table 5. List of genes located in the vicinity of trait-associated SNPs markers.

Marker	Gene full name	Gene abbreviation
BS00083630	cytochrome P450 709B2	CYP709B2
BS00038820	MALE DISCOVERER 2	MDIS2
BS00066143	STAY-GREEN	STAY-GREEN
BS00089954	LOC109781215	-
BS00021704	phosphatidylinositol 4-phosphate 5-kinase 9	PIP5K9
BS00078124	monosaccharide-sensing 2	MSSP2
BS00064146	LOC109732503	-
BS00101408	LOC109736307	-
BS00050057	LOC109755160	-
BS00107837	LOC109753414	-
BS00000006	beta-amylase	-
BS00076622	3beta-hydroxysteroid-dehydrogenase/decarboxylase	3BETAHSD/D3
BS00024921	disease resistance RPM1	RPM1

CONCLUSION

The genetic diversity and population structure of a set of local and international wheat accessions were investigated. These accessions were successfully assigned to different groups depending on their genetic background. This study revealed the genetic structure of adapted and imported wheat accessions, which could be used to select potential wheat accessions for local breeding programs. In addition, GWAS analysis was used to identify some genes that are related to wheat resistance to salinity stress. Molecular markers that could be used to select salinity-tolerant accessions could be integrated into national genetic improvement programs. The SNP genotyping assay is a very potential technology that could be efficiently applied to detect some genes that control wheat response to salinity stress.

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تحليل التركيب العشيري والارتباط على مستوى الجينوم لتحمل الملوحة في قمح الخبز بإستخدام المعلمات الجزيئية SNP و SSR و SCoT

[63]

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الموجـــــز

القمح هو غذاء ثابت وأساسي في دول العالم النامي، حيث من المتوقع أن ينمو الطلب عليه بشكل كبير في المستقبل؛ في الوقت نفسه، من المتوقع أن تؤدي التغيرات المناخية إلى تقليل المنزرع من القمح في المستقبل القريب. تعتبر الملوحة من النتائج الرئيسية لتغير المناخ، مما يؤثر سلبًا على المساحة المزروعة في العالم وبالتالي يؤثر على إنتاج القمح العالمي. أهدافنا في هذه الدراسة هي دراسة التركيب العشيري للعديد من أصناف القمح المصرية والدولية وتحديد العوامل الوراثية التي تتحكم في استجابة إجهاد الملوحة لقمح الخبز. بالإضافة إلى ذلك، حاولنا تحديد الجينات التي تتحكم في بعض القياسات الزراعية الهامة للقمح تحت ضغط الملوحة. تتكون مجموعة الأصول الوراثية للقمح من 70 نوع من القمح تم الحصول عليها من مصر وسوريا وإيران. تم إجراء تقييم تحمل الملوحة على مدار عامي 2018 و 2019 في الحقل وفي الصوبة. تم إجراء تحليل ارتباط الجينوم (GWAS) وتحليل البنية العشيرية بإستخدام ستة SCoT، وخمسة SSR و 93 معلمات SNP. أظهر تحليل التركيبة العشيرية بإستخدام تردد الأليل والتحليل الوراثي أن مدخلات القمح المدروسة تنتمي إلى أربع مجموعات عشائرية. حيث ، بالنسبة للجزء الأكبر، يتم تجميع الأنواع المصرية والسورية والإيرانية اعتمادًا على بلدهم الأصلي. كشف تحليل GWAS عن 13 معلم SNP كانت مرتبطة بشكل كبير مع صفات القمح المورفولوجية الزراعية أثناء إجهاد الملوحة. كانت هذه مرتبطة ارتباطًا وثيقًا بالجينات المعروفة بصلتها المباشرة باستجابة القمح لإجهاد الملوحة مثل جينات 2CYP709B و 2MDIS و STAY-GREEN و 9PIP5K و 2MSSP. كشفت هذه الدراسة عن التركيب الوراثي أصناف القمح المكيفة والمستوردة ، والتي يمكن استخدامها لاختيار أنواع القمح المحتملة لبرامج التربية المحلية. بالإضافة إلى ذلك ، يعد اختبار التنميط الجيني SNP تقنية محتملة للغاية يمكن تطبيقها بكفاءة لاكتشاف الجينات التي تتحكم في استجابة قمح الخبز لإجهاد الملوحة.