CONSTRUCTION OF HIGH DENSITY GENETIC MAP FOR BREAD WHEAT THROUGH GENOME WIDE ASSOCIATION ANALYSIS

By

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B. Sc. Agric. Sc. (Biotechnology), AzharUniv. (2010)
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ABSTRACT

Alsamman Mahmoud Mohammed. Construction of High Density Genetic Map for Bread Wheat Through Genome Wide Association Analysis, Unpublished P.H.D. Thesis, Department of Genetics, Faculty of Agriculture, Ain- Shams University, 2021.

Wheat (*Triticum aestivum*) 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 to identify the genetic factors controlling the salinity stress response of bread wheat. In addition, 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 were 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 technique that could be efficiently applied to detect genes that control bread wheat response to salinity stress.

Key Words: Wheat, Salinity, SNP genotyping, SSR, SCoT, GWAS.

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LIST OF ABBREVIATION

AFLP	**:**	Amplified fragment length polymorphism
CTAB	**:**	Cetyltrimethylammonium bromide
DArTs	**:**	Diversity arrays technology markers
DEGs	**:**	Differentially expressed genes
DF	**:**	Days to 50% of flowering
EC	**:**	Electrical conductivity
GWAS	**:**	Genome wide association analysis
ISSR	**:**	Inter simple sequence repeats
MAS	**:**	Markers assisted selection
NS	**:**	Number of spikes
NSL	**:**	Number of spikelets
NT	**:**	Number of tillers
PH	**:**	Plant hight
Q-RT-PCR	**:**	Quantitative reverse transcription PCR
QTL	**:**	Quantitative trait loci
RAPD	**:**	Randomly amplified polymorphism DNA
RILs	**:**	Recombinant inbred lines
RNA	**:**	Non-encoding RNAs
RT-PCR	**:**	Reverse transcription polymerase chain reaction
SCoT	**:**	Start codon targeted polymorphism
SH	**:**	Spike height
SNP	**:**	Single nucleotide polymorphism
SSR	**:**	Simple sequence repeats
STR	**:**	Salinity tolerance rate
UPGMA	**:**	Unweighted pair group method with arithmetic mean
RILs	**:**	Recombinant inbred lines
DArTs	**:**	Diversity arrays technology markers

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 km2 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 by multi-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 analyses 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 **(Vivodík *et al.,* 2020)**, 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)**. These 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 with 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.

The major objectives of this study are to investigate the population structure of several Egyptian, Syrain and Iranian wheat accessions and to identify some markers associated with salt stress tolerance in bread wheat. In addition, to identify some genes that control some important agronomic parameters of wheat under salinity stress.

REVIEW OF LITERATURE

Varieties identification is an important step in wheat breeding programs to manage and improve wheat germplasm resources. This step could be performed through a strong and efficient molecular technique, selecting elite varieties contains many valuable genes for multiple agronomic characteristics and managing the association between these agronomic traits. PCR-based molecular markers offered some valuable tools for studying the genetic polymorphism that distinguishes several wheat genotypes. The degree of polymorphism generated by these markers is high and more reliable because environmental conditions or developmental stages do not affect it. Furthermore, the use of PCR technology has enabled the production of a large number of types of molecular markers that have proved useful not only in characterizing crop accessions, but also in mapping genomes and to develop markers linked to a particular trait.

2.1 SCoT marker analysis

The assessment of genetic variability in the current germplasm collection is one of the primary objectives of breeding programs, as this may assist in the selection of cultivars and genotypes with higher diversity and improved performance under particular conditions. Molecular markers provide useful information on crop breeding, particularly in studies on genetic variability and genetic relationships between several crop species.

In particular, the PCR is used to study the , random amplified polymorphic DNA (RAPD), amplification fragment length polymorphism (AFLP), and inter-simple sequence repeats (ISSR). Furthermore, these techniques, due to their high repeatability and polymorphism, and highly informative, are appropriate for the genetic diversity studies in various plants species **(Bornet and Branchard, 2001; Moradkhani *et al.,* 2015)**. Many new alternative and competent marker technologies have been proposed in recent years. SCoT polymorphisms are reproducible markers that are focused on the short conserved region of the plant genomic regions surrounding the beginning (or initiation) of the ATG translation codon **(Collard and Mackill, 2009)**. This technique was used to assess genetic variability and population structure, cultivar identification, genetic linkage mapping and cultivar fingerprinting in several plant species, such as wheat, rice, check pea, sugarcane and grape (**Guo *et al.,* 2012; Amirmoradi *et al.,* 2012; Hamidi *et al.,* 2014; Que *et al.,* 2014**).

Etminan *et al.* (2016) assesed the applicability of SCoT and ISSR marker analyses for genetic evaluation of some durum wheat genotypes. They used a mini-core collection of durum wheat samples including 18 landraces and 25 breeding lines. The genetic diversity was evaluated using 15 ISSR and six SCoT PCR primers. The results of the genetic polymorphism observed were 98.70% and 100% for ISSR and SCoT, respectively, indicating that these markers were useful in detecting genetic differences in the collection

of durum wheat. The comparison of genetic variations in breeding lines and land races based on genetic variables was higher than in breeding lines. Although the cluster analysis , based on both markers, grouped the genotypes into five groups, the dendrogram obtained from SCoT offered the best clustering tool. Inter-population variation, measured on the basis of two marker systems, reflects that a significant portion of the cumulative genetic diversity refers to variation between two sets of genotypes. They concluded that the results confirmed a high degree of genetic variation among the durum wheat mini-core collection, in specific among landraces groups, which may be of interest to potential breeding programs.

Guo *et al.* (2016) assessed the phylogenetic relationships of Triticum and Thinopyrum species generated by CDDP and SCoT markers. They studied the phylogenetic relationships among 7 accessions of Thinopyrum species (2 *Th. Intermedium*, 2 *Th. bessarabicum*, 1 *Th. elongatum*, and 2 *Th. ponticum*), 11 accessions of Triticum species (5 *T. aestivum*, 2 *T. timopheevii*, 2 *Aeglips tauschii*, 1 *T. monococcum*, and 1 *T. turgidum*) and one accession of *Hordeum vulgare* using 10 CDDP and 17 SCoT and markers. The average number of obtained bands was 6.6 and 8.5 among the species for CDDP and SCoT markers, respectively. They generated cluster analysis among Thinopyrum species, Triticum species and Hordeum in order to produce dendrograms based on the genetic data obtained by the SCoT and CDDP markers. Their findings showed that, based on the

genetic relationships between Thinopyrum species, Triticum species and *H. vulgare*, SCoT markers were in accordance with the results of CDDP markers. Furthermore, their findings showed that the species Thinopyrum and Triticum were the nearest to each other, while *H. vulgare* was relatively far from both genera.

Yan *et al.* (2016) attempted to associate between EST-SSR and SCoT Markers and rust traits in orchardgrass (*Dactylis glomerata L.*). Using 75 orchardgrass accessions they used 18 EST-SSR and 21 SCoT markers to evaluate genetic diversity and investigate potential marker-trait associations to rust disease. A total of 164 and 289 bands were obtained for the markers EST-SSR and SCoT, of which 148 (90.24%) and 272 (94.12%) were polymorphic, respectively. They reported less genetic variance existed among populations (12.43%) than within populations (87.57%). Both the findings of a UPGMA cluster analysis and a population structure analysis were associated with the geographic distribution of the accessions.Using two years of rust trait data and 410 PCR bands from the EST-SSR and SCoT markers, 20 band panels were obtained which are associated with rust trait. They suggested that, these bands could be used in breeding programs to avoid great losses of orchardgrass caused by rust using lineage selection.

Heidari *et al.* (2017) evaluated the genomic variability in 17 durum wheat genotypes using SCoT, CBDP, and ISSR marker analyses. There results revealed that, ISSR primers produced 130 bands throughout

durum wheat genotypes with an mean of 8.12 bands per primer, while CBDP and SCoT primers provided 66 and 99 polymorphic bands with an mean of 5.5 and 7.07 bands per primer, accordingly. The values of the genetic variables obtained for all the three marker systems suggested the high efficacy of these markers in the detection of genetic variation in durum wheat. There results showed that ISSR markers with a mean of 83.46% polymorphism were an effective marker system for detecting genetic variation between genetic materials. SCoT and CBDP, on the other hand, were relatively less powerful tools for assessing genetic variation in the studied collection. They suggested that genetic research utilizing gene-targeted markers including CBDP and SCoT would be much more effective for crop improvement programs.

Abdel-Lateif and Hewedy (2018) used ISSR and SCoT analysis to study the genetic diversity of some wheat Egyptian cultivars. Their analysis included eight cultivars of Egyptian wheat (Misr-2, Giza-168, Sakha-94, Giza-171, Sakha-93, Shandweel-1, Sids-1 and Gemmiza-9) and six SCoT PCR primers. SCoT PCR analysis generated a total of 32 PCR bands, where 19 (59%) bands were polymorphic. The mean polymorphic band was 3.16. The results showed that genetic heterogeneity was successfully identified in eight Egyptian wheat cultivars using ISSR and SCoT markers. They pointed out that ISSR markers displayed higher polymorphism compared to SCoT markers and can be used in wheat breeding programs to study genetic

variability. Additionally, the results suggested good sources of diversity which will allow breeders to assess genetic diversity and possibly recognize economically significant traits such as salt stress.

Pour-Aboughadareh *et al.* (2018) applied SCoT analysis to evaluate the genetic variability among some Triticum species and Aegilops. They studied a set of 180 accessions of four species belongs to Triticum and eight species of Aegilops. They assessed the genetic variability using 15 SCoT markers. These markers produced 166 bands, of which 164 were polymorphic with 98.79% of polymorphism. Analysis of genetic variability and inter-population differentiation revealed high genetic diversity among the investigated populations. The analysis showed high genetic diversity in *Ae. cylindrica, T. boeoticum, Ae. Umbellulata* and *T. durum*, low diversity in *Ae. Caudata, Ae. speltoides* and *Ae. crassa,* and a similar relationship among *T. aestivum, Ae. tauschii, T. durum, T. boeoticum,* and *T. urartu*. Cluster analysis showed 180 accessions clustered into eight homogeneous clades and eleven sub-groups. The accessions of *T. durum* and *T. aestivum* were clustered together, and wheat accessions contain C and U genomes were clustered into the same clade. Their results support the theory that the two diploid ancestors of *T. aestivum* are *Ae. tauschii* and *T. urartu* and the possible donors of C and U genomes for other Aegilops species are *Ae. Umbellulata* and *Ae. caudata.* They concluded that, SCoT technique is useful and can be used to determine the genetic relationship among wheat

germplasm.

Pavia *et al.* (2019) reported the use of SCoT marker (8 primers) analysis in studying drought resistance in Iberian wheat cultivars during germination. The goals of their research was to investigate early drought stress resistance and to assess the genetic diversity of four bread wheat cultivars, including three modern elite germplasm lines (Jordão, Antequera, and Roxo) and an ancient Portuguese cultivar ('Mestiço') over five water potentials. Their findings showed that while the drawbacks of bulk analysis are noted, low intra-cultivar variability is expected and the genetic variation between cultivars can be evaluated. SCoT markers showed a polymorphic bands averaging 64.6%. Mestiço displayed a significant genetic difference than the cultivars of the elite. They related this to the origin of Mestiço, an ancient Portuguese wheat cultivar that was not subjected to a modern breeding program.

El-Moneim (2020) evaluated the genetic diversity and gene expression in some Egyptian wheat genotypes using ISSR and SCoT and *TaWRKY* gene expression. He used ten PCR primers (5 SCoT and 5 ISSR) to assess the genetic diversity among nine Egyptian wheat genotypes (Misr 2, Giza 168, Misr 1, Shandaweel 1, Sids 12, Sakha 95, Misr 3, Bani Seuf 7 and Sohag 4). The PCR analysis produced 141 bands, of which 72 (87.5%) and 69 (81.1%) polymorphic bands were detected by ISSR and SCoT, respectively. ISSR developed higher levels of polymorphism, suggesting its effectiveness

in separating closely related germplasms. ISSR markers displayed a higher degree of genetic polymorphism than SCoT markers, where SCoT primers 1 and 12 and ISSR primers HB-11,15 and 98-A recorded the maximum values of genetic variation parameters compared to all other primers studied. Generally, the SCoT and ISSR markers have shown their efficacy in discriminating studied wheat genotypes by producing a variety of unique and specific bands. Any of these bands may be used as markers that are correlated with drought tolerance in Egyptian wheat. The Shandaweel 1 genotype showed the maximum number of unique markers (18) and the maximum *TaWRKY* gene expression. On the other hand, the minimum number of unique markers (2) was generated in Misr 3, and revealed a low *TaWRKY* expression. He concluded that these markers could be used to measure drought tolerance in wheat, and revealed a high genetic divergence between genotypes.

2.2 SSR marker analysis

Molecular markers provide an infinite number of markers to compare different genotypes under a wide variety of environmental conditions, and provide data that can be objectively analyzed. SSRs have been widely used by codominant markers across studies because they are easily polymorphic, multi-allic, highly reproducible, and have a broad genome coverage. Impressively, SSRs, otherwise known as microsatellite markers, have been found to be efficient in studying genetic diversity, and genomic polymor-

phism as well as producing informative genetic maps in different germplasms (**Miah *et al.,* 2013; Shirnasabian *et al.,* 2014**). Therefore, SSR analysis may also play a significant role in determining the main salt tolerance controlling genes, which would be used in marker assisted selection for salt tolerance in different genotypes (**El-Hendawy *et al.,* 2019**).

Singh *et al.* (2018) Identified and developed SSR markers using known salt responsive genes in wheat. Their study identified 161 SSR motifs in 94 candidate genes for wheat salt tolerance. These SSR motifs were scattered almost evenly on the three subgenomes of wheat; 35.7% in B, 29.8% in A, and 34.4% in D subgenome. They identified 30 polymorphic SSR markers, selected for initial screening validation out of the 65 candidate genes. In a panel of wheat genotypes including salt tolerant and susceptible lines these markers were used to assess genetic diversity. Those markers averaged 2,83 alleles / locus. Phylogenetic analysis identified four clusters where salt susceptible genotypes were mainly found in clusters I and III, while the remaining two clusters represented high and moderate salt tolerant genotypes. Analysis of population structure yielded three subpopulations, subpopulation 1 contained most of the salt tolerant, while subpopulation 2 contained most of the susceptible genotypes. In addition, they found that the transferability of SSR markers to related wheat species was considerably higher.

Wheat wild relatives are possible sources of useful genetic materials

for development in wheat. Information of the genetic variability of wild relative wheat species is essential to its sustainability and utilization. **Salehi *et al.* (2018)** studied the genetic variability of intra and inter species of *Triticum monococcum* ssp. aegilopoides, *Aegilops cylindrica* and Aegilops tauschii native to western and northern Iran. In their study, thirty SSR markers corresponding to the genomes A, B , C and D belongs to wheat were used, and 20 were shown to be polymorphic between and within species. The SSR markers produced a total of 180 alleles in 21 genotypes, with a mean of 9 alleles per locus. For all loci the genetic variation varied from 0.74-0.90 with a mean of 0.83. Some SSR markers were linked with a specific genome, for example, the Xgwm205 marker displayed the greatest genetic variation and could be amplified in the A, D and CD genomes of *T. monococcum, Ae. tauschii* and *Ae. cylindrica*, respectively. Additionally, some markers produced two times the number of bands in *Ae. cylindrica* (CD) than that of *Ae. tauschii* (D). The neighbor-joining system dendrogram grouped the genotypes of the three species into three distinct groups. It can be inferred that SSR markers can be useful not only in the classification of wild wheat species with genomes A, D and C, but also in the evaluation of genetic diversity of genotypes within these species.

Comprehending the mechanistic basis of salt tolerance is important in order to increase crop yields under salinity stress. **Liu *et al.* (2018)** investigated QTLs for associated salinity-tolerance traits using correlation

analysis through SSR analysis. They treated 227 wheat varieties with artificial seawater in germination and seedling stages and evaluated different agronomic parameters as criteria to evaluate salt tolerance. They used a total of 546 pairs of SSR primers with efficient genome coverage for mapping wheat salt tolerance.They identified 24 loci that are related to salinity tolerance on 17 wheat chromosomes, of which 18 loci were unreported. Out of the total SSR markers used, 44 unlinked loci, one on each arm of the 21 wheat chromosomes, were selected to evaluate the structure of the wheat population. The result of the structure at K = 2 was the best separator with the highest delta k value. Population structure analysis highlighted two sub-populations, one comprising mainly modern cultivars, and the other was mainly landraces. Comparing the frequencies comprising various numbers of beneficial alleles in landraces and modern cultivars, they have observed that there is significant genetic flexibility in landraces for salt tolerance improvement using breeding programs.

Ghaedrahmati *et al.* (2018) identified several QTLs that are associated with salt tolerance traits wheat using SSR markers. They used a population of 254 recombinant inbred lines (RILs), generated from a cross between Sabalan × Roshan. These lines were evaluated in glasshouse during the seedling stage to investigate QTLs linked to salinity-tolerance related agronomic traits. A genetic linkage map was constructed using 225 diversity arrays technology markers (DArTs) and 14 SSRs markers that

covered a total of 1,099.7 cM. They found 31 QTLs associated with salinity tolerance in 13 wheat chromosomes, which account for more than 50% of the overall variance in phenotypic traits. Most of the QTLs identified were 3B and 5B chromosomes. SSR markers gwm626 and gwm540 were closely related to various QTLs under regulation and stress conditions and described 21.1% and 8.1% of the overall phenotypic variation, respectively. Some of these QTLs linked to genomic regions previously identified with wheat salt tolerance.

Synthetic hexaploid wheat is considered to be an excellent tool of transferring genetic variations, particularly many traits present in the D genome of Aegilops tauschii Cosson accessions (2n=2x=14, DD) to cultivated wheat (2n=6x=42, AABBDD) in order to improve its performance. **Zhang *et al.* (2018a)** aimed to evaluate 102 SSR markers associated with agronomic traits of plant height, top internode length, spike number per plant and spike length in a natural population composed of 86 synthetic hexaploid wheats and 42 common wheats. The SSR analysis generated a total of 660 alleles , where the number of alleles per locus ranged from 3 to 11 with a mean of 4.6 alleles. The structure analysis and cluster analysis revealed that studied collection composed of three subpopulations. There was a strong difference between synthetic hexaploid wheat and common wheat, suggesting that the genetic history of most synthetic hexaploid wheat varieties differs considerably relative to common wheat varieties.

The generalized linear model method was used to perform the association analysis between SSR markers and agronomic traits. They reported 20 and 17 loci to be associated with the studied agronomic traits in 2015 and 2016, respectively (p-value<0.01).

Rahmani *et al.* (2018) studied the genetic diversity and population structure of some Iranian wheat cultivars and lines SSR analysis. In their study, they used 20 SSR primers to assess the genetic diversity of 49 wheat cultivars and 99 lines. Of the primers evaluated, 19 generated polymorphism among the cultivars and lines studied, and 67 alleles were amplified in total. The maximum number of alleles per locus was 7 (Xgwm47), while the minimum was 1 (Xgwm44) with an average of 3.5. The genetic variation within cultivars and lines (89%) compared to among cultivars and lines (11%) based on the analysis of molecular variance. The phylogenetic analysis clustered the cultivars and lines in five groups, where the similarity coefficients varied from 0.40 to 1 with an average of 0.70. Geographic origin was the key factor controlling clustering, where cultivars with same origin clustered in the same cluster. They concluded that, the narrow genetic base of the Iranian wheat germplasm can show the high degree of genetic similarity observed between cultivars. However lines in divergent groups might theoretically be used as parents in wheat breeding programmes, based on the genetic distance between different groups.

Marzario *et al.* (2018) collected an ex situ durum wheat collection

from southern italy and conducted a molecular genotyping and agronomic phenotyping. They used 44 of SSR molecular markers to assess the genetic diversity for 136 accessions to characterize the gene pool of their origin and to generate comparisons with 28 Italian varieties of known pedigree. Phenotyping was performed for 12 morpho-physiological parameters. The forty-four SSR markers were distributed across the A and B durum wheat genomes and generated a total of 242 alleles through the 164 durum wheat genotypes. The population structure analysis and discriminant analysis of principal components revealed six groups, where the distribution of accessions demonstrated the genetic base and breeding procedures used in their development. They concluded that, coupling an efficient statistical analysis and comparison of pedigree varieties, a limited number of molecular markers and little phenotyping can provide adequate knowledge on the genetic structure of durum wheat germplasm for a rapid screening and identifying accessions for breeding programs.

Mahmoud *et al.* (2020) reported the molecular and generation mean analysis during wheat germination and seedling stage for salinity tolerance. P1, P2, F1 , F2, BC1 and BC2 populations of three crosses were used for the generation mean analyses for salt tolerance in wheat. At germination and seedling stage, the genotypes were assessed for seven traits under control and salinity stress (150.0 mM NaCl). Analysis of the molecular markers revealed that only three pairs of SSR primers produced polymorphic

bands from the genotypes studied. The SSR-2215bp polymorphic band only appeared in the tolerant genotypes. Such markers can be known as unique salt tolerance markers. The identified markers in this study would enable marker-assisted screening to select for salt tolerance in wheat segregating populations. Their findings indicated that the two parents, Line-6 and Sakha-8, produced salt-tolerant alleles that could be used to develop this trait in the wheat breeding programs.

Elshafei *et al.* (2019) reported the use of SSR analysis for the identification of molecular markers correlated with salinity tolerance in wheat and the evaluation of genetic diversity and morphological variability of wheat genotypes grown on saline soil. They used seven genotypes of bread wheat cultivated at Siwa Oasis and Ashmon, Menofeya Governorate, which were evaluated for their agronomic attributes under salinity stress during the 2016/2017 winter. Due to variations in salinity levels at both locations, significant difference were identified for all traits between the genotypes studied, where certain lines developed the high yields of grains. In their study, 33 SSR primers resulted in the identification of one to three alleles per primer, with an average of 1,36. The use of 31 EST-SSR markers resulted in the identification of 38 polymorphic alleles, varying from one to five, with an average of 1.23 per locus. Cluster analysis using information from SSR and EST-SSR divided the 11 wheat genotypes into three clusters.

Al-Ashkar *et al.* (2020) used a set of biochemical and morpho-

physiological parameters and SSR marker analysis to reveal phenotypic and genetic variability of 18 wheat genotypes under salinity stress using multivariate analysis. Genotypes were tested for salinity stress at 150 mM NaCl for 43 days. There findings revealed that the proportional change in genetic variance was strong for all parameters, genetic gain (>20%) and heritability (> 60%). For cluster analysis, genotypes were grouped into three groups: resistant, intermediate, and sensitive, comprising five, six, and seven genotypes. The morphological and genetic differences were strongly associated on the basis of the Mantel test. Of the 23 SSR markers that displayed polymorphism, 17 were correlated with nearly all the parameters tested. They concluded that, on the basis of the molecular marker-phenotypic trait association was found, and the markers were very useful for the identification of tolerant and sensitive genotypes. They considered these markers as a valuable tool for salt tolerance by marker-assisted selection.

ncRNA is the key regulator of eukaryotic where nucleotide variations in its sequence, may impact transcriptional and post-transcriptional gene regulation. ncRNA-derived markers can thus prove to be useful in QTL mapping, molecular breeding, and association studies of phenotype dissection. **Bhandawat *et al.* (2020)** reported a total of 661 SSRs located in pre-miRNA (15), lncRNA (621), and small nuclear RNA (25). The laboratory validation using selected wheat genotypes revealed 46 out of the developed SSRs with 100% amplification success. The genetic variability

evaluation of forty-eight Indian wheat genotypes was assessed using a 36 ncRNA-SSRs markers. An average of two alleles per SSR locus with a number of alleles ranged from 1 to 4 . The polymorphism genetic parameters indicated that ncRNA-SSRs exhibit higher polymorphism relative to genic SSRs but lower polymorphism compared to genomic SSRs. According to Jaccard's dissimilarity, the average genetic dissimilarity among genotypes of wheat was observed to be 0.29. Their study of ncRNA-SSRs in wheat was the first study that will be useful for molecular breeding and genetic enhancement of wheat.

2.3 SNP genotyping and GWAS analyses

The two major methods adopted to dissect the genetic bases of complex traits are the genome-wide association studies (GWAS) and quantitative trait loci (QTL) mapping **(Risch and Merikangas, 1996)**. In addition to the QTL mapping, GWAS provides a high-resolution, cost-effective way to explore genes and classify molecular markers. The SNP genotyping technique uses high throughput technologies to screen the whole plant genome for genetic variation in nucleotides, which could be responsible for phenotypic diversity. The interaction between SNP markers and GWAS analysis provide a useful tools for the detection of genes underlying complex agronomic traits. Such technologies enables researchers to study thousands of genetic variants and conduct complex statistical analyses to interpret a variety of important agronomic traits. Additionally, it provides breeders with hundreds of reliable genetic markers could be used as marker-assisted selection tools

in breeding programs. In addition, in July 2014, a chromosome-based bread wheat genome was released **(Consortium Internatddonal Wheat Genome Sequencing, 2014)**, while thousands of genes linked to essential agronomic traits are available in public databases, offering developing countries an enormous opportunity to quickly scan and preserve valuable wheat varieties resources and enhance their wheat breeding systems.

Sheoran *et al.* (2019) used GWAS analysis Indian spring wheat to uncover genomic regions associated with 36 agro-morphological traits in a diverse panel of 404 genotypes. They used an SNP chip array that contains 35,143 SNPs covers covering 4364.79 cM of the wheat genome. A comprehensive genome-wide association using a compressed mixed linear model identified 146 SNPs (-log10 P - 4) correlated with 23 traits and clarified 3.7–47.0% of phenotypic variance. The gene annotation and stage-specific gene expression data mined and confirmed 38 putative candidate genes. They observed strong co-localized loci for spike length, glume pubescence, plant height, and awn color on chromosome 1B, where five putative candidate genes were annotated.Their research led to the discovery of previously unreported loci for certain less studied traits in addition to the refined of several chromosomal regions with recognised loci associated with traits. In addition, their analysis provides useful knowledge on the genetic loci and their possible genes that underlie characteristics such as awn characters that are known to be significant contributors to yield development.

REVIEW OF LITERATURE

Fusarium head blight is a major wheat disease that has caused billions in losses in recent years. While major breeding efforts have been made on several continents, there are no wheat cultivars with disease immunity. Resistance is influenced by several genetic loci and is further complicated by the effect of the environment in the manifestation of the disease phenotype. **Tessmann *et al.* (2019)** reported the utilizing of GWAS technology in studying Fusarium head blight in a soft red winter wheat. Their objective was to assess the phenotypic response to Fusarium head blight in a wide, diverse soft red winter wheat population and to classify promising QTLs correlated with FHB resistance based according to GWAS analysis. They genotyped 250 lines using 90,000 SNPs. Traits evaluated were plant height, heading date, Fusarium head blight rating, incidence, severity, index, deoxynivalenol, and Fusarium-damaged kernels. The GWAS reported 16 SNPs associated with multiple chromosomes disease traits. The significance of the association SNP ranges from -2.14 to 4.01% and it distributes across chromosomes 4A, 5B and 6B. Their research has shown that even small-effect QTL can theoretically minimise disease levels and thus be useful in breeding programs.

Beyer *et al.* (2019) used GWAS analysis to identify loci and candidate genes controlling root traits in wheat. They evaluated 201 wheat accessions for five root traits. They found accessions with almost no branching and accessions of up to 132 cm of branching. The average

seminal root length ranged from 70 to 248 cm and the variance was 3.5-fold. A total of 20,881 polymorphic SNPs were chosen for GWAS analysis after filtering and imputation. The gene annotations for identified marker-trait associations revealed several genes linked to seminal axis root length (63 genes), branching (24 genes), total seminal root length (4 genes), root dry matter (8 genes), and root diameter (20 genes). These genes belong to known gene families such as aquaporin, chalcone synthase, peroxidase, chymotrypsin inhibitor, amino acid transporters, zinc fingers, and cinnamoyl-CoA reductase. Their research developed a phenotype-genotype relationship in the historical wheat population group and offered valuable knowledge of potential genetic elements influencing root characteristics.

Internationally, *ex situ* genebanks are responsible for the preservation of seeds to prevent the loss of plant genetic resources. Regular evaluation of their germination ability is essential to any gene bank, and any decrease below a certain level influences their regeneration period. The longevity of the seed varies between different species and is a quantitative trait. In this regard, **Arif and Börner (2020)** performed GWAS analysis based on SNP genotyping to study seed longevity in wheat. Using the SNP-based GWAS analysis to cover genomic regions, they attempt to provide new insights into the inheritance of this phenotype. They used 15,000 SNPs to evaluate 207 spring wheat accessions, some of which were thoroughly investigated for agronomic characteristics, longevity, dormancy and pre-harvest sprouting.

A total of 72 marker trait associations were identified that could be limited to 24 QTLs based on the similarity of the markers to each other. They also estimated that a 12.8% increase in seed longevity could be accomplished with the pyramiding of favourable alleles. Their research highlighted the significance of dense genetic maps to identify novel loci for seed longevity, covering the otherwise unidentified genome regions. In addition, such researchers will help curators of genebanks and plant breeders.

The detection of grain yield loci and related traits and the dissection of the genetic architecture are critical for enhancement of yield via marker-assisted selection. **Li *et al.* (2019)** assessed the genetic architecture of grain yield in bread wheat using GWAS. On a diverse panel of 166 elite wheat varieties from China, two GWAS techniques were used to detect stable loci and examine associations between grain yield and underlying agronomic factors. For GWAS of grain yield and related characteristics, a total of 326,570 SNP markers they selected from the wheat 90 K and 660 K SNP arrays, providing a physical distance of 14,064.8 Mb. One hundred and twenty common loci were identified using Haplotype-GWAS and SNP-GWAS, of which two were potentially active genes underlying plant height and kernel weight, seventy-eight were possibly novel and eight were at close locations to the quantitative trait loci found in recombinant inbreed line populations in previous research. Twelve wheat loci were found in eight chromosomes, of which 714.4–725.8 Mb on chromosome 3A was

strongly correlated with several studied agronomic traits. They concluded that the SNP markers found could be used for pyramiding beneficial alleles in the production of high-yield wheat varieties. Their research has shown that both GWAS approaches and high-density genetic markers are effective ways of defining grain yield loci and associated traits and have offered new insights into the genetic grain yield architecture.

Comprehensive analysis is required to achieve a better understanding of the genetic structure of the local wheat varieties. **Basile *et al.* (2019)** reported the use of GWAS analysis to evaluate Argentinean hexaploid wheat collection for adaptation and yield components. They used a group of 102 Argentinian hexaploid wheat cultivars genotyped with an array of 35.000 SNPs, grown from two to six years in three different locations, to understand the genetic basis and the interaction of yield related traits. There results revealed the genetic structure of the collected samples composed of four subpopulations, representing the background of the germplasm used in the major breeding programs in Argentina. On the basis of GWAS, ninety-seven chromosome regions were reported associated with plant height, heading date, grain number per spike, 1,000 grain weight, and fruiting performance at harvest time. Fifteen markers associated with improved fruiting efficiency at harvest values were found, of which eleven demonstrated substantial effects at all three locations evaluated. In addition, they stated that the *Ppd-D1* gene was suggested as the key determinant of the life cycle of Argentinian

wheat cultivars.

Most modern wheat cultivars have been selected on the basis of yield-related indices measured under optimum fertiliser and irrigation inputs and few have the potential to resolve medium constraining factors such as salinity. **Yu *et al.* (2020)** assessed haplotype QTL variability in Chinese wheat accessions for salt tolerance using pedigree-based kinship and marker-based analyses. In their research, a panel of 307 wheat accessions used in Chinese breeding programs and released during different periods after 1940, were introduced to a GWAS study to analyse the genetic basis of salinity tolerance. A number of 402,176 SNPs with a mean density of 0.49 Mb were used in GWAS to detect QTL for salinity tolerance. Their results reveal that, marker-based and pedigree-based kinship analyses have shown that desirable haplotypes have been introduced in certain exotic cultivars as well as in a small range of Chinese landraces since the 1940s. However, the increase in salinity resistance throughout modern breeding is not as clear as that of yield. They indicated that, there is a need to refocus resources on local landraces with elevated degrees of salinity resistance and rare favourable alleles that have not been used for breeding.

2.4 Genes conferring salinity tolerance in wheat

Salinity is a significant environmental stress that seriously affects the productivity of corps around the world. All plants undergo numerous quantitative and qualitative changes at different levels of plant differentiation,

from morphological to molecular, to overcome the negative salt stress effect **(Terletskaya *et al.,* 2019)**.

Plant exposure to excess salt causes ion imbalance causing ion toxicity, and water deficit caused by hyperosmotic stress. Plants follow various forms of salt-tolerant processes, such as osmolyte and polyamine synthesis, reducing the amount of reactive oxygen species by antioxidant protection processes, and transporting ions and their compartmentalization **(Huang *et al.,* 2012)**.

Yousfi *et al.* (2016) utilized physiological responses and gene expression to study salinity and water stress of different durum wheat genotypes. They reported that, the genes associated assessed included two transcription factors *TaDREB1A* and *TaDREB2B* for the dehydration responsive element binding, two other for the unique Na^+/H^+ vacuolar antiporter (*TaNHX1*) , and one for the cytosolic and plastidic glutamine synthetase (*TaGS1* and *TaGS2*). Strong association resulted between the genotype and growing conditions for growth, nitrogen content, and these genes expression. Generally, higher expression of *TaGS1, TaGS2, TaDREB2B* and to a lesser extent of *TaNHX1* was associated with improved genotypic efficiency in photosynthetic metabolism of growth, nitrogen, and carbon under salinity and water stress.

Through the gene chip expression study, **Al-Mashhadani *et al.* (2016)** detected and cloned *TaNIP* gene in a salt-tolerant wheat mutant

RH8706-49 under salt stress in local breeding program. In order to detect the *TANIP* salt-tolerant gene, the Q-RT-PCR technology was used to detect its expression under salinity stress in some genotypes of wheat through plant breeding program. Results have shown that the gene band is absent in salt-sensitive genotypes under salinity and non-salinity conditions. The amount and expression of *TaNIP* gene is positively correlated with salt level in the salt tolerant genotype. They reported that the selected salt-tolerant genotype had approximately the same amount and expression of the *TANIP* gene in all salinity environments, while there were no amounts and expressions of the *TANIP* gene in sensitive cultivar.

Xiong *et al.* (2017) used RNAseq analysis to study biological pathways and candidate genes associated with salinity tolerance in a wheat mutant. They reported that some mutations in sodium ion transport-related genes could directly contribute to salinity tolerance. In addition, differentially expressed gene analysis suggested that the oxidation-reduction process homeostasis is important for tolerance of saline. Key genes for salinity tolerance, such as genes encoding polyamine oxidase, arginine decarboxylase, hormone-related genotypes showed higher expression compared to control in salt-treated genotypes, indicating that these genes can play a significant role in salinity tolerance.

MYB transcription factors are a broad family of proteins associated with plant growth and stress response. **Yu *et al.* (2017)** studied the ability

of wheat salinity-induced R2R3-MYB transcription factor *TaSIM* to confer salinity tolerance in *Arabidopsis thaliana*. They used gene transformation technology to transfer *TaSIM* to *Arabidopsis thaliana* using recombinant yeast plasimd. They reported the characterization of *TaSIM* and identified of its expression patterns under a variety of abiotic stresses in various wheat tissues. The gene expression analysis has showed that *TaSIM* was induced by high salinity, drought, abscisic acid treatment, and low temperature. The results suggested that *TaSIM* has a potential for genetic modification of wheat to improve its tolerance for salt stress.

Plants have developed effective defence mechanisms against stress-induced oxidative damage, including the important role of glutathione S-transferases (GSTs). This vast class of proteins has been reported to increase under temperature and saline stress in a number of crops. **Bacu *et al.* (2017)** Studied the specific characteristics of expression in different wheat cultivars display under three different concentrations of NaCl, 50, 100, and 200mM. These cultivars were planted in Hoagland culture, and total RNA was retrieved from fresh leaves gathered at 0,3,6,10,24, and 72 hrs after the treatment with saline solution. Local cultivar Dajti, previously assessed as resistant to salt and temperature stress, was used to control the transcription of the *GSTF1* gene in the leaves. Total RNA was retrieved after one week, 30 days and 45 days. The RT-PCRs were carried out using *GSTF1*-specific primers. In conclusion, during the time of exposure to

saline conditions, the transcription of *GSTF1* at Dajti cultivar is reduced, does not depend on the salt concentration and is not affected by prolonged temperature stress.

High-affinity potassium transporters (*HKTs*) is responsible for the homeostasis of potassium and sodium ions in crops under salt stress. While some reports challenge the assumption that Na^+ exclusion leads to an improvement in salinity tolerance, *HKTs* have emerged as crucial components of tolerance to salt stress. **Kumar *et al.* (2017)** assessed the variations in cytosine methylation and their impacts on the expression of *HKT* genes in different wheat genotypes under salinity stress. They observed an increase in cytosine methylation, that is genotype-specific and tissue-specific and induced by NaCl stress. This increased cytosine methylation lowered the expression of *TaHKT2;1* and *TaHKT2;3* in the root and shoot tissues of some wheat cultivars, thus leads to better salt tolerance. Additionally, there results showed that, although *TaHKT1;4* was expressed only in roots and was less regulated by stress in salt-tolerant genotypes, it was not regulated by variations in cytosine methylation.

Mitogen-activated protein kinase (*MAPK*) cascades are triggered by a highly conserved signalling pathway and play an important role in the growth and response of plants under environmental stress. **AL-Jobori and AL-Waiely (2017)** studied the expression of the *MAPK1* and *MAPK4* genes in four wheat genotypes under salinity stress using RT-PCR. Wheat

genotypes were grown in hydroponic system for 2-3 weeks and were treated with four levels of salinity including 0 (control), 100, 150 and 200 mM NaCl. RNA was extracted from leaf tissues and primers pair (*MAPK1* and *MAPK4*) were used. There results indicated that the *MAPK1* and *MAPK4* regulated plant ability to tolerate to salinity. The expression analysis showed that the local genotypes Uruk and Furat were salt tolerant and Axad9 and Iba99 genotypes were sensitive to salinity.

Ubiquitin/26S Proteasome System (UPS) is an essential controlling mechanism for protein degradation in plants. UPS degrades a broad range of proteins in the cytoplasm and nucleus and is engaged in several processes, such as cell cycle, signal transduction, stress responses and other processes **(Craig *et al.,* 2009; Dreher and Callis, 2007) . Zhang *et al.* (2017)** extracted a wheat gene named TaPUB1 , which encodes a novel protein containing a U-box domain, WD-40 repeats, and the precursor RNA processing 19p (*Prp19*) superfamily. Results of RT-PCR showed that, *TaPUB1* transcript was cumulatively up-regulated by high salinity, drought and phytohormones, which suggested that it could play a role in the plant response to abiotic-related defense. They studied this genes regulation under salinity stress by in *Nicotiana benthamiana* by overexpressed to assess its function in the regulation of the salt stress response. *N. benthamiana* mutants with constitutively overexpressed *TaPUB1* showed a higher less growth inhibition, germination rate, and an increase in the photosynthetic

capacity compared to wild-type under salinity stress environment. These results indicated an increased tolerance of *TaPUB1*-mutant plants to salt stress compared to wild type.

Several gene families play an important role in controlling wheat response to salinity stress. **Yarra (2019)** summarized the importance of some candidate genes (*TaNHX1, TaNHX2* and *TaNHX3*) that belong to wheat *NHX* gene family for enhancing the salt tolerance. He stated that, the role of vacuolar *NHX* antiporters in plants is characterised and expressed in heterologous systems to enhance the tolerance of salinity stress. Where a total of three *NHX* vacuolar genes have been identified from the wheat genome, among which, *TaNHX2* plays a critical role in overcoming salinity bad effect on plant growth. Author has suggested potential prospects for engineering the higher plant genome with wheat *NHX* genes to influence a sustainable food production in areas affected with salinity.

Amirbakhtiar *et al.* (2019) studied the root gene expression of Iranian bread wheat salt tolerant cultivar under salt stress. They compared the abundance of wheat genes, of which 5,128 genes were expressed differently due to salt stress. A panel of novel genes that are differentially expressed under salinity have been identified and a model is proposed for salt stress response in this salt-tolerant wheat cultivar. Coding genes for Ca^{+2} transporters such as *Ta.ANN4, Ta.ACA7, Ta.NCL2* that regulate the concentrations of cytosolic Ca^{+2} have been shown to be up-regulated.

Cytosolic Ca^{+2} is the primary secondary messenger molecule in plants under stress conditions, including salinity stress. Additionally, they highlighted the potential of several transcription factors such as *NAC, MYB, bHLH, bZIPs, WRKY,* and *AP2/ERF*. There achieved results could be beneficial for a deeper understanding and developing of salt tolerance in wheat.

The basic leucine zipper (bZIP) is among the largest and most diverse transcription factor (TFs) families. They are believed to play an important role in both stress and plant development processes. **Agarwal *et al.* (2019)** identified 191 bZIP transcription factors in bread wheat. Expression analysis during different stress conditions, different varieties, developmental stages, and gene ontology enrichment analysis showed their potential roles in abiotic stress and crop developmental responses. They *TabZIP* gene, which is a member of bZIP family to study its role under various abiotic stress conditions. They reported differential expression of *TabZIP* in several abiotic stress conditions like salinity, heat, and dehydration demonstrated the potential role of bZIP in different stress mitigation mechanism. Additionally, arabidopsis mutants with overexpressing *TabZIP* demons-tarted improved tolerance to salinity, heat, drought, and oxidative stress. Finally, they concluded that, *TabZIP* can be used as a possible gene for enhancing salinity, heat, drought and other abiotic stress tolerance and could be useful in improving the crop production under stress environments.

The cytochrome P450 monooxygenase genes (CYPs) are involved

as one of the largest gene families in plants in diverse biological processes including biotic and abiotic stress response. **Wang *et al.* (2020)** used RNA extraction, transcriptional profiling, and transcriptome sequencing to study genes belong to this family controlling wheat salinity tolerance. They found that *TaCYP81D5* and *BdCYP81D1* genes have important role in wheat salinity tolerance process. The introduction of salinity stress could up-regulate *TaCYP81D5*, but in plants treated with a reactive oxygen species synthesis inhibitor, the effect was abolished. Expressing *TaCYP81D5* increased salinity tolerance at both seedling and wheat reproductive stages by accelerating ROS scavenging. Although knockout of *TaCYP81D5* alone showed no effect on salinity tolerance, knockdown of *BdCYP81D1* or all of the cluster's *TaCYP81D* members caused the sensitivity to salt stress.

To elaborate the inter-specific similarity and resistance mechanism difference between the wheat and the barley against salinity stress, **Zeeshan *et al.* (2020)** hydroponically grown some tolerant and sensitive wheat and barely genotypes under a greenhouse condition with 100 mM NaCl. Secondary metabolites, Glutathione, and genes associated with Na^+ transport, detoxification, and plant protection were evaluated to discriminate between species / cultivar difference in responding to salinity stress. Expression of the *HKT1, HKT2, SOS1, AKT1,* and *NHX1* genes was significantly differentiated between salinity tolerant and sensitive wheat cultivars.In addition, glutathione homeostasis and upregulation of the transcription

factor *TaWRKY10* played a key role in salt-tolerant wheat cultivars. This new finding may help to improve salinity tolerance in wheat and barley cultivars.

Alla *et al.* (2020) assessed the reducing the impacts of salinity on production of wheat, antioxidants and selenium homeostasis ROS. Wheat seeds were treated with 15 M Se and NaCl at 75, 150, and 225 mM. There results demonstrated that NaCl strongly decreased growth of wheat seedlings, K/Na ratio, K^+ , ascorbic acid , soluble sugars and glutathione. Additionally, activities of ascorbate peroxidase, catalase, peroxidase, and the reduced glutathione reductase, and ribulose-1.5-bisphosphate carboxylase/oxygenase (Rubisco) have been inhibited. Similarly, they detected decreases in the expression of salt overly sensitive (*SOS1*), the alternative oxidase (*AOX*), and sodium hydrogen antiporter (*NHX1*) genes. The concentration of NaCl on wheat was associated with its impact. However, soaking wheat grains at Se mitigated salinity injury. There findings suggest that Se improves the resistance of wheat to NaCl stress by enhancing antioxidants and by over-expression of Na-manipulating genes to deal with harsh environments.

Tiwari *et al.* (2020) reported the assessment of physiological traits and expression of *SOS3* and *NHX* genes under salinity stress in bread wheat. Hydroponic experiments in a phytotron were performed to determine the effect of salinity on physiological traits and to analyse gene expression using qRT-PCR analysis. A wheat genotype, was grown under various

salt levels of 100, 200 and 250 mM NaCl in hydroponic. Several plant parameters were documented during the experiment, including carotenoid content, chlorophyll content, root area, relative water content and root diameter. They concluded that, salinity stress has negatively impacted several physiological traits including chlorophyll and carotenoid contents, relative water content, and root traits. Additionally, gene expression analysis using qRT-PCR revealed that, genes correlated with sodium ion homeostasis (*NHX* and *SOS3*) were signficantely up-regulated in root tissues of wheat under salinity stress.

MATERIAL AND METHODS

3.1 Material

This study was carried out in the green house of Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC), and the field experiment at faculty of environmental agricultural sciences, Suez university in Arish , Sinai, Egypt, during the period from 2018 to 2020. The studied germplasm panel consisted of 70 bread wheat (*Triticum aestivum*) accessions were 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 as shown in Table (1).

3.2 Methods

3.2.1 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 of 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 replicates using Alpha Lattice design. In the field, an irrigation system of dripping water was installed and the field was irrigated one time every two

Table (1): Identity No (IN), ICARDA bank Identity No (IBIN), seed identity No (SIN), and country of seventy bread wheat genotypes used in this study.

IN	IBIN	SIN	Country	IN	IBIN	SIN	Country
WA1	98815	SEEDICAR2562	Syria	WA35	122010	SEEDICAR881	Iran
WA2	98819	SEEDICAR2462	Syria	WA37	122033	SEEDICAR848	Iran
WA3	98824	SEEDICAR2488	Syria	WA38	122050	SEEDICAR18170	Iran
WA4	98825	SEEDICAR2455	Syria	WA39	122072	SEEDICAR18192	Iran
WA5	98826	SEEDICAR2513	Syria	WA40	122113	SEEDICAR18229	Iran
WA6	107098	SEEDICAR17853	Iran	WA41	122114	SEEDICAR18230	Iran
WA7	121880	SEEDICAR828	Iran	WA42	122115	SEEDICAR18231	Iran
WA8	121885	SEEDICAR959	Iran	WA43	122116	SEEDICAR18232	Iran
WA9	121887	SEEDICAR961	Iran	WA44	122129	SEEDICAR18244	Iran
WA10	121890	SEEDICAR964	Iran	WA45	-	WAEGY1242	Egypt
WA11	121903	SEEDICAR976	Iran	WA46	-	WAEGY1243	Egypt
WA12	121913	SEEDICAR936	Iran	WA47	-	WAEGY1244	Egypt
WA13	121919	SEEDICAR942	Iran	WA48	-	WAEGY1245	Egypt
WA14	121935	SEEDICAR910	Iran	WA49	-	WAEGY1246	Egypt
WA15	121937	SEEDICAR912	Iran	WA50	-	WAEGY1247	Egypt
WA16	121945	SEEDICAR919	Iran	WA51	-	WAEGY1248	Egypt
WA17	121947	SEEDICAR921	Iran	WA52	-	WAEGY1249	Egypt
WA18	121963	SEEDICAR889	Iran	WA53	-	WAEGY1250	Egypt
WA19	121974	SEEDICAR899	Iran	WA54	-	WAEGY1251	Egypt
WA20	121976	SEEDICAR901	Iran	WA55	-	WAEGY1252	Egypt
WA21	121977	SEEDICAR902	Iran	WA56	-	WAEGY1253	Egypt
WA22	121987	SEEDICAR854	Iran	WA57	-	WAEGY1254	Egypt
WA23	121990	SEEDICAR861	Iran	WA58	-	WAEGY1255	Egypt
WA24	121991	SEEDICAR862	Iran	WA59	-	WAEGY1256	Egypt
WA25	121994	SEEDICAR865	Iran	WA60	-	WAEGY1257	Egypt
WA26	121995	SEEDICAR866	Iran	WA61	-	WAEGY1258	Egypt
WA27	121996	SEEDICAR867	Iran	WA62	-	WAEGY1259	Egypt
WA28	121998	SEEDICAR869	Iran	WA63	-	WAEGY1260	Egypt
WA29	121999	SEEDICAR870	Iran	WA64	-	WAEGY1261	Egypt
WA30	122000	SEEDICAR871	Iran	WA65	-	WAEGY1262	Egypt
WA31	122001	SEEDICAR872	Iran	WA66	-	WAEGY1263	Egypt
WA32	122006	SEEDICAR877	Iran	WA67	-	WAEGY1264	Egypt
WA34	122009	SEEDICAR880	Iran	WA68	-	WAEGY1265	Egypt
WA36	122012	SEEDICAR19129	Iran	WA69	-	WAEGY1266	Egypt
WA33	122007	SEEDICAR878	Iran	WA70	-	WAEGY1267	Egypt

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 about 344, and 904 ppm, respectively, and 848 ppm at depths of more than 60 cm. The morpho-agronomic traits such as 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 to 5,844 ppm (pH 8). The plant salinity tolerance rate (STR) was measured in the range of one (normal) to five (dead). Throughout the trial, plant performances were scored three times, at an interval of two weeks.

3.2.2 DNA extraction

DNA was extracted from young leaves of four to six week old seedlings using the Cetyltrimethylammonium Bromide (CTAB) method **(Rogers and Bendich, 1989)** as following:

1. After chilling in liquid nitrogen at room temperature the plant samples were prepared by grinding tissue in a mortar and pestle.
2. For every 100 mg homogenized tissue a 500 µł of CTAB extraction buffer was used. Mixture was thoroughly blended with a vortex. The homogenate was incubated for 30 minutes to a 60 °C bathroom.
3. After incubation, the homogenate was centrifuged at 14,000 x g for 5

minutes.

4. The supernatant was transferred to a new tube, where a 5 µł of RNase solution A was added and incubated at 37 ° C for 20 minutes.
5. An equal amount of chloroform / isoamyl alcohol (24:1) was added and mixed with a vortex for 5 seconds and centrifuged at 14,000 x g for 1 minute to separate the phases. The upper aqueous phase was transferred to a new tube. This step was repeated until the upper phase was clear.
6. The upper aqueous phase was transferred to a new tube. DNA was precipitated by adding a 0.7-volume of cold isopropanol and incubating at -20 °C for 15 minutes.
7. Samples were centrifuged at 14,000 x g for 10 minutes. The supernatant was decanting without disturbing the pellet and washed away with a 500 µł of ice cold ethanol (70%) . Ethanol was decanted, and residual ethanol was removed by drying at room temperature.
8. The pellets were dried long enough to remove alcohol, but without completely drying up the DNA. DNA was dissolved in a 20 µł of TE buffer (10 mM Tris, pH 8, 1 mM EDTA).

Agarose gel electrophoresis was performed with 1% agarose (Bioline) dissolved in 1x TBE buffer and stained by ethidium bromide staining (Sigma) to test the extracted genomic DNA. A 10 µł of each sample was

loaded while the electrophoresis conditions were set at 100 V for 30 min.

Ten-X Tris-Boric-EDTA (TBE) was prepared according to **Sambrook *et al.* (1989)** as following:

Reagent	**Quantity**	**Final Concentration**
Tris base	121.1 g	1 M
Boric Acid	61.8 g	1 M
EDTA	73.4 g	0.02 M
pH was adjusted to	8.3	

3.2.3 Molecular markers analyses

Through PCR-based analyses, five SSR and six SCoT primers were applied (Table 2). SSR and SCoT markers were designed according to **Somers *et al.* (2004)** and **Collard *et al.* (2009)**, respectively. The reactions of SSR and SCoT PCR analyses were performed in a 15 µł volume of reaction contained a 5 ng of DNA, a 10 pmol of each primer, a 2 mM of dNTPs, a 25 mM of MgCl2, a 0.1 unit of Taq DNA polymerase, and a 10X of PCR buffer. The SSR PCR program included an initiation step of 95 °C (5 min) and for 35 cycles, 95 °C (15 sec), 55 °C (15 sec) and 72 °C (30 sec). The final step was 72 °C (5 min). 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)**. The SCoT PCR program included an initiation step of 94 °C (3 min), followed by 36 cycles of 95 °C (50 sec), 50 °C (1 min) and 72 °C (2 min). The final step was 72 °C (5 min). 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.

Table (2): 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	-

The SNP panel of DarT® company (Triticarte Pty. Ltd. Australia) was applied to the 44 Syrian and Iranian wheat accessions. The DNAs were sent to a marker genotyping as a supplier for profit-oriented service. A 93 SNP marker loci (Table 3) were used for GWAS analysis. The BLAST software **(Altschul *et al.,* 1997)** was used to locate SNP markers on wheat chromosomes **(Consortium International Wheat Genome Sequencing, 2014)**.

3.2.4 Statistical and genetical 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 the 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)**.

Table (3): Sequences and code names of DNA primers used for the SNP genotyping of wheat genotypes, where targeted SNPs are surrounded by square brackets.

Name	Sequence
AX-86163814	TTCTGTTAGGCATGG[R]AACTCTTCTCTGTTT
AX-86167869	TCACTTGTCACTGCC[K]GTGCTCAAAGTCATC
AX-94382081	TTTGCGAAAGGGGCT[S]AAGCTAGTAGTTCGG
AX-94392216	TCCATTTGTACCTAA[K]CTGTGTAGTTGGTAA
AX-94401211	CACCTACAAGCTAAT[R]ATAAGGAAGCAGTTA
AX-94406983	ATCTTTACCACCTGG[S]CTTCTTGCTTTCTAT
AX-94415898	TAACACACACAGTTG[R]TGCTTAAACTGATTC
AX-94431524	GATAAGGTGCATGAA[R]GTCGTCTGATCTACT
AX-94442305	GTCATCTAGTAAGGA[R]ACCAAATCACTCATC
AX-94446956	GGAATTGTGGGTCGA[K]CAAGAGATGGTTCGT
AX-94454241	GTTCTTGTGCTTGAG[Y]GTCTTAAGCAACCGC
AX-94457966	ATACACTGAGATTTC[Y]TGGAGATGTTGGTCG
AX-94486277	TACAATGCATAGAAC[K]AGTGGTTATGTTGTG
AX-94488939	AGACTTAAATGGACT[M]CCAGAGGCACTTCTT
AX-94527869	CCTATAGGTACACTG[Y]AGGATGCGAAACTTA
AX-94529943	GCGATACACATGCCC[Y]GCCATCCGCGGATGA
AX-94540417	TGTATTCTGTTCTGA[S]ATCGTTTACACAGGA
AX-94544363	GAAGGAGGTGACCAG[R]AAGAAGAACGAGACG
AX-94545917	CTGAACCCTTCCTGT[Y]AATTGTTTCCGAGTA
AX-94558874	CCAGCAGCTTCATTC[S]TCACCGGCCAGGTCA
AX-94559367	CCCTGAGGGAGTGCT[Y]CAAGGGAGGGGGTTT
BS00049977	AAAGGAATTTCCTGG[Y]GTAGTACATTAGGAT
BS00000006	TCCCGCAGTGGGTGC[K]GAATGTCGGTGCGAG
BS00018707	AAGTCCAAAATCCGC[R]ATTCTTGGGTTCATG
BS00021704	TCACTTTTCAGTGCC[Y]GCTTTACCGTTGCAG
BS00021745	CACGACAGAAGCAAC[R]CGTTTGCGAGGTTTG
BS00022411	ATCTATGACTATCTA[K]GAATTTGTATCTCCT
BS00022625	TTTCTTTTTGTTGTG[R]GCTTGTTTCGTATGC
BS00022653	TGTAGTTTATGCTTA[M]TCACTTTGGCTGAAA
BS00023673	TTGCCGGCTGATGGA[Y]CTTAAAAGCGGCACT
BS00024548	TTGCCATCCATATTT[R]CATGCCCCATGAATA
BS00024786	CTCCCCATTCAGTCC[Y]GACAAATGTAAATAT
BS00024921	TCACAACAAGCGCAC[R]CAAAATTAGCAGCAC
BS00025017	GAGCAGACTGTAGAG[C/T]TTTTACAATGGCAAG
BS00030651	TGACCGGACCCTGTA[Y]GCCGACGAGATTTTG

Table (3): Continue.

Name	Sequence
AX-86163814	TTCTGTTAGGCATGG[R]AACTCTTCTCTGTTT
AX-86167869	TCACTTGTCACTGCC[K]GTGCTCAAAGTCATC
AX-94382081	TTTGCGAAAGGGGCT[S]AAGCTAGTAGTTCGG
AX-94392216	TCCATTTGTACCTAA[K]CTGTGTAGTTGGTAA
AX-94401211	CACCTACAAGCTAAT[R]ATAAGGAAGCAGTTA
AX-94406983	ATCTTTACCACCTGG[S]CTTCTTGCTTTCTAT
AX-94415898	TAACACACACAGTTG[R]TGCTTAAACTGATTC
AX-94431524	GATAAGGTGCATGAA[R]GTCGTCTGATCTACT
AX-94442305	GTCATCTAGTAAGGA[R]ACCAAATCACTCATC
AX-94446956	GGAATTGTGGGTCGA[K]CAAGAGATGGTTCGT
AX-94454241	GTTCTTGTGCTTGAG[Y]GTCTTAAGCAACCGC
AX-94457966	ATACACTGAGATTTC[Y]TGGAGATGTTGGTCG
AX-94486277	TACAATGCATAGAAC[K]AGTGGTTATGTTGTG
AX-94488939	AGACTTAAATGGACT[M]CCAGAGGCACTTCTT
AX-94527869	CCTATAGGTACACTG[Y]AGGATGCGAAACTTA
AX-94529943	GCGATACACATGCCC[Y]GCCATCCGCGGATGA
AX-94540417	TGTATTCTGTTCTGA[S]ATCGTTTACACAGGA
AX-94544363	GAAGGAGGTGACCAG[R]AAGAAGAACGAGACG
AX-94545917	CTGAACCCTTCCTGT[Y]AATTGTTTCCGAGTA
AX-94558874	CCAGCAGCTTCATTC[S]TCACCGGCCAGGTCA
AX-94559367	CCCTGAGGGAGTGCT[Y]CAAGGGAGGGGGTTT
BS00049977	AAAGGAATTTCCTGG[Y]GTAGTACATTAGGAT
BS00000006	TCCCGCAGTGGGTGC[K]GAATGTCGGTGCGAG
BS00018707	AAGTCCAAAATCCGC[R]ATTCTTGGGTTCATG
BS00021704	TCACTTTTCAGTGCC[Y]GCTTTACCGTTGCAG
BS00021745	CACGACAGAAGCAAC[R]CGTTTGCGAGGTTTG
BS00022411	ATCTATGACTATCTA[K]GAATTTGTATCTCCT
BS00022625	TTTCTTTTTGTTGTG[R]GCTTGTTTCGTATGC
BS00022653	TGTAGTTTATGCTTA[M]TCACTTTGGCTGAAA
BS00023673	TTGCCGGCTGATGGA[Y]CTTAAAAGCGGCACT
BS00024548	TTGCCATCCATATTT[R]CATGCCCCATGAATA
BS00024786	CTCCCCATTCAGTCC[Y]GACAAATGTAAATAT
BS00024921	TCACAACAAGCGCAC[R]CAAAATTAGCAGCAC
BS00025017	GAGCAGACTGTAGAG[C/T]TTTTACAATGGCAAG
BS00030651	TGACCGGACCCTGTA[Y]GCCGACGAGATTTTG
BS00031140	ACATACAGACCACTA[Y]TAAAACCAAAAATAC
BS00031178	TATGTTGTCTCCTTT[Y]CATTCATTTGTCATG
BS00032039	CCCGGTGATTTCACT[K]TAACATGAGTAAGGA

Table (3): Continue.

Name	Sequence
BS00040283	CTGCTCCATCATCTC[R]TGGTCCAGGTGAAGA
BS00040798	TGGATCGATGCGCTG[R]TGTTTACTGCATTTT
BS00042105	CAACAACTTCATTCG[Y]CCGCTCGCTAGGGGT
BS00043169	CCCTATATGTGCGAC[A/C]GTTGATTTCTTTTGT
BS00033795	CAGCGCCGTCGCTTC[Y]AGGAGATCCAGCCCG
BS00035234	TAGTGCAAACTGAGT[R]TACTGGGTTCAAAAG
BS00037020	ACAACCCCCATTGGA[K]AGGGATTTCTAAAGA
BS00038820	GATAGCATACTGCCT[Y]GAGCAAATGCACAAG
BS00039211	GAGCTAGTAGTGATG[T/C]ATTGGTCAGATCGAT
BS00040283	CTGCTCCATCATCTC[R]TGGTCCAGGTGAAGA
BS00040798	TGGATCGATGCGCTG[R]TGTTTACTGCATTTT
BS00042105	CAACAACTTCATTCG[Y]CCGCTCGCTAGGGGT
BS00043169	CCCTATATGTGCGAC[A/C]GTTGATTTCTTTTGT
BS00033795	CAGCGCCGTCGCTTC[Y]AGGAGATCCAGCCCG
BS00035234	TAGTGCAAACTGAGT[R]TACTGGGTTCAAAAG
BS00037020	ACAACCCCCATTGGA[K]AGGGATTTCTAAAGA
BS00038820	GATAGCATACTGCCT[Y]GAGCAAATGCACAAG
BS00039211	GAGCTAGTAGTGATG[T/C]ATTGGTCAGATCGAT
BS00031140	ACATACAGACCACTA[Y]TAAAACCAAAAATAC
BS00031178	TATGTTGTCTCCTTT[Y]CATTCATTTGTCATG
BS00032039	CCCGGTGATTTCACT[K]TAACATGAGTAAGGA

RESULTS AND DISCUSSIONS

4.1 Field performance of the wheat collection under salinity stress

Forty-four foreign international accessions (Syrian and Iranian genotypes) were chosen for the assessment of salinity tolerance over the years of 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 performance of these accessions under salinity stress was evaluated according to seven agronomic traits (Table 4). The average value of PH, NT, DF, NS, SH, NSL, STR was 77.88, 5.15, 13.77, 5.07, 5.96, 11.00 and 3.02, where accessions of WA-41, WA-3, WA-9, WA-7, WA-6, WA-27, and WA-37 have the maximum values according to these field measurements, respectively. The clustering heatmap analysis (Figure 1A) revealed clear relationship between PH and DF, and SH and NSL. On the other hand, STR was linked to NT and NS. The association between PH and DF under salinity stress was previously reported in wheat (**Nia *et al.*,2012**). The effect of STR on NT and NS could indicate the high effect of salinity on these parameters. Such findings may suggest the relation between these field parameters and notify wheat researchers that they are highly correlated under salinity stress. The principle component analysis (PCA) revealed that some wheat accessions (WA-8, WA-3, WA-7, WA-30, and WA-16) performed differently according to filed measurements 1B).

This could be indicate their variable performance under salinity stress.

4.2 Genetic polymorphism of the PCR markers

Studying the genetic diversity of local and international genotypes of wheat could benefit local breeding programs by enriching their genetic resources with more adaptive and stable genotypes. PCR-based techniques such as SCoT and SSR could provide different but complementary information regarding wheat evolutionary adaptation to environment. In this regard, a total number of 61 PCR-bands were revealed using SSR and SCoT primers, where SCoT analysis provided a higher number of bands (46 bands) compared to SSR analysis (15 bands) as shown in Figures (2 and 3) and Table (5). The maximum number of bands was obtained from SCoT-05 primer (10 bands). Additionally, the total number of polymorphic bands was 48 bands, where SCoT-10, and SCoT-01 primers revealed the maximum number of polymorphic bands (eight bands). The PCR primers of SCoT-02 and SSR-01 revealed the maximum percentage of polymorphism (100%) (Table 5 and Figure 2 and 3). On the other hand, out of the 91 SNP primers used for SNP genotyping, only 20 makers were monomorphic (Table 6).

Etminan *et al.* (2016) used six SCoT primers to study the genetic diversity of several durum wheat genotypes, where they obtained 54 PCR bands with a polymorphism percentage of 100%. SCoT analysis was used to assess the genetic variability of some Egyptian wheat cultivars, where 32 bands with a 59% of polymorphism percentage using six SCoT

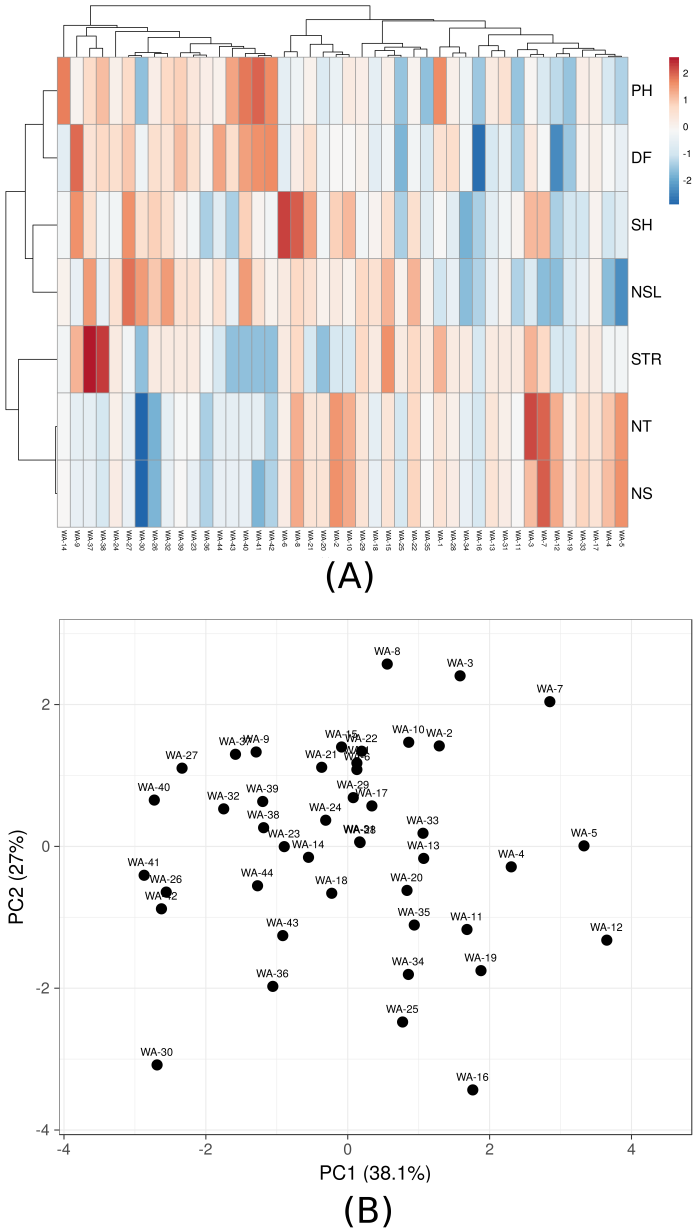

Fig. (1): The clustering heatmap analysis (A) and principle component analysis (B) of the studied wheat accessions according to their field performance under salinity stress.

primers were detected **(Abdel-Lateif and Hewedy, 2018)**. In addition, 14 SCoT primers were used to study the population structure of 17 durum wheat genotypes, that generated a total of 118 bands with a polymorphism percentage of 83.24% **(Heidari *et al.,* 2017)**. SCoT was used to identify the allelic variation among multiple olive genotypes, a moderate ability of SCoT markers to detect genetic variation compared to other molecular analyses was recorded **(Alsamman *et al.,* 2017)**. The genetic diversity of 480 bread wheat accessions, chosen from 15 European countries or geo-graphic groups, were genotyped using 39 polymorphic SSR primers. These SSRs generated 635 PCR bands with a 72% of polymorphism, where the number of bands was ranged from 40 to 4 bands **(Roussel *et al.,* 2005)**.

4.3 Genetic diversity and population structure

In this study, our aim is to identify the population structure of several genotypes collected from different geographical areas. SSR, SCoT and SNP markers were used to compare genetical to geographical origin of the used genotypes (Figures 4, 5 and 6).

Population structure analysis through STRUCTURE is widely conducted using Markov Chain Monte Carlo (MCMC), which uses genetic allele frequencies to allocate individuals to different groups **(Pritchard *et al.,* 2000)**. Analysis of population structure involved allocating each individual to a group in a population, and reporting the number of clusters was done.

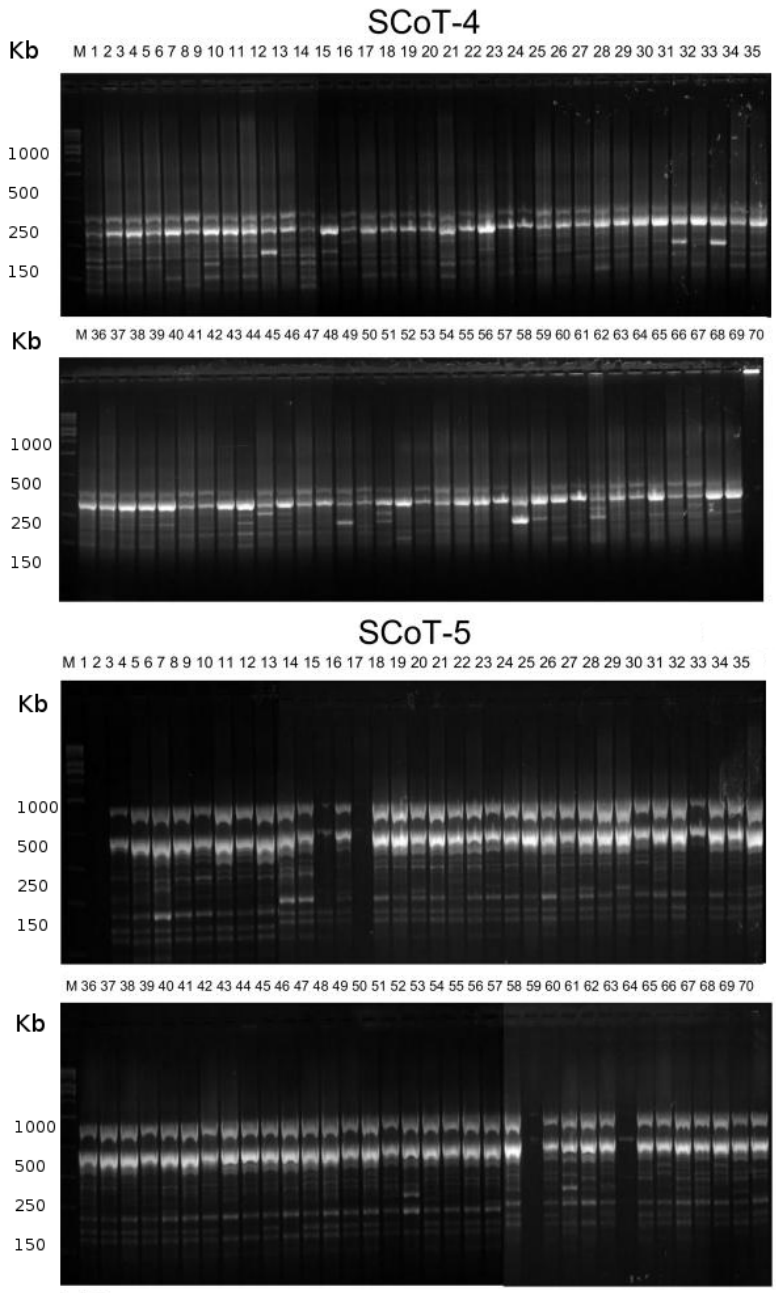

Fig. (2): Gel electrophoresis profiles of the 70 studied wheat genotypes studied using SCoT primers.

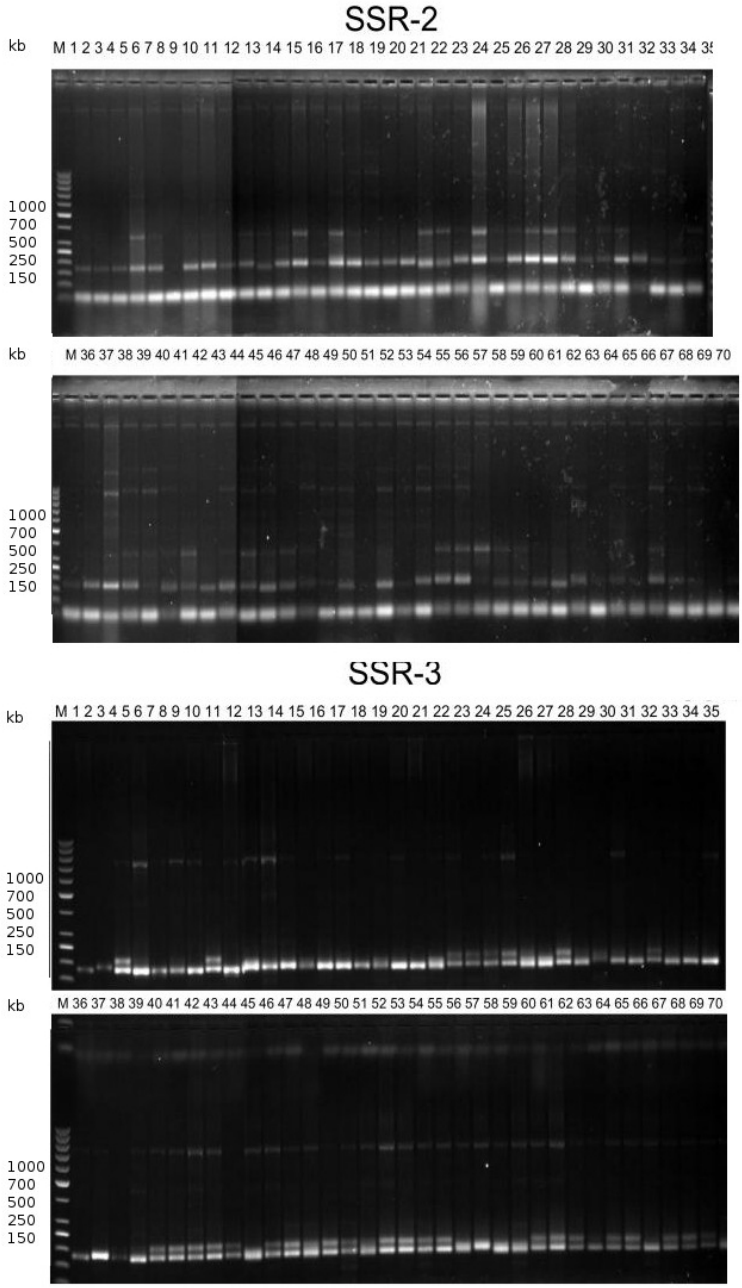

Fig. (3): Gel electrophoresis profiles of the 70 studied wheat genotypes using SSR primers.

Table (4): Field performance of the bread wheat collection under salinity stress according to plant hieght (PH), number of tillers (NT), days to 50% of flowering (DF), number of spikes (NS), spike height (SH), number of spikelets (NSL), and salinity tolerance rate (STR).

Plant No.	PH	NT	DF	NS	SH	NSL	STR
WA-1	87.67	5.33	15	5.33	5.5	9.33	3.67
WA-2	72.67	6.67	12	6.67	6.67	11	2.33
WA-3	75	7.67	14	6	7	9.67	3.67
WA-4	69.67	6	11.67	6	4.67	8.67	2.67
WA-5	67	6.67	10.33	6.67	5	8	2.67
WA-6	71.67	5	10	5	8.67	11	3
WA-7	70	7.33	11.67	7.33	7	8.67	3.33
WA-8	73.33	6.33	13.33	6.33	8.33	11.67	3.33
WA-9	75.33	4.33	24.67	4.33	7.67	10	3.67
WA-10	77.33	6.33	12	6.33	7	10.67	2.33
WA-11	65.67	4.67	7.67	4.67	5.67	9	3
WA-12	67.67	6.33	5.33	6.33	4.33	8.67	2.33
WA-13	77.33	5.33	9	5.33	5	10	3
WA-14	88.33	4.67	10	4.67	5.33	10.67	2.67
WA-15	76.33	5.33	11.33	5.33	6.33	12.33	4
WA-16	66	4	4.67	4	4.33	9	2.33
WA-17	76	5.33	13	5.33	5.67	10.67	3
WA-18	71.67	4.33	10	4.33	5.67	11.33	3
WA-19	65.67	5	7.33	5	4.67	9.67	2.33
WA-20	70.33	5.33	11	5.33	5.33	10.67	2
WA-21	75.67	5.33	15.33	5.33	7.33	11.33	2.33
WA-22	75.33	5.67	11.33	5.67	6.33	12.33	3.33
WA-23	78	4.67	15.33	4.33	5.33	11.33	3
WA-24	76	5	15.33	5	5.33	11.33	3
WA-25	65.67	4	6.67	4	4.33	10.67	3
WA-26	76.67	3.33	15.33	3.33	6.67	12	3
WA-27	80.33	4.33	17.67	4.33	7.67	13.33	2.67
WA-28	76.33	5	15.67	5	5	10	3
WA-29	76.67	5.33	12.67	5.33	5.33	11.67	3.33
WA-30	65.33	2.67	11	2.67	6.33	12.67	2
WA-31	79.33	5	10.67	5	5.33	10.67	3
WA-32	81	4.33	14.67	4.33	6.33	12.67	3
WA-33	76.67	5.67	12.33	5.67	4.67	10	3
WA-34	73.33	4.33	11.33	4.33	4	8.67	3
WA-35	65.33	4.67	9.33	4.67	5	10.67	3
WA-36	76.67	3.67	12.33	3.67	4.33	10.67	2.67
WA-37	80.33	4.33	14.67	4.33	6.33	12.67	5
WA-38	83.33	4	16.33	4	5.33	10	4.67
WA-39	81.67	4.67	18.33	4.67	5.67	11.33	3
WA-40	89	4.33	20.67	4.33	6.67	12.67	2
WA-41	90.33	4.33	21.67	3.33	5.67	11.33	2
WA-42	87.67	3.67	22.33	3.67	5.33	10.67	2
WA-43	85.33	4.33	15.67	4.33	4.33	10	2
WA-44	75.67	4.33	20.33	4.33	5	11.33	2.33
Max.	90.33	7.67	24.67	7.33	8.67	13.33	5
Average	77.88	5.15	13.77	5.07	5.96	11.00	3.02
Max. Plant	WA-41	WA-3	WA-9	WA-7	WA-6	WA-27	WA-37

Table (5): The primer name (PN),total number of bands (TB), monomorphic bands (MB), polymorphic bands (PB) and polymorphism percentage as revealed by SCoT and SSR marker analyses.

PN	TB	MB	PB	Polymorphism %
SCoT-01	9	1	8	88.89%
SCoT-02	6	0	6	100.00%
SCoT-03	5	1	4	80.00%
SCoT-04	7	1	6	85.71%
SCoT-05	10	3	7	70.00%
SCoT-10	9	1	8	88.89%
SSR-01	3	0	3	100.00%
SSR-02	2	2	0	0.00%
SSR-03	2	1	1	50.00%
SSR-04	6	2	4	66.67%
SSR-05	2	1	1	50.00%
Total	61	13	48	78.69%

Table (6): The primer name (PN), missing percentage (MP), polymorphism status (PS) [polymorphic (P) or monomorphic (M)] , and nucleotide percentage (NP) as revealed by SNP analysis.

PN	MP	PS	NP							
			R	K	A	C	Y	G	T	M
BS00076248	0	P	4.55	0	6.82	0	0	88.64	0	0
AX-94527869	0	P	0	0	0	95.45	0	0	4.55	0
AX-94559367	0	M	0	0	0	100	0	0	0	0
BS00044720	0	P	0	0	93.18	0	0	6.82	0	0
BS00035234	2.27	P	2.33	0	86.05	0	0	11.63	0	0
BS00050057	0	P	0	0	0	0	0	90.91	9.09	0
AX-94454241	2.27	P	0	0	0	74.42	0	0	25.58	0
BS00076192	20.45	M	0	0	0	0	0	100	0	0
BS00100939	0	M	0	0	0	0	0	0	100	0
BS00060686	0	P	0	2.27	0	0	0	9.09	88.64	0
BS00105878	0	P	0	0	0	22.73	0	0	77.27	0
AX-94406983	4.55	P	0	0	0	83.33	0	16.67	0	0
BS00097126	2.27	M	0	0	0	0	0	0	100	0
BS00024548	0	M	0	0	100	0	0	0	0	0
BS00039211	0	P	0	0	0	11.36	0	0	88.64	0
BS00058591	0	P	0	0	0	75	0	0	25	0
AX-94457966	0	M	0	0	0	0	0	0	100	0
BS00024921	0	P	0	0	79.55	0	0	20.45	0	0
BS00031178	0	P	0	0	0	27.27	0	0	72.73	0
BS00042105	0	M	0	0	0	100	0	0	0	0
BS00040798	2.27	P	0	0	58.14	0	0	41.86	0	0
BS00021745	0	M	0	0	100	0	0	0	0	0
BS00082503	0	P	0	0	31.82	0	0	68.18	0	0
BS00074083	0	P	0	0	11.36	0	0	88.64	0	0
BS00070791	0	P	0	0	0	77.27	0	0	22.73	0
AX-94415898	0	P	0	0	88.64	0	0	11.36	0	0
BS00000006	2.27	P	0	0	0	0	0	67.44	32.56	0
BS00063425	0	P	0	0	0	70.45	0	0	29.55	0
BS00022625	4.55	P	0	0	26.19	0	0	73.81	0	0
BS00076622	0	P	0	0	0	11.36	0	0	88.64	0
BS00104432	0	P	0	0	54.55	45.45	0	0	0	0
AX-94488939	0	P	0	0	63.64	36.36	0	0	0	0
BS00089403	0	M	0	0	0	100	0	0	0	0
BS00071558	0	P	0	0	0	88.64	0	0	11.36	0
BS00075815	0	M	0	0	0	100	0	0	0	0
BS00107837	0	P	43.18	0	9.09	0	0	47.73	0	0
BS00083630	0	P	0	0	0	0	0	27.27	72.73	0
AX-94392216	0	P	0	0	0	0	0	20.45	79.55	0
BS00106043	0	P	0	0	0	84.09	0	0	15.91	0
AX-94529943	0	P	0	0	0	15.91	0	0	84.09	0
BS00089597	0	M	0	0	0	0	0	0	100	0
BS00033795	2.27	P	0	0	0	37.21	6.98	0	55.81	0
BS00022411	0	M	0	0	0	0	0	0	100	0

Table (5): Continue.

PN	MP	PS	NP							
			R	K	A	C	Y	G	T	M
BS00049370	0	P	0	0	97.73	0	0	2.27	0	0
AX-94545917	0	P	0	0	0	68.18	2.27	0	29.55	0
BS00040283	0	P	0	0	72.73	0	0	27.27	0	0
BS00101408	0	P	0	0	27.27	70.45	0	0	0	2.27
BS00070903	15.91	P	0	0	64.86	0	0	35.14	0	0
BS00038820	0	P	0	0	0	88.64	0	0	11.36	0
BS00022653	0	P	0	0	63.64	36.36	0	0	0	0
BS00077716	0	P	0	0	0	95.45	0	0	4.55	0
AX-94558874	0	P	0	0	0	93.18	0	6.82	0	0
BS00018707	0	P	0	0	0	31.82	2.27	0	65.91	0
AX-94442305	0	P	0	0	81.82	0	0	18.18	0	0
AX-94382081	0	M	0	0	0	0	0	100	0	0
BS00021704	0	P	0	0	0	40.91	0	0	59.09	0
AX-86167869	0	M	0	0	0	0	0	100	0	0
AX-94446956	0	P	0	0	0	0	0	50	50	0
BS00064691	0	P	0	0	0	0	0	2.27	97.73	0
BS00107766	0	P	0	0	0	97.73	0	0	2.27	0
BS00046264	2.27	P	0	0	0	55.81	0	0	44.19	0
BS00030651	0	P	0	0	0	34.09	0	0	65.91	0
BS00089954	0	P	2.27	0	27.27	0	0	70.45	0	0
BS00078124	0	P	0	0	0	77.27	0	0	22.73	0
BS00077891	0	M	0	0	0	0	0	100	0	0
BS00049977	0	P	0	0	0	59.09	0	0	40.91	0
AX-94540417	0	P	0	0	0	72.73	0	27.27	0	0
BS00046963	13.64	P	2.63	0	57.89	0	0	39.47	0	0
BS00109036	0	P	0	0	0	13.64	0	0	86.36	0
BS00080749	0	P	0	0	88.64	11.36	0	0	0	0
BS00031140	0	P	0	0	0	45.45	0	0	54.55	0
AX-94486277	0	P	0	0	0	0	0	29.55	70.45	0
BS00024786	0	P	0	0	0	47.73	0	0	52.27	0
BS00025017	0	P	0	0	0	81.82	0	0	18.18	0
BS00050109	0	M	0	0	0	100	0	0	0	0
BS00064146	0	P	2.27	0	27.27	0	0	70.45	0	0
BS00043169	2.27	M	0	0	100	0	0	0	0	0
BS00073116	0	P	0	0	0	0	0	72.73	27.27	0
BS00076033	0	P	0	0	72.73	0	0	27.27	0	0
BS00023673	2.27	P	0	0	0	74.42	2.33	0	23.26	0
BS00071183	0	P	0	0	90.91	0	0	9.09	0	0
BS00032039	2.27	P	0	0	0	0	0	95.35	4.65	0
AX-94401211	0	P	2.27	0	90.91	0	0	6.82	0	0
BS00049818	0	P	0	0	0	93.18	0	0	6.82	0
BS00050993	0	P	0	0	95.45	4.55	0	0	0	0
BS00044237	0	P	0	0	0	4.55	0	0	95.45	0
BS00057851	0	M	0	0	0	100	0	0	0	0
BS00066143	0	P	0	0	0	77.27	0	0	22.73	0
AX-86163814	0	M	0	0	0	0	0	0	100	0
BS00084133	0	P	0	0	0	27.27	0	0	72.73	0
BS00037020	0	M	0	0	0	0	0	100	0	0

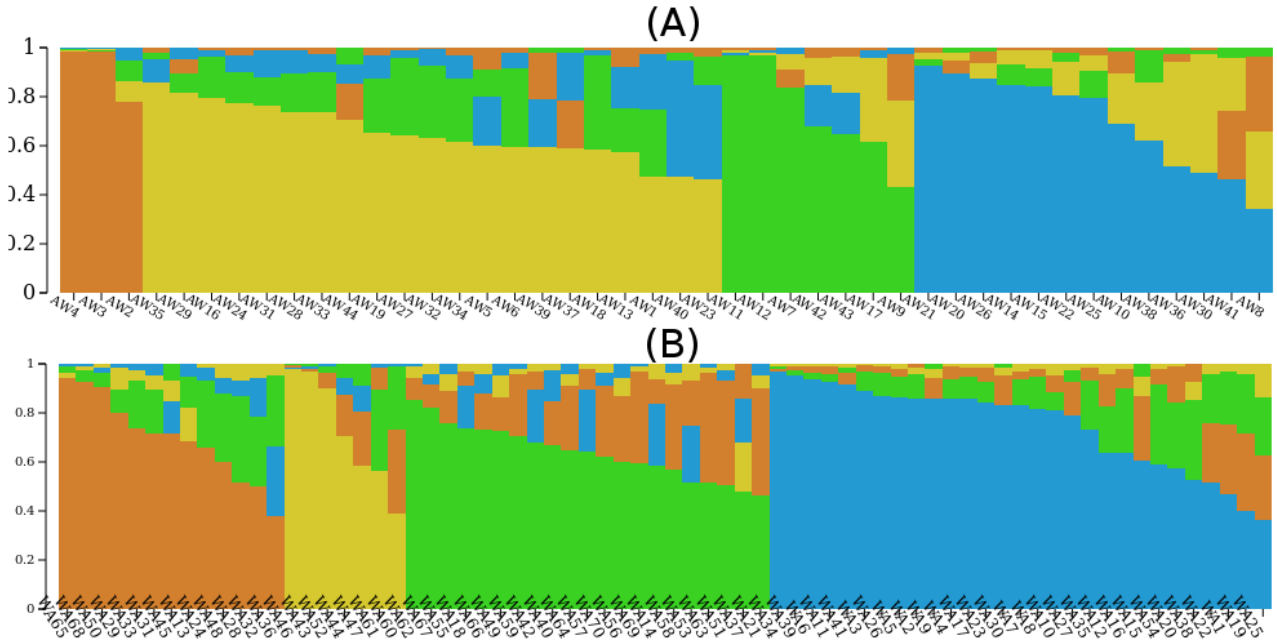

Fig. (4): Analysis of the population structure based on the allele frequencies using STRUCTURE software for the 70 studied wheat genotypes used in this study. (A) The population structure of the 44 foreign genotypes using SNP genotyping analysis. (B) The population structure of the 70 local and foreign genotypes using SCoT and SSR analyses.

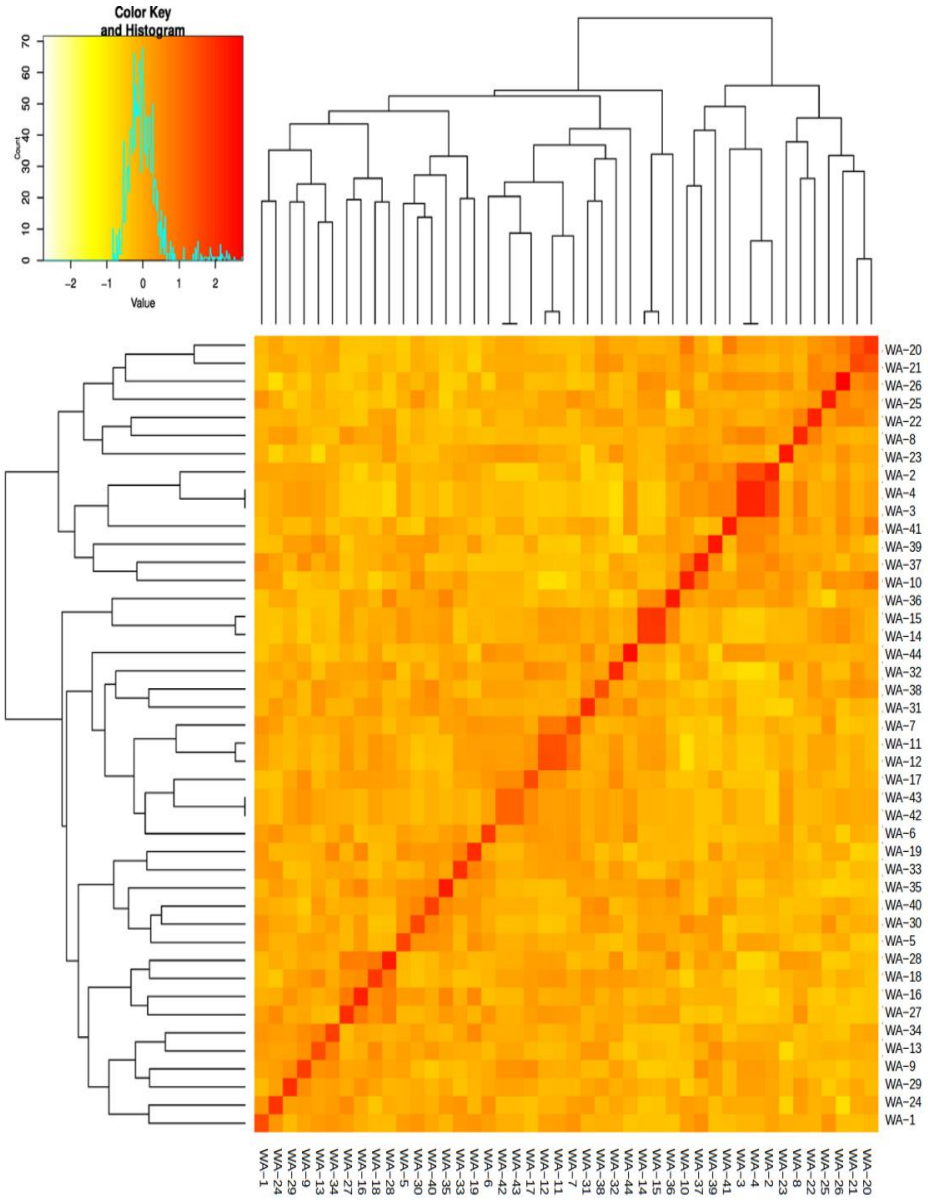

Fig. (5): Analysis of the population structure kinship based on the alleles frequencies of SNP markers generated by GAPIT software through VanRaden algorithm for the 44 foreign wheat genotypes used in this study.

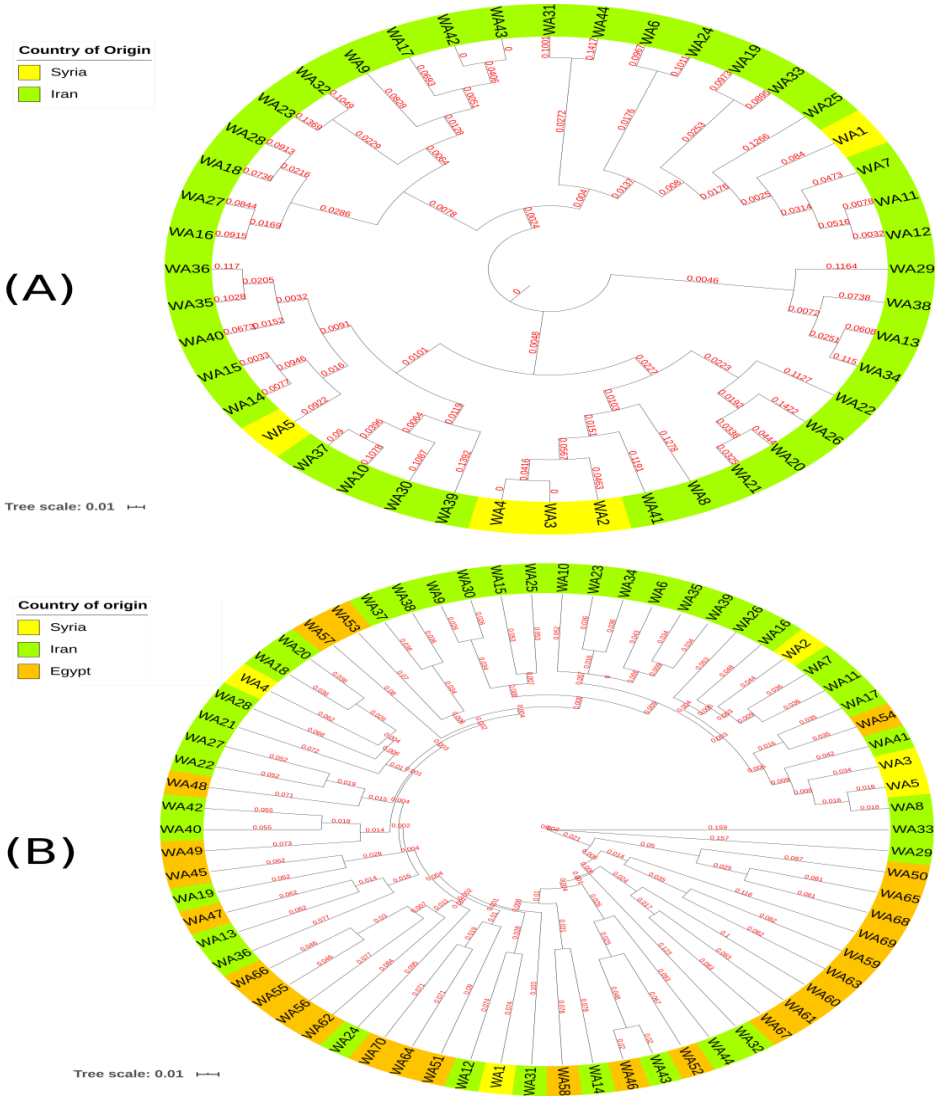

Fig. (6): The phylogenetic diversity for the studied wheat genotypes used in this study. (A) The phylogenetic tree using SNP genotyping analysis for the 44 foreign gentypes. (B) The phylogenetic tree of the 70 local and foreign genotypes using SCoT and SSR analyses.

RESULTS AND DISCUSSIONS

Population structure analysis 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)**.

Analysis of the population structure using allele frequencies of the Egyptian, Syrian and Iranian wheat genotypes indicated that these genotypes were belong to four different population groups (Figure 4). Where, for the most portion, Egyptian, Syrian and Iranian genotypes were clustered depending on their country of origin. On the other hand, some genotypes showed a type of genetic migration, which could be caused by varietal adaptation. The kinship population structure based on the allele frequencies of SNP markers generated by GAPIT software for the foreign genotypes showed a high divergence, where mutation rate among genotypes drived from different geographical regions, where it clustred into two different groups depending on its origin (Figure 5).

Similar results were retrieved using the phylogenetic analysis (Figure 6). Most genotypes were almost clustered, depending on their geographical origin, although some Egyptian genotypes were clustered with the Iranian and Syrian genotypes, which could indicated their source of origin. Such analysis could indicated a number of potential foreign

genotypes that could be successfully adapted in the Egyptian environment through local breeding programmes.

In this regard, **Würschum *et al.* (2013)** used SNP and SSR markers to asses the genetic diversity and population structure in 172 elite European winter wheat. There results revealed that, no clear population structure appears to be present in the panel of 172 elite wheat lines with both, SNPs and SSRs, which could indicated a high genome similarity and low genetic variation among the studied genotypes. They linked such result to the breeding history of European wheat, where local genotypes improving by line breeding is mostly depending on a constant exchange of germplasm between breeding programs.

Bhatta *et al.* (2018) used 35,939 high-quality SNPs to evaluate 139 synthetic hexaploid wheat genotypes. The population structure analysis revealed that wheat genotypes could be clustered into two subgroups (Delta K = 2), that mainly characterized by geographical location of durum parents and growth habit of the crop (spring and winter type). Further population structure analysis of durum and *Ae.* parents separately identified two subgroups, mainly based on type of used parents. Population differentiation between spring and winter samples using analysis of molecular variance indicated low genetic variance between populations and the remainder within populations.

Mourad *et al.* (2020) detected genetic diversity and a population

structure of a core collection of spring wheat (103 spring wheat genotypes) which represented five different continents using SNP genotyping analysis . Significant variations were found within and among the subpopulations and one subpopulation was found to be the most diverse one based on the different allelic patterns. The STRUCTURE analysis software was used to identify the number of subpopulations in the studied genotypes, where the largest Delta K value was observed at K = 3 suggested the presence of three subpopulations in the studied genotypes. The first subpopulation (48 genotypes) contained all of the genotypes from Australia, Germany, Greece, and Kenya while, the second subpopulation (46 genotypes) contained the genotypes from Algeria, Ethiopia, and Tunisia. The genotypes from Egypt, Afghanistan, Canada, Iran, Kazakhstan, Morocco, Saudi Arabia, and Oman were distributed among the third subpopulation.

Kumar *et al.* (2020) characterized the genetic diversity and population structure in 483 Indian spring wheat (*Triticum aestivum*) genotypes using array based SNP markers using 13,557 SNPs. To study population structure in the panel of 483 genotypes, Delta K values were used to infer the number of subpopulations. The obtained suitable value of K from the plot between number of clusters (K) against Delta K (where K = 2) showed the maximum value. They indicated that these two obtained subpopulations could include all of the 483 genotypes with a high probability. The two sub-populations comprised of 106 and 377 genotypes. The molecular

variance analysis revealed that a 2% of variation was observed among subpopulations, while the rest of the variation of 98% was observed within subpopulations. They cocluded that, the identified two subpopulations reflected the natural adaptation and selection history for traits of interest, where one subpopulation comprised genotypes that were mostly the result of breeding selection.

4.4 GWAS analysis of wheat morpho-agronomic traits

SNP genotyping was used to detect genes that are related to wheat response to salinity stress. Generated salinity-associated markers may be used for MAS programs where high tolerance genotypes could be selected for breeding programs.The statistical correlation between the 93 used SNP markers and the seven measured agronomic traits of wheat was calculated (Figure 7). Additionally, the gene annotation analysis using all wheat genome and BLAST analysis revealed several genes that were near to these SNPs markers (Table 7). GWAS analysis revealed several SNP markers that were associated with the studied agronomic traits (Tables 8 - 14). The effects of these SNPs variation were ranged from -3.8 (BS00066143 marker in PH trait) to 5 (BS00049370 marker in PH trait) (Table 12). Thirteen SNP markers showed significant values (p.value > 0.05) that were distributed across the chromosomes of 7B (3 markers), 6A (3 markers), 5A (2 markers), 2B (1 marker), 2A (1 marker), 5B (1 marker), 3B (1 marker), and 1B (1 marker) (Table 15 and Figure 7). The

genetic variation effects of these markers on the studied wheat traits were ranged from -3.85 (BS00066143 marker in PH trait) to 4.16 (BS00038820 marker in PH trait). Five different markers were correlated with STR trait (BS00064146, BS00101408, BS00089954, BS00000006, and BS00076622 markers). The effects of these markers on STR trait was ranged from -0.56 (BS00076622 marker) to 0.469 (BS00064146 and BS00101408 markers). DF trait was correlated with four SNP markers (BS00024921, BS00083630, BS00078124, and BS00038820 markers), where their effects were ranged from -2.544 (BS00078124 marker) to 2.526 (BS00038820, and BS00024921 markers). Some SNP markers showed correlations with multiple traits such as BS00038820 (DF and PH traits), BS00107837 (NS and NT traits), and BS00089954 (NS and STR traits) markers. In this regard, **Shamaya *et al.* (2017)** used SNP genotyping to detect SNPs markers related to salinity tolerance in durum Afghani wheat using bulked segregant analysis from the cross of Jandaroi × AUS-14740, where they focused on markers that were associated with third leaf Na+ concentration in the durum wheat of Afghani landraces. They found two SNP markers to be strongly associated with Na+ concentration in the wheat leaves. The first marker, Xm5511 located on the long arm of chromosome 3B, was associated only with the third leaf Na+ concentration and with neither third leaf K+ concentration nor the K+ /Na+ ratio. In contrast, the second marker, Xm564 was identified in the distal region of the long arm of chromosome 4B, found to had a strong association

with the studied traits .

Table (7): Some SNP and SSR markers which were nearest to genes located on wheat genome as revealed by BLAST analysis.

Marker	Chr	Start	End	Gene	NCBI-ID
AX-86163814	1A	391256938	391256838	*DREB2A*	XP_020156298.1
AX-86167869	6A	90032550	90032650	*EIF5*	XP_020166798.1
AX-94382081	7B	599423437	599423337	*LOC109771663*	XP_020185950.1
AX-94392216	1D	11498575	11498675	*RPP133*	XP_020197782.1
AX-94401211	6B	46960354	46960454	*RGA2*	XP_020198550.1
AX-94406983	2B	752491221	752491121	*GAUT10*	XP_020167967.1
AX-94415898	1A	37501036	37501136	*Rf1*	XP_020153657.1
AX-94442305	2A	5636987	5637087	*LOC109735770*	XP_020150559.1
AX-94446956	1D	457256690	457256590	*LOC109774774*	XP_020189126.1
AX-94454241	1D	10717834	10717734	*WNK2*	XP_020156699.1
AX-94486277	4B	673203228	673203328	*26S-protease-sub7A*	XP_020196032.1
AX-94488939	5A	664484671	664484771	*EPS15*	XP_020164947.1
AX-94527869	7A	191266504	191266404	*MRE11A*	XP_020149197.1
AX-94529943	6D	437805746	437805846	*LOC109783299*	XP_020197502.1
AX-94545917	4B	25834875	25834775	*PSMB2*	XP_020157284.1
AX-94558874	5A	25838427	25838327	*TRH-At2g29150*	XP_020156862.1
AX-94559367	6A	111530936	111530836	*GSTU6*	XP_020167939.1
BS00000006	5A	706240246	706240365	*beta-amylase*	XP_020197275.1
BS00018707	4B	95108658	95108797	*CRK37*	XP_020148269.1
BS00021704	6A	611851132	611851563	*PIP5K9*	XP_020195896.1
BS00021745	7D	629831315	629830671	*SPBC800.10c*	XP_020183037.1
BS00022411	1B	629158750	629159325	*LOC109753548*	XP_020168046.1
BS00022625	1B	163096846	163096405	*FLBR*	XP_020185250.1
BS00022653	4B	526928651	526928412	*APK3*	XP_020176710.1
BS00023673	7A	484221305	484221405	*IAA21*	XP_020176439.1
BS00024548	3A	701852713	701852813	*THIM*	XP_020155192.1
BS00024786	7A	79542753	79542853	*IN-At3g16190*	XP_020192027.1
BS00024921	2A	733091224	733091124	*RPM1*	XP_020163558.1
BS00025017	5D	551059395	551059325	*LOC109770517*	XP_020184810.1
BS00030651	3B	764693976	764694076	*GPDHC1*	XP_020147131.1
BS00031140	2A	241087161	241087061	*LOC109782409*	XP_020196621.1
BS00031178	6A	51409130	51409030	*STARD7*	XP_020185689.1
BS00032039	1B	660528542	660528642	*RPP13*	XP_020170913.1
BS00033795	6A	402473488	402473588	*tropomyosin-2*	XP_020165653.1
BS00035234	7B	711362265	711362365	*Acyl-A-enzyme-19*	XP_020175237.1

Table (6): Continue.

Marker	Chr	Start	End	Gene	NCBI-ID
BS00037020	4B	595271088	595271188	*LOC109765313*	XP_020179701.1
BS00038820	2B	64988340	64988240	*MDIS2*	XP_020170708.1
BS00039211	2D	74981700	74981770	*LOC109786089*	XP_020200257.1
BS00040283	7B	709255965	709256065	*chaperone_dnaJ_11*	XP_020160795.1
BS00040798	3A	528555243	528555143	*ICR1*	XP_020158184.1
BS00042105	4B	616274576	616274476	*DDB_G0270170*	XP_020164890.1
BS00043169	7D	629449685	629449615	*OMT2*	XP_020148883.1
BS00044237	6B	192349755	192349655	*USP2*	XP_020160607.1
BS00044720	2D	78793669	78793739	*BTBD1*	XP_020170912.1
BS00046264	6B	704974232	704974332	*ETR3*	XP_020171652.1
BS00046963	6B	150665120	150665020	*F26K9_60*	XP_020161913.1
BS00049370	2D	12978069	12977999	*LOC109776520*	XP_020190752.1
BS00049977	3A	688688770	688688670	*ARF4*	XP_020197968.1
BS00050057	5B	658370071	658370171	*LOC109755160*	XP_020169633.1
BS00050109	3A	680749708	680749608	*DEK*	XP_020186407.1
BS00050993	7B	36490152	36490052	*CYP71E1*	XP_020167088.1
BS00057851	5B	629930641	629930541	*HNRNPA2B1*	XP_020172898.1
BS00058591	5A	459003197	459003097	*AHL17*	XP_020179086.1
BS00060686	1B	675320377	675320277	*SKIP25*	XP_020176527.1
BS00063425	5A	419868519	419868619	*LOC109787609*	XP_020201733.1
BS00064146	7B	655818477	655818377	*LOC109732503*	XP_020147258.1
BS00064691	5D	496067082	496067152	*formin20*	XP_020166911.1
BS00066143	5A	533072063	533072163	*STAY-GREEN*	XP_020172097.1
BS00070791	7B	642475363	642475463	*receptor12*	XP_020156262.1
BS00070903	5A	416170551	416170651	*LOC109755320*	XP_020169824.1
BS00071183	3B	823762843	823762943	*LOC109766200*	XP_020180563.1
BS00071558	7A	626897256	626897156	*LOC109762474*	XP_020176925.1
BS00073116	5D	546864019	546864119	*ZMYND15*	XP_020181418.1
BS00074083	7B	62687149	62687249	*LOC109778722*	XP_020192894.1
BS00075815	5B	536047052	536046952	*cap3C*	XP_020186567.1
BS00076033	4B	609515871	609515971	*LOC109772166*	XP_020186444.1
BS00076192	1B	1779897	1779797	*RGA1*	XP_020162922.1
BS00076248	3B	53567426	53567326	*FRS5*	XP_020197126.1
BS00076622	7B	717202778	717202678	*3BETAHSD/D3*	XP_020166331.1
BS00077716	4A	597693265	597693165	*FRS5*	XP_020160726.1
BS00077891	7A	647308676	647308576	*polyubiquitin-A*	XP_020161941.1
BS00078124	6A	617182650	617182750	*MSSP2*	XP_020146664.1
BS00080749	2D	72171503	72171433	*SODCC.3*	XP_020166289.1
BS00082503	1D	412219294	412219194	*GLU-D1-2B*	XP_020162496.1
BS00083630	6A	5604316	5604416	*CYP709B2*	XP_020152506.1
BS00084133	5D	550441990	550441920	*KH-_At4g18375*	XP_020174731.1
BS00089403	4D	505433671	505433571	*LOC109762625*	XP_020177085.1
BS00089597	5D	552040073	552040143	*LOC109746940*	XP_020161627.1
BS00089954	3B	543718728	543718628	*LOC109781215*	XP_020195405.1
BS00097126	2D	27651413	27651343	*LOC109769300*	XP_020183640.1
BS00101408	7B	657662587	657662487	*LOC109736307*	XP_020151122.1
BS00104432	5A	636413881	636413981	*PTC52*	XP_020156117.1
BS00105878	3B	750361390	750361290	*LOC109757330*	XP_020171744.1
BS00106043	5B	27460098	27459998	*TRH-At5g06060*	XP_020188695.1
BS00107766	4A	599846343	599846443	*Af-acyl-CoA*	XP_020188656.1
BS00107837	1B	674821532	674821632	*LOC109753414*	XP_020167905.1
BS00109036	6B	663531485	663531385	*LOC109743956*	XP_020158640.1
WMS136	1A	6423427	6425847	*CSLA9*	XP_020197194.1
Xgwm219	6B	674842296	674844477	*LOC109774880*	XP_020189236.1

Table (8): The GWAS result of SNP markers for DF trait.

SNP	Chr	Position	P.value	MAF	nobs	R-without	R-with	Effect
BS00083630	6A	5604416	0.02	0.2727	44	0.0842	0.2189	-2.372
BS00024921	2A	733091124	0.0208	0.2045	44	0.0842	0.217	2.5246
BS00078124	6A	617182750	0.0363	0.2273	44	0.0842	0.1919	-2.5437
BS00038820	2B	64988240	0.0473	0.1136	44	0.0842	0.1803	2.5256
BS00022625	1B	163096405	0.05	0.2727	44	0.0842	0.1779	2.1307
BS00084133	5D	550441920	0.0745	0.2727	44	0.0842	0.1611	-2.6326
BS00066143	5A	533072163	0.0779	0.2273	44	0.0842	0.1593	-1.9033
BS00021704	6A	611851563	0.0891	0.4091	44	0.0842	0.1538	2.0547
BS00049370	2D	12977999	0.0895	0.0227	44	0.0842	0.1537	3.6862
BS00046963	6B	150665020	0.1043	0.4205	44	0.0842	0.1476	-1.722
BS00040283	7B	709256065	0.1645	0.2727	44	0.0842	0.1302	-1.4413
AX-94540417	1B	431456742	0.2166	0.2727	44	0.0842	0.1203	-1.3913
BS00031140	2A	241087061	0.2166	0.4545	44	0.0842	0.1203	1.2716
BS00049977	3A	688688670	0.2431	0.4091	44	0.0842	0.1164	-1.2144
BS00030651	3B	764694076	0.2588	0.3409	44	0.0842	0.1143	-1.098
AX-94446956	1D	457256590	0.2797	0.5	44	0.0842	0.1117	-1.1254
BS00025017	5D	551059325	0.2887	0.1818	44	0.0842	0.1107	-1.6757
AX-94442305	2A	5637087	0.3428	0.1818	44	0.0842	0.1053	-1.058
AX-94529943	6D	437805846	0.3515	0.1591	44	0.0842	0.1046	-1.1414
BS00076248	3B	53567326	0.3579	0.0909	44	0.0842	0.104	-1.1646
AX-94454241	1D	10717734	0.3692	0.2614	44	0.0842	0.1031	-1.0088
BS00076033	4B	609515971	0.3999	0.2727	44	0.0842	0.1008	0.799
BS00107837	1B	674821632	0.403	0.3068	44	0.0842	0.1006	1.0714
BS00104432	5A	636413981	0.4161	0.4545	44	0.0842	0.0997	-0.7634
BS00058591	5A	459003097	0.4203	0.25	44	0.0842	0.0994	0.8027
BS00035234	7B	711362365	0.4209	0.1364	44	0.0842	0.0994	-1.277
BS00031178	6A	51409030	0.4311	0.2727	44	0.0842	0.0987	0.7638
BS00023673	7A	484221405	0.4582	0.25	44	0.0842	0.0971	-0.7552
AX-94415898	1A	37501136	0.4688	0.1136	44	0.0842	0.0965	-1.1693
BS00018707	4B	95108797	0.5	0.3295	44	0.0842	0.0948	0.6378
BS00050993	7B	36490052	0.5195	0.0455	44	0.0842	0.0939	-1.2007
BS00044237	6B	192349655	0.5334	0.0455	44	0.0842	0.0932	0.9842
BS00109036	6B	663531385	0.5537	0.1364	44	0.0842	0.0924	-0.7269
BS00071183	3B	823762943	0.5576	0.0909	44	0.0842	0.0922	-0.7806
BS00032039	1B	660528642	0.558	0.0568	44	0.0842	0.0922	-0.923
BS00050057	5B	658370171	0.5679	0.0909	44	0.0842	0.0918	0.6999
BS00089954	3B	543718628	0.5827	0.2841	44	0.0842	0.0912	-0.5777
BS00080749	2D	72171433	0.5901	0.1136	44	0.0842	0.091	0.6995
AX-94488939	5A	664484771	0.618	0.3636	44	0.0842	0.09	-0.513
BS00105878	3B	750361290	0.6222	0.2273	44	0.0842	0.0899	0.5647
BS00064691	5D	496067152	0.6225	0.0227	44	0.0842	0.0898	-1.0783
BS00046264	6B	704974332	0.6353	0.4432	44	0.0842	0.0894	-0.5031
BS00076622	7B	717202678	0.639	0.1136	44	0.0842	0.0893	-0.7326
BS00106043	5B	27459998	0.6436	0.1591	44	0.0842	0.0892	-0.5397

Table (7): Continue.

SNP	Chr	Position	P.value	MAF	nobs	R-without	R-with	Effect
BS00073116	5D	546864119	0.6585	0.2727	44	0.0842	0.0887	-0.4562
BS00074083	7B	62687249	0.6757	0.1136	44	0.0842	0.0883	-0.5255
BS00082503	1D	412219194	0.679	0.3182	44	0.0842	0.0882	-0.4797
BS00071558	7A	626897156	0.6867	0.1136	44	0.0842	0.088	-0.5184
AX-94486277	4B	673203328	0.7296	0.2955	44	0.0842	0.087	-0.3248
BS00024786	7A	79542853	0.7362	0.4773	44	0.0842	0.0868	-0.3215
AX-94558874	5A	25838327	0.7562	0.0682	44	0.0842	0.0864	-0.4586
AX-94545917	4B	25834775	0.7589	0.3068	44	0.0842	0.0864	0.3043
AX-94406983	2B	752491121	0.765	0.1818	44	0.0842	0.0863	-0.3462
BS00070903	5A	416170651	0.783	0.375	44	0.0842	0.086	-0.3522
BS00070791	7B	642475463	0.7893	0.2273	44	0.0842	0.0859	-0.3523
AX-94392216	1D	11498675	0.7991	0.2045	44	0.0842	0.0857	0.2846
BS00107766	4A	599846443	0.8258	0.0227	44	0.0842	0.0853	0.513
BS00064146	7B	655818377	0.8297	0.2841	44	0.0842	0.0853	-0.2979
BS00101408	7B	657662487	0.8297	0.2841	44	0.0842	0.0853	-0.2979
BS00040798	3A	528555143	0.8381	0.4205	44	0.0842	0.0852	0.2
BS00077716	4A	597693165	0.8735	0.0455	44	0.0842	0.0848	0.2675
BS00039211	2D	74981770	0.8748	0.1136	44	0.0842	0.0848	0.1971
BS00063425	5A	419868619	0.8749	0.2955	44	0.0842	0.0848	0.1901
BS00060686	1B	675320277	0.876	0.1023	44	0.0842	0.0848	-0.1939
BS00022653	4B	526928412	0.877	0.3636	44	0.0842	0.0848	0.1755
BS00033795	6A	402473588	0.9116	0.4091	44	0.0842	0.0845	-0.1175
AX-94401211	6B	46960454	0.9198	0.0795	44	0.0842	0.0844	-0.1378
BS00000006	5A	706240365	0.9306	0.3295	44	0.0842	0.0844	-0.0832
AX-94527869	7A	191266404	0.9352	0.0455	44	0.0842	0.0844	0.1546
BS00044720	2D	78793739	0.9734	0.0682	44	0.0842	0.0842	0.052
BS00049818	6D	451020060	0.9868	0.0682	44	0.0842	0.0842	0.0218
AX-86163814	1A	391256838	1	0	44	NA	NA	NA
AX-86167869	6A	90032650	1	0	44	NA	NA	NA
AX-94382081	7B	599423337	1	0	44	NA	NA	NA
AX-94457966	7B	11253456	1	0	44	NA	NA	NA
AX-94559367	6A	111530836	1	0	44	NA	NA	NA
BS00021745	7D	629830671	1	0	44	NA	NA	NA
BS00022411	1B	629159325	1	0	44	NA	NA	NA
BS00024548	3A	701852813	1	0	44	NA	NA	NA
BS00037020	4B	595271188	1	0	44	NA	NA	NA
BS00042105	4B	616274476	1	0	44	NA	NA	NA
BS00043169	7D	629449615	1	0	44	NA	NA	NA
BS00050109	3A	680749608	1	0	44	NA	NA	NA
BS00057851	5B	629930541	1	0	44	NA	NA	NA
BS00075815	5B	536046952	1	0	44	NA	NA	NA
BS00076192	1B	1779797	1	0	44	NA	NA	NA
BS00077891	7A	647308576	1	0	44	NA	NA	NA
BS00089403	4D	505433571	1	0	44	NA	NA	NA
BS00089597	5D	552040143	1	0	44	NA	NA	NA
BS00097126	2D	27651343	1	0	44	NA	NA	NA
BS00100939	2B	29991102	1	0	44	NA	NA	NA

Table (9): The GWAS result of SNP markers for NS trait.

SNP	Chr	Position	P.value	MAF	nobs	R-without	R-with	Effect
BS00107837	1B	674821632	0.0252	0.3068	44	0.0865	0.2102	-0.5638
BS00089954	3B	543718628	0.0453	0.2841	44	0.0865	0.1842	0.4302
BS00024921	2A	733091124	0.0678	0.2045	44	0.0865	0.1671	-0.3751
BS00049977	3A	688688670	0.0716	0.4091	44	0.0865	0.1648	0.3773
BS00076033	4B	609515971	0.073	0.2727	44	0.0865	0.164	-0.3428
BS00031140	2A	241087061	0.0902	0.4545	44	0.0865	0.1554	-0.3411
BS00109036	6B	663531385	0.1111	0.1364	44	0.0865	0.1472	0.3859
BS00050993	7B	36490052	0.1122	0.0455	44	0.0865	0.1468	0.6075
BS00104432	5A	636413981	0.1176	0.4545	44	0.0865	0.1449	0.2826
BS00066143	5A	533072163	0.1313	0.2273	44	0.0865	0.1407	0.3261
BS00064146	7B	655818377	0.1457	0.2841	44	0.0865	0.1368	0.3828
BS00101408	7B	657662487	0.1457	0.2841	44	0.0865	0.1368	0.3828
AX-94529943	6D	437805846	0.1739	0.1591	44	0.0865	0.1303	0.3097
BS00071183	3B	823762943	0.186	0.0909	44	0.0865	0.1278	-0.3645
BS00018707	4B	95108797	0.2083	0.3295	44	0.0865	0.1238	0.2183
BS00084133	5D	550441920	0.23	0.2727	44	0.0865	0.1204	0.3673
BS00022625	1B	163096405	0.246	0.2727	44	0.0865	0.1181	-0.2237
BS00076248	3B	53567326	0.2599	0.0909	44	0.0865	0.1163	0.3172
BS00060686	1B	675320277	0.2615	0.1023	44	0.0865	0.1161	-0.2953
BS00021704	6A	611851563	0.2714	0.4091	44	0.0865	0.1149	-0.261
BS00039211	2D	74981770	0.2969	0.1136	44	0.0865	0.112	0.2661
BS00070903	5A	416170651	0.3188	0.375	44	0.0865	0.1097	0.229
AX-94392216	1D	11498675	0.3255	0.2045	44	0.0865	0.1091	-0.2169
BS00040283	7B	709256065	0.3428	0.2727	44	0.0865	0.1075	-0.1827
BS00082503	1D	412219194	0.3588	0.3182	44	0.0865	0.1061	0.2185
BS00022653	4B	526928412	0.3588	0.3636	44	0.0865	0.1061	-0.1841
AX-94540417	1B	431456742	0.3638	0.2727	44	0.0865	0.1057	0.1993
BS00078124	6A	617182750	0.3747	0.2273	44	0.0865	0.1049	0.1974
AX-94401211	6B	46960454	0.3801	0.0795	44	0.0865	0.1045	0.2673
BS00070791	7B	642475463	0.3973	0.2273	44	0.0865	0.1032	-0.2241
AX-94454241	1D	10717734	0.4472	0.2614	44	0.0865	0.0999	0.152
BS00105878	3B	750361290	0.4475	0.2273	44	0.0865	0.0999	0.1694
BS00025017	5D	551059325	0.4529	0.1818	44	0.0865	0.0996	0.2419
BS00031178	6A	51409030	0.4571	0.2727	44	0.0865	0.0993	-0.1423
BS00040798	3A	528555143	0.5061	0.4205	44	0.0865	0.0967	-0.1259
BS00063425	5A	419868619	0.5165	0.2955	44	0.0865	0.0962	0.1424
BS00106043	5B	27459998	0.5202	0.1591	44	0.0865	0.0961	-0.1493
AX-94545917	4B	25834775	0.5379	0.3068	44	0.0865	0.0953	-0.1129
AX-94446956	1D	457256590	0.5665	0.5	44	0.0865	0.0941	0.11
BS00107766	4A	599846443	0.5941	0.0227	44	0.0865	0.093	-0.2733
BS00038820	2B	64988240	0.5983	0.1136	44	0.0865	0.0929	0.1388
BS00044720	2D	78793739	0.6153	0.0682	44	0.0865	0.0923	-0.1555
BS00080749	2D	72171433	0.6292	0.1136	44	0.0865	0.0919	-0.1242
AX-94442305	2A	5637087	0.6389	0.1818	44	0.0865	0.0916	-0.1012

Table (8): Continue.

SNP	Chr	Position	P.value	MAF	nobs	R-without	R-with	Effect
BS00073116	5D	546864119	0.6592	0.2727	44	0.0865	0.091	0.0874
BS00049818	6D	451020060	0.6603	0.0682	44	0.0865	0.0909	-0.1379
BS00046963	6B	150665020	0.673	0.4205	44	0.0865	0.0906	-0.0867
BS00023673	7A	484221405	0.7293	0.25	44	0.0865	0.0892	-0.0678
BS00074083	7B	62687249	0.7372	0.1136	44	0.0865	0.0891	0.0863
BS00030651	3B	764694076	0.7446	0.3409	44	0.0865	0.0889	0.0575
BS00071558	7A	626897156	0.7491	0.1136	44	0.0865	0.0888	-0.08
AX-94527869	7A	191266404	0.7687	0.0455	44	0.0865	0.0885	-0.1201
BS00064691	5D	496067152	0.7725	0.0227	44	0.0865	0.0884	0.1465
BS00076622	7B	717202678	0.7813	0.1136	44	0.0865	0.0882	0.0948
BS00044237	6B	192349655	0.7947	0.0455	44	0.0865	0.088	-0.0948
AX-94488939	5A	664484771	0.7982	0.3636	44	0.0865	0.088	0.0511
AX-94558874	5A	25838327	0.8058	0.0682	44	0.0865	0.0879	-0.0753
AX-94415898	1A	37501136	0.8317	0.1136	44	0.0865	0.0875	0.0717
BS00035234	7B	711362365	0.8323	0.1364	44	0.0865	0.0875	0.0685
BS00050057	5B	658370171	0.8425	0.0909	44	0.0865	0.0874	-0.0526
BS00083630	6A	5604416	0.8517	0.2727	44	0.0865	0.0873	0.035
BS00033795	6A	402473588	0.8571	0.4091	44	0.0865	0.0872	-0.0377
BS00049370	2D	12977999	0.8574	0.0227	44	0.0865	0.0872	-0.0923
BS00058591	5A	459003097	0.8739	0.25	44	0.0865	0.087	-0.0321
BS00000006	5A	706240365	0.9133	0.3295	44	0.0865	0.0867	0.0199
BS00024786	7A	79542853	0.9223	0.4773	44	0.0865	0.0867	-0.0179
BS00046264	6B	704974332	0.9272	0.4432	44	0.0865	0.0866	-0.0187
BS00077716	4A	597693165	0.9352	0.0455	44	0.0865	0.0866	0.03
AX-94406983	2B	752491121	0.9382	0.1818	44	0.0865	0.0866	-0.0166
BS00032039	1B	660528642	0.9449	0.0568	44	0.0865	0.0866	0.0237
AX-94486277	4B	673203328	0.9844	0.2955	44	0.0865	0.0865	-0.0034
AX-86163814	1A	391256838	1	0	44	NA	NA	NA
AX-86167869	6A	90032650	1	0	44	NA	NA	NA
AX-94382081	7B	599423337	1	0	44	NA	NA	NA
AX-94457966	7B	11253456	1	0	44	NA	NA	NA
AX-94559367	6A	111530836	1	0	44	NA	NA	NA
BS00021745	7D	629830671	1	0	44	NA	NA	NA
BS00022411	1B	629159325	1	0	44	NA	NA	NA
BS00024548	3A	701852813	1	0	44	NA	NA	NA
BS00037020	4B	595271188	1	0	44	NA	NA	NA
BS00042105	4B	616274476	1	0	44	NA	NA	NA
BS00043169	7D	629449615	1	0	44	NA	NA	NA
BS00050109	3A	680749608	1	0	44	NA	NA	NA
BS00057851	5B	629930541	1	0	44	NA	NA	NA
BS00075815	5B	536046952	1	0	44	NA	NA	NA
BS00076192	1B	1779797	1	0	44	NA	NA	NA
BS00077891	7A	647308576	1	0	44	NA	NA	NA
BS00089403	4D	505433571	1	0	44	NA	NA	NA
BS00089597	5D	552040143	1	0	44	NA	NA	NA
BS00097126	2D	27651343	1	0	44	NA	NA	NA
BS00100939	2B	29991102	1	0	44	NA	NA	NA

Table (10): The GWAS result of SNP markers for NSL trait.

SNP	Chr	Position	P.value	MAF	nobs	R-without	R-with	Effect
BS00050057	5B	658370171	0.0566	0.0909	44	0.1462	0.2286	0.6702
BS00066143	5A	533072163	0.0574	0.2273	44	0.1462	0.2281	-0.578
BS00073116	5D	546864119	0.0645	0.2727	44	0.1462	0.2235	-0.5474
BS00024786	7A	79542853	0.072	0.4773	44	0.1462	0.2193	-0.4922
BS00083630	6A	5604416	0.075	0.2727	44	0.1462	0.2177	-0.5026
BS00024921	2A	733091124	0.0927	0.2045	44	0.1462	0.2097	0.5075
AX-94540417	1B	431456742	0.1172	0.2727	44	0.1462	0.201	-0.4984
BS00076033	4B	609515971	0.1244	0.2727	44	0.1462	0.1989	0.4141
BS00030651	3B	764694076	0.1276	0.3409	44	0.1462	0.198	-0.4191
BS00033795	6A	402473588	0.128	0.4091	44	0.1462	0.1979	-0.4594
BS00049370	2D	12977999	0.1447	0.0227	44	0.1462	0.1935	0.8837
BS00049818	6D	451020060	0.2107	0.0682	44	0.1462	0.1808	-0.4671
BS00040283	7B	709256065	0.2173	0.2727	44	0.1462	0.1798	-0.3584
BS00025017	5D	551059325	0.2185	0.1818	44	0.1462	0.1796	-0.5472
BS00018707	4B	95108797	0.2293	0.3295	44	0.1462	0.1781	-0.3214
BS00044237	6B	192349655	0.2526	0.0455	44	0.1462	0.175	-0.5103
AX-94446956	1D	457256590	0.2671	0.5	44	0.1462	0.1733	0.3247
AX-94558874	5A	25838327	0.2744	0.0682	44	0.1462	0.1725	0.4565
BS00032039	1B	660528642	0.29	0.0568	44	0.1462	0.1708	-0.4704
BS00039211	2D	74981770	0.3313	0.1136	44	0.1462	0.1669	-0.3432
BS00046963	6B	150665020	0.3315	0.4205	44	0.1462	0.1669	-0.2859
BS00109036	6B	663531385	0.3421	0.1364	44	0.1462	0.166	-0.3286
BS00022653	4B	526928412	0.3426	0.3636	44	0.1462	0.1659	0.3042
AX-94392216	1D	11498675	0.3477	0.2045	44	0.1462	0.1655	0.2965
BS00074083	7B	62687249	0.3562	0.1136	44	0.1462	0.1649	-0.3268
BS00107837	1B	674821632	0.3629	0.3068	44	0.1462	0.1643	0.3277
AX-94401211	6B	46960454	0.3656	0.0795	44	0.1462	0.1641	-0.3492
BS00022625	1B	163096405	0.3658	0.2727	44	0.1462	0.1641	0.2708
BS00038820	2B	64988240	0.3789	0.1136	44	0.1462	0.1632	0.3081
BS00082503	1D	412219194	0.3805	0.3182	44	0.1462	0.163	-0.2866
AX-94406983	2B	752491121	0.3814	0.1818	44	0.1462	0.163	0.2861
BS00104432	5A	636413981	0.3901	0.4545	44	0.1462	0.1624	0.2267
BS00031178	6A	51409030	0.4058	0.2727	44	0.1462	0.1613	0.2266
AX-94442305	2A	5637087	0.4078	0.1818	44	0.1462	0.1612	-0.2589
BS00070903	5A	416170651	0.4311	0.375	44	0.1462	0.1598	-0.2839
BS00064146	7B	655818377	0.4594	0.2841	44	0.1462	0.1582	0.2889
BS00101408	7B	657662487	0.4594	0.2841	44	0.1462	0.1582	0.2889
BS00071183	3B	823762943	0.4793	0.0909	44	0.1462	0.1571	0.2647
BS00058591	5A	459003097	0.4869	0.25	44	0.1462	0.1568	-0.1943
BS00064691	5D	496067152	0.527	0.0227	44	0.1462	0.1549	-0.3892
BS00077716	4A	597693165	0.5283	0.0455	44	0.1462	0.1549	0.2982
BS00105878	3B	750361290	0.5383	0.2273	44	0.1462	0.1545	-0.1983
AX-94527869	7A	191266404	0.5518	0.0455	44	0.1462	0.1539	0.3185
BS00021704	6A	611851563	0.5574	0.4091	44	0.1462	0.1537	-0.1959

Table (9): Continue.

SNP	Chr	Position	P.value	MAF	nobs	R-without	R-with	Effect
BS00070791	7B	642475463	0.5771	0.2273	44	0.1462	0.153	-0.2068
BS00107766	4A	599846443	0.5792	0.0227	44	0.1462	0.1529	0.3632
BS00076622	7B	717202678	0.5942	0.1136	44	0.1462	0.1524	-0.2336
BS00031140	2A	241087061	0.6046	0.4545	44	0.1462	0.1521	0.1485
BS00044720	2D	78793739	0.6731	0.0682	44	0.1462	0.1501	0.1849
BS00049977	3A	688688670	0.6835	0.4091	44	0.1462	0.1498	-0.1182
BS00046264	6B	704974332	0.6861	0.4432	44	0.1462	0.1498	-0.1204
AX-94415898	1A	37501136	0.6891	0.1136	44	0.1462	0.1497	0.1809
BS00050993	7B	36490052	0.7044	0.0455	44	0.1462	0.1494	0.1982
AX-94545917	4B	25834775	0.7085	0.3068	44	0.1462	0.1493	0.1042
BS00106043	5B	27459998	0.7138	0.1591	44	0.1462	0.1492	0.1201
BS00078124	6A	617182750	0.714	0.2273	44	0.1462	0.1492	0.1216
BS00080749	2D	72171433	0.7258	0.1136	44	0.1462	0.1489	0.1278
AX-94486277	4B	673203328	0.7563	0.2955	44	0.1462	0.1483	0.0819
AX-94529943	6D	437805846	0.7839	0.1591	44	0.1462	0.1479	-0.0939
BS00040798	3A	528555143	0.7841	0.4205	44	0.1462	0.1479	0.0754
BS00076248	3B	53567326	0.7854	0.0909	44	0.1462	0.1479	-0.0963
BS00023673	7A	484221405	0.7947	0.25	44	0.1462	0.1477	0.0742
BS00000006	5A	706240365	0.799	0.3295	44	0.1462	0.1477	-0.0683
AX-94488939	5A	664484771	0.8375	0.3636	44	0.1462	0.1472	0.0592
BS00035234	7B	711362365	0.8598	0.1364	44	0.1462	0.1469	-0.0784
BS00084133	5D	550441920	0.8835	0.2727	44	0.1462	0.1467	0.0595
BS00060686	1B	675320277	0.9305	0.1023	44	0.1462	0.1464	-0.0304
AX-94454241	1D	10717734	0.931	0.2614	44	0.1462	0.1464	-0.0272
BS00063425	5A	419868619	0.951	0.2955	44	0.1462	0.1463	-0.0209
BS00071558	7A	626897156	0.9745	0.1136	44	0.1462	0.1463	0.0115
BS00089954	3B	543718628	0.9938	0.2841	44	0.1462	0.1462	0.0023
AX-86163814	1A	391256838	1	0	44	NA	NA	NA
AX-86167869	6A	90032650	1	0	44	NA	NA	NA
AX-94382081	7B	599423337	1	0	44	NA	NA	NA
AX-94457966	7B	11253456	1	0	44	NA	NA	NA
AX-94559367	6A	111530836	1	0	44	NA	NA	NA
BS00021745	7D	629830671	1	0	44	NA	NA	NA
BS00022411	1B	629159325	1	0	44	NA	NA	NA
BS00024548	3A	701852813	1	0	44	NA	NA	NA
BS00037020	4B	595271188	1	0	44	NA	NA	NA
BS00042105	4B	616274476	1	0	44	NA	NA	NA
BS00043169	7D	629449615	1	0	44	NA	NA	NA
BS00050109	3A	680749608	1	0	44	NA	NA	NA
BS00057851	5B	629930541	1	0	44	NA	NA	NA
BS00075815	5B	536046952	1	0	44	NA	NA	NA
BS00076192	1B	1779797	1	0	44	NA	NA	NA
BS00077891	7A	647308576	1	0	44	NA	NA	NA
BS00089403	4D	505433571	1	0	44	NA	NA	NA
BS00089597	5D	552040143	1	0	44	NA	NA	NA
BS00097126	2D	27651343	1	0	44	NA	NA	NA
BS00100939	2B	29991102	1	0	44	NA	NA	NA

Table (11): The GWAS result of SNP markers for NT trait.

SNP	Chr	Position	P.value	MAF	nobs	R-without	R-with	Effect
BS00107837	1B	674821632	0.0137	0.3068	44	0.1579	0.2982	-0.6376
BS00024921	2A	733091124	0.0562	0.2045	44	0.1579	0.2395	-0.3999
BS00109036	6B	663531385	0.063	0.1364	44	0.1579	0.235	0.461
BS00076033	4B	609515971	0.074	0.2727	44	0.1579	0.2289	-0.3459
BS00031140	2A	241087061	0.0792	0.4545	44	0.1579	0.2263	-0.3599
BS00049977	3A	688688670	0.0953	0.4091	44	0.1579	0.2195	0.3526
BS00089954	3B	543718628	0.1037	0.2841	44	0.1579	0.2164	0.3509
AX-94529943	6D	437805846	0.1149	0.1591	44	0.1579	0.2127	0.3685
BS00066143	5A	533072163	0.1221	0.2273	44	0.1579	0.2105	0.3388
BS00084133	5D	550441920	0.125	0.2727	44	0.1579	0.2097	0.4762
BS00104432	5A	636413981	0.1392	0.4545	44	0.1579	0.2059	0.2715
BS00018707	4B	95108797	0.1691	0.3295	44	0.1579	0.1993	0.245
BS00050993	7B	36490052	0.1753	0.0455	44	0.1579	0.1981	0.5217
BS00076248	3B	53567326	0.1911	0.0909	44	0.1579	0.1952	0.3701
BS00071183	3B	823762943	0.1944	0.0909	44	0.1579	0.1947	-0.3619
BS00060686	1B	675320277	0.1995	0.1023	44	0.1579	0.1938	-0.3411
BS00031178	6A	51409030	0.2489	0.2727	44	0.1579	0.1868	-0.2248
BS00070903	5A	416170651	0.2649	0.375	44	0.1579	0.1849	0.263
BS00064146	7B	655818377	0.2666	0.2841	44	0.1579	0.1847	0.2961
BS00101408	7B	657662487	0.2666	0.2841	44	0.1579	0.1847	0.2961
BS00021704	6A	611851563	0.2668	0.4091	44	0.1579	0.1847	-0.2672
AX-94392216	1D	11498675	0.3167	0.2045	44	0.1579	0.1796	-0.2243
BS00039211	2D	74981770	0.3254	0.1136	44	0.1579	0.1788	0.2536
BS00078124	6A	617182750	0.3397	0.2273	44	0.1579	0.1776	0.2169
AX-94540417	1B	431456742	0.3765	0.2727	44	0.1579	0.1748	0.1973
BS00063425	5A	419868619	0.3814	0.2955	44	0.1579	0.1744	0.1972
AX-94545917	4B	25834775	0.3991	0.3068	44	0.1579	0.1732	-0.1584
BS00082503	1D	412219194	0.4179	0.3182	44	0.1579	0.1721	0.1947
BS00022625	1B	163096405	0.4281	0.2727	44	0.1579	0.1714	-0.1561
BS00040283	7B	709256065	0.436	0.2727	44	0.1579	0.171	-0.1527
BS00040798	3A	528555143	0.4642	0.4205	44	0.1579	0.1694	-0.1408
AX-94454241	1D	10717734	0.4775	0.2614	44	0.1579	0.1688	0.1456
BS00105878	3B	750361290	0.485	0.2273	44	0.1579	0.1684	0.1581
AX-94401211	6B	46960454	0.4853	0.0795	44	0.1579	0.1684	0.213
BS00022653	4B	526928412	0.493	0.3636	44	0.1579	0.168	-0.1412
BS00073116	5D	546864119	0.5098	0.2727	44	0.1579	0.1673	0.133
BS00038820	2B	64988240	0.5325	0.1136	44	0.1579	0.1663	0.166
BS00023673	7A	484221405	0.542	0.25	44	0.1579	0.1659	-0.1217
BS00044720	2D	78793739	0.6029	0.0682	44	0.1579	0.1637	-0.1632
AX-94527869	7A	191266404	0.6071	0.0455	44	0.1579	0.1636	-0.2111
BS00106043	5B	27459998	0.6132	0.1591	44	0.1579	0.1634	-0.1187
BS00070791	7B	642475463	0.6133	0.2273	44	0.1579	0.1634	-0.1357
BS00049818	6D	451020060	0.6307	0.0682	44	0.1579	0.1629	-0.1505
BS00046963	6B	150665020	0.6459	0.4205	44	0.1579	0.1625	-0.0958

Table (10): Continue.

SNP	Chr	Position	P.value	MAF	nobs	R-without	R-with	Effect
BS00071558	7A	626897156	0.6628	0.1136	44	0.1579	0.162	-0.1111
BS00064691	5D	496067152	0.6796	0.0227	44	0.1579	0.1616	0.2093
BS00025017	5D	551059325	0.689	0.1818	44	0.1579	0.1614	0.1298
BS00080749	2D	72171433	0.6917	0.1136	44	0.1579	0.1613	-0.1033
AX-94558874	5A	25838327	0.6938	0.0682	44	0.1579	0.1613	-0.1217
AX-94442305	2A	5637087	0.7796	0.1818	44	0.1579	0.1596	-0.0613
BS00076622	7B	717202678	0.7833	0.1136	44	0.1579	0.1596	0.0944
BS00107766	4A	599846443	0.8036	0.0227	44	0.1579	0.1593	-0.1281
BS00050057	5B	658370171	0.8082	0.0909	44	0.1579	0.1592	-0.0644
BS00000006	5A	706240365	0.8103	0.3295	44	0.1579	0.1592	0.0446
BS00058591	5A	459003097	0.8122	0.25	44	0.1579	0.1592	-0.0486
AX-94406983	2B	752491121	0.8311	0.1818	44	0.1579	0.1589	-0.0468
BS00044237	6B	192349655	0.8469	0.0455	44	0.1579	0.1587	-0.0703
AX-94486277	4B	673203328	0.8557	0.2955	44	0.1579	0.1587	-0.032
BS00074083	7B	62687249	0.863	0.1136	44	0.1579	0.1586	0.0449
BS00035234	7B	711362365	0.8894	0.1364	44	0.1579	0.1584	0.0454
BS00049370	2D	12977999	0.8975	0.0227	44	0.1579	0.1583	-0.0659
BS00030651	3B	764694076	0.9054	0.3409	44	0.1579	0.1582	0.0215
AX-94488939	5A	664484771	0.9109	0.3636	44	0.1579	0.1582	0.0227
AX-94446956	1D	457256590	0.9204	0.5	44	0.1579	0.1582	0.0196
BS00077716	4A	597693165	0.9226	0.0455	44	0.1579	0.1581	0.036
BS00033795	6A	402473588	0.9251	0.4091	44	0.1579	0.1581	0.0199
AX-94415898	1A	37501136	0.9437	0.1136	44	0.1579	0.1581	0.024
BS00024786	7A	79542853	0.946	0.4773	44	0.1579	0.158	0.0126
BS00032039	1B	660528642	0.9721	0.0568	44	0.1579	0.158	-0.0121
BS00046264	6B	704974332	0.9771	0.4432	44	0.1579	0.158	-0.006
BS00083630	6A	5604416	0.9969	0.2727	44	0.1579	0.1579	-0.0008
AX-86163814	1A	391256838	1	0	44	NA	NA	NA
AX-86167869	6A	90032650	1	0	44	NA	NA	NA
AX-94382081	7B	599423337	1	0	44	NA	NA	NA
AX-94457966	7B	11253456	1	0	44	NA	NA	NA
AX-94559367	6A	111530836	1	0	44	NA	NA	NA
BS00021745	7D	629830671	1	0	44	NA	NA	NA
BS00022411	1B	629159325	1	0	44	NA	NA	NA
BS00024548	3A	701852813	1	0	44	NA	NA	NA
BS00037020	4B	595271188	1	0	44	NA	NA	NA
BS00042105	4B	616274476	1	0	44	NA	NA	NA
BS00043169	7D	629449615	1	0	44	NA	NA	NA
BS00050109	3A	680749608	1	0	44	NA	NA	NA
BS00057851	5B	629930541	1	0	44	NA	NA	NA
BS00075815	5B	536046952	1	0	44	NA	NA	NA
BS00076192	1B	1779797	1	0	44	NA	NA	NA
BS00077891	7A	647308576	1	0	44	NA	NA	NA
BS00089403	4D	505433571	1	0	44	NA	NA	NA
BS00089597	5D	552040143	1	0	44	NA	NA	NA
BS00097126	2D	27651343	1	0	44	NA	NA	NA
BS00100939	2B	29991102	1	0	44	NA	NA	NA

Table (12): The GWAS result of SNP markers for PH trait.

SNP	Chr	Position	P.value	MAF	nobs	R-without	R-with	Effect
BS00066143	5A	533072163	0.0304	0.2273	44	0.0059	0.1313	-3.8526
BS00038820	2B	64988240	0.0446	0.1136	44	0.0059	0.113	4.1609
BS00024921	2A	733091124	0.0538	0.2045	44	0.0059	0.1042	3.417
AX-94392216	1D	11498675	0.059	0.2045	44	0.0059	0.1	3.531
BS00022625	1B	163096405	0.085	0.2727	44	0.0059	0.0836	3.0551
BS00021704	6A	611851563	0.1016	0.4091	44	0.0059	0.0758	3.2287
BS00033795	6A	402473588	0.102	0.4091	44	0.0059	0.0756	2.8797
BS00030651	3B	764694076	0.1177	0.3409	44	0.0059	0.0695	-2.5174
BS00078124	6A	617182750	0.1265	0.2273	44	0.0059	0.0665	-3.001
BS00049370	2D	12977999	0.1415	0.0227	44	0.0059	0.0619	5.1169
BS00083630	6A	5604416	0.1446	0.2727	44	0.0059	0.061	-2.3843
BS00032039	1B	660528642	0.1757	0.0568	44	0.0059	0.0532	-3.4991
BS00046264	6B	704974332	0.1968	0.4432	44	0.0059	0.0488	-2.2602
BS00082503	1D	412219194	0.2035	0.3182	44	0.0059	0.0475	-2.4272
BS00018707	4B	95108797	0.2043	0.3295	44	0.0059	0.0474	1.9835
BS00071558	7A	626897156	0.2086	0.1136	44	0.0059	0.0466	2.6644
AX-94401211	6B	46960454	0.2114	0.0795	44	0.0059	0.0461	-2.7974
BS00107837	1B	674821632	0.2289	0.3068	44	0.0059	0.0431	2.533
BS00073116	5D	546864119	0.2569	0.2727	44	0.0059	0.0388	-1.9301
BS00040283	7B	709256065	0.2749	0.2727	44	0.0059	0.0364	-1.8479
BS00046963	6B	150665020	0.2944	0.4205	44	0.0059	0.034	-1.7988
BS00063425	5A	419868619	0.3103	0.2955	44	0.0059	0.0322	2.0251
AX-94486277	4B	673203328	0.3157	0.2955	44	0.0059	0.0316	-1.556
BS00031140	2A	241087061	0.3166	0.4545	44	0.0059	0.0315	1.68
BS00050057	5B	658370171	0.3767	0.0909	44	0.0059	0.0258	1.768
BS00077716	4A	597693165	0.391	0.0455	44	0.0059	0.0246	2.3476
BS00025017	5D	551059325	0.399	0.1818	44	0.0059	0.024	-2.1671
BS00104432	5A	636413981	0.4045	0.4545	44	0.0059	0.0236	-1.2821
BS00049977	3A	688688670	0.4093	0.4091	44	0.0059	0.0232	-1.3977
BS00050993	7B	36490052	0.4202	0.0455	44	0.0059	0.0224	-2.4564
AX-94454241	1D	10717734	0.4444	0.2614	44	0.0059	0.0208	-1.4111
BS00089954	3B	543718628	0.4506	0.2841	44	0.0059	0.0204	1.2989
BS00071183	3B	823762943	0.4682	0.0909	44	0.0059	0.0193	-1.5745
AX-94488939	5A	664484771	0.4743	0.3636	44	0.0059	0.0189	-1.2072
AX-94406983	2B	752491121	0.4905	0.1818	44	0.0059	0.018	-1.3126
AX-94527869	7A	191266404	0.4947	0.0455	44	0.0059	0.0177	-2.1208
BS00000006	5A	706240365	0.5445	0.3295	44	0.0059	0.0152	-0.9508
BS00076248	3B	53567326	0.55	0.0909	44	0.0059	0.015	1.2246
BS00105878	3B	750361290	0.555	0.2273	44	0.0059	0.0147	1.1079
BS00074083	7B	62687249	0.5668	0.1136	44	0.0059	0.0142	-1.1738
BS00070791	7B	642475463	0.6121	0.2273	44	0.0059	0.0124	-1.0894
AX-94446956	1D	457256590	0.6282	0.5	44	0.0059	0.0119	-0.8212
BS00049818	6D	451020060	0.6361	0.0682	44	0.0059	0.0116	1.0057

Table (11): Continue.

SNP	Chr	Position	P.value	MAF	nobs	R-without	R-with	Effect
BS00024786	7A	79542853	0.6392	0.4773	44	0.0059	0.0115	-0.7328
BS00076033	4B	609515971	0.6596	0.2727	44	0.0059	0.0108	-0.6815
BS00060686	1B	675320277	0.6741	0.1023	44	0.0059	0.0104	0.8513
BS00044720	2D	78793739	0.6778	0.0682	44	0.0059	0.0103	-1.0594
BS00058591	5A	459003097	0.7109	0.25	44	0.0059	0.0094	0.6006
BS00040798	3A	528555143	0.7206	0.4205	44	0.0059	0.0092	-0.5741
BS00035234	7B	711362365	0.7274	0.1364	44	0.0059	0.009	-0.8989
BS00022653	4B	526928412	0.7304	0.3636	44	0.0059	0.0089	-0.6431
BS00070903	5A	416170651	0.7384	0.375	44	0.0059	0.0087	0.7023
BS00106043	5B	27459998	0.7446	0.1591	44	0.0059	0.0086	-0.6212
AX-94558874	5A	25838327	0.7877	0.0682	44	0.0059	0.0078	-0.6482
BS00080749	2D	72171433	0.799	0.1136	44	0.0059	0.0076	0.5398
AX-94442305	2A	5637087	0.8033	0.1818	44	0.0059	0.0075	-0.452
AX-94545917	4B	25834775	0.8112	0.3068	44	0.0059	0.0074	0.3886
AX-94540417	1B	431456742	0.8222	0.2727	44	0.0059	0.0072	-0.4093
BS00064146	7B	655818377	0.8226	0.2841	44	0.0059	0.0072	0.5096
BS00101408	7B	657662487	0.8226	0.2841	44	0.0059	0.0072	0.5096
BS00039211	2D	74981770	0.8231	0.1136	44	0.0059	0.0072	0.4562
BS00044237	6B	192349655	0.8295	0.0455	44	0.0059	0.0071	0.5486
BS00064691	5D	496067152	0.8376	0.0227	44	0.0059	0.007	-0.7246
BS00084133	5D	550441920	0.8709	0.2727	44	0.0059	0.0066	-0.3825
BS00109036	6B	663531385	0.8966	0.1364	44	0.0059	0.0064	-0.2601
BS00023673	7A	484221405	0.9122	0.25	44	0.0059	0.0062	-0.183
BS00076622	7B	717202678	0.9407	0.1136	44	0.0059	0.0061	0.1881
BS00107766	4A	599846443	0.9526	0.0227	44	0.0059	0.006	0.2243
AX-94415898	1A	37501136	0.9624	0.1136	44	0.0059	0.006	0.1234
AX-94529943	6D	437805846	0.9802	0.1591	44	0.0059	0.0059	0.0496
BS00031178	6A	51409030	0.9858	0.2727	44	0.0059	0.0059	0.0282
AX-86163814	1A	391256838	1	0	44	NA	NA	NA
AX-86167869	6A	90032650	1	0	44	NA	NA	NA
AX-94382081	7B	599423337	1	0	44	NA	NA	NA
AX-94457966	7B	11253456	1	0	44	NA	NA	NA
AX-94559367	6A	111530836	1	0	44	NA	NA	NA
BS00021745	7D	629830671	1	0	44	NA	NA	NA
BS00022411	1B	629159325	1	0	44	NA	NA	NA
BS00024548	3A	701852813	1	0	44	NA	NA	NA
BS00037020	4B	595271188	1	0	44	NA	NA	NA
BS00042105	4B	616274476	1	0	44	NA	NA	NA
BS00043169	7D	629449615	1	0	44	NA	NA	NA
BS00050109	3A	680749608	1	0	44	NA	NA	NA
BS00057851	5B	629930541	1	0	44	NA	NA	NA
BS00075815	5B	536046952	1	0	44	NA	NA	NA
BS00076192	1B	1779797	1	0	44	NA	NA	NA
BS00077891	7A	647308576	1	0	44	NA	NA	NA
BS00089403	4D	505433571	1	0	44	NA	NA	NA
BS00089597	5D	552040143	1	0	44	NA	NA	NA
BS00097126	2D	27651343	1	0	44	NA	NA	NA
BS00100939	2B	29991102	1	0	44	NA	NA	NA

Table (13): The GWAS result of SNP markers for SH trait.

SNP	Chr	Position	P.value	MAF	nobs	R-without	R-with	Effect
BS00050057	5B	658370171	0.0217	0.0909	44	0.0491	0.185	0.7332
BS00021704	6A	611851563	0.0316	0.4091	44	0.0491	0.1673	-0.5927
BS00033795	6A	402473588	0.0581	0.4091	44	0.0491	0.1397	-0.4617
AX-94442305	2A	5637087	0.0635	0.1818	44	0.0491	0.1358	-0.4627
BS00050993	7B	36490052	0.0951	0.0455	44	0.0491	0.1187	0.7321
BS00031140	2A	241087061	0.1391	0.4545	44	0.0491	0.1033	-0.3341
AX-94540417	1B	431456742	0.145	0.2727	44	0.0491	0.1017	-0.363
BS00024786	7A	79542853	0.1815	0.4773	44	0.0491	0.0931	-0.2787
BS00064146	7B	655818377	0.2171	0.2841	44	0.0491	0.0865	0.3639
BS00101408	7B	657662487	0.2171	0.2841	44	0.0491	0.0865	0.3639
BS00070791	7B	642475463	0.218	0.2273	44	0.0491	0.0864	-0.3693
AX-94545917	4B	25834775	0.2487	0.3068	44	0.0491	0.0817	0.2368
BS00040283	7B	709256065	0.2508	0.2727	44	0.0491	0.0814	-0.2487
BS00074083	7B	62687249	0.2527	0.1136	44	0.0491	0.0812	-0.3395
BS00076622	7B	717202678	0.2605	0.1136	44	0.0491	0.0801	-0.4481
BS00049370	2D	12977999	0.2618	0.0227	44	0.0491	0.0799	0.6883
BS00105878	3B	750361290	0.2908	0.2273	44	0.0491	0.0764	-0.2673
BS00030651	3B	764694076	0.2943	0.3409	44	0.0491	0.076	-0.2077
BS00049977	3A	688688670	0.2961	0.4091	44	0.0491	0.0758	0.2462
BS00038820	2B	64988240	0.2973	0.1136	44	0.0491	0.0756	0.3167
BS00049818	6D	451020060	0.3445	0.0682	44	0.0491	0.0709	-0.3507
BS00083630	6A	5604416	0.3523	0.2727	44	0.0491	0.0702	-0.1956
BS00040798	3A	528555143	0.3596	0.4205	44	0.0491	0.0695	0.1972
BS00000006	5A	706240365	0.3631	0.3295	44	0.0491	0.0692	-0.1889
BS00078124	6A	617182750	0.3678	0.2273	44	0.0491	0.0689	0.2234
AX-94392216	1D	11498675	0.3785	0.2045	44	0.0491	0.068	-0.2196
AX-94406983	2B	752491121	0.3979	0.1818	44	0.0491	0.0665	0.2028
AX-94558874	5A	25838327	0.398	0.0682	44	0.0491	0.0665	0.2986
BS00071183	3B	823762943	0.4106	0.0909	44	0.0491	0.0656	-0.2566
BS00084133	5D	550441920	0.425	0.2727	44	0.0491	0.0646	-0.2794
BS00044237	6B	192349655	0.4339	0.0455	44	0.0491	0.064	0.3364
BS00031178	6A	51409030	0.4417	0.2727	44	0.0491	0.0635	0.1676
AX-94488939	5A	664484771	0.4458	0.3636	44	0.0491	0.0632	-0.1737
BS00066143	5A	533072163	0.4605	0.2273	44	0.0491	0.0623	-0.1792
BS00035234	7B	711362365	0.4974	0.1364	44	0.0491	0.0603	-0.2522
BS00104432	5A	636413981	0.518	0.4545	44	0.0491	0.0592	0.1295
BS00073116	5D	546864119	0.5193	0.2727	44	0.0491	0.0592	-0.1442
AX-94527869	7A	191266404	0.5377	0.0455	44	0.0491	0.0583	0.2933
BS00044720	2D	78793739	0.5389	0.0682	44	0.0491	0.0582	-0.2166
BS00032039	1B	660528642	0.5457	0.0568	44	0.0491	0.0579	-0.2418
BS00107766	4A	599846443	0.5666	0.0227	44	0.0491	0.0571	-0.3395
BS00046963	6B	150665020	0.5948	0.4205	44	0.0491	0.0559	-0.1241
AX-94486277	4B	673203328	0.6152	0.2955	44	0.0491	0.0552	0.096

Table (12): Continue.

SNP	Chr	Position	P.value	MAF	nobs	R-without	R-with	Effect
SNP	Chr	Position	P.value	MAF	nobs	R-without	R-with	Effect
BS00022625	1B	163096405	0.6159	0.2727	44	0.0491	0.0552	0.1059
BS00080749	2D	72171433	0.6238	0.1136	44	0.0491	0.0549	-0.1436
BS00071558	7A	626897156	0.6538	0.1136	44	0.0491	0.054	-0.1262
BS00089954	3B	543718628	0.6599	0.2841	44	0.0491	0.0538	0.1052
BS00107837	1B	674821632	0.6902	0.3068	44	0.0491	0.0529	0.1089
BS00060686	1B	675320277	0.6984	0.1023	44	0.0491	0.0527	-0.1165
BS00076248	3B	53567326	0.7119	0.0909	44	0.0491	0.0524	0.1205
BS00022653	4B	526928412	0.7221	0.3636	44	0.0491	0.0522	0.0777
BS00064691	5D	496067152	0.7328	0.0227	44	0.0491	0.0519	0.2027
BS00058591	5A	459003097	0.7365	0.25	44	0.0491	0.0518	0.078
AX-94529943	6D	437805846	0.7564	0.1591	44	0.0491	0.0514	0.0779
BS00070903	5A	416170651	0.7568	0.375	44	0.0491	0.0514	-0.0783
BS00106043	5B	27459998	0.8135	0.1591	44	0.0491	0.0505	-0.0624
BS00077716	4A	597693165	0.8144	0.0455	44	0.0491	0.0504	-0.1006
BS00046264	6B	704974332	0.8486	0.4432	44	0.0491	0.05	0.0438
BS00082503	1D	412219194	0.8813	0.3182	44	0.0491	0.0496	-0.0405
BS00018707	4B	95108797	0.8895	0.3295	44	0.0491	0.0496	0.0264
BS00076033	4B	609515971	0.9064	0.2727	44	0.0491	0.0494	-0.0251
BS00109036	6B	663531385	0.9158	0.1364	44	0.0491	0.0494	0.0283
AX-94446956	1D	457256590	0.92	0.5	44	0.0491	0.0494	0.0214
AX-94401211	6B	46960454	0.9209	0.0795	44	0.0491	0.0493	-0.0349
BS00023673	7A	484221405	0.9213	0.25	44	0.0491	0.0493	0.0217
AX-94454241	1D	10717734	0.922	0.2614	44	0.0491	0.0493	-0.0216
BS00025017	5D	551059325	0.9222	0.1818	44	0.0491	0.0493	-0.036
BS00024921	2A	733091124	0.9377	0.2045	44	0.0491	0.0493	-0.0177
BS00063425	5A	419868619	0.9706	0.2955	44	0.0491	0.0491	-0.0089
AX-94415898	1A	37501136	0.9737	0.1136	44	0.0491	0.0491	-0.0128
BS00039211	2D	74981770	0.9889	0.1136	44	0.0491	0.0491	-0.004
AX-86163814	1A	391256838	1	0	44	NA	NA	NA
AX-86167869	6A	90032650	1	0	44	NA	NA	NA
AX-94382081	7B	599423337	1	0	44	NA	NA	NA
AX-94457966	7B	11253456	1	0	44	NA	NA	NA
AX-94559367	6A	111530836	1	0	44	NA	NA	NA
BS00021745	7D	629830671	1	0	44	NA	NA	NA
BS00022411	1B	629159325	1	0	44	NA	NA	NA
BS00024548	3A	701852813	1	0	44	NA	NA	NA
BS00037020	4B	595271188	1	0	44	NA	NA	NA
BS00042105	4B	616274476	1	0	44	NA	NA	NA
BS00043169	7D	629449615	1	0	44	NA	NA	NA
BS00050109	3A	680749608	1	0	44	NA	NA	NA
BS00057851	5B	629930541	1	0	44	NA	NA	NA
BS00075815	5B	536046952	1	0	44	NA	NA	NA
BS00076192	1B	1779797	1	0	44	NA	NA	NA
BS00077891	7A	647308576	1	0	44	NA	NA	NA
BS00089403	4D	505433571	1	0	44	NA	NA	NA
BS00089597	5D	552040143	1	0	44	NA	NA	NA
BS00097126	2D	27651343	1	0	44	NA	NA	NA
BS00100939	2B	29991102	1	0	44	NA	NA	NA

Table (14): The GWAS result of SNP markers for STR trait.

SNP	Chr	Position	P.value	MAF	nobs	R-without	R-with	Effect
BS00064146	7B	655818377	0.0146	0.2841	44	-0.0081	0.1564	0.4692
BS00101408	7B	657662487	0.0146	0.2841	44	-0.0081	0.1564	0.4692
BS00076622	7B	717202678	0.0239	0.1136	44	-0.0081	0.131	-0.5613
BS00089954	3B	543718628	0.0367	0.2841	44	-0.0081	0.1098	0.3187
BS00000006	5A	706240365	0.0436	0.3295	44	-0.0081	0.1015	-0.2689
BS00070791	7B	642475463	0.05	0.2273	44	-0.0081	0.095	-0.3766
AX-94415898	1A	37501136	0.0723	0.1136	44	-0.0081	0.0779	0.4378
BS00109036	6B	663531385	0.0758	0.1364	44	-0.0081	0.0757	-0.3072
BS00071558	7A	626897156	0.0797	0.1136	44	-0.0081	0.0735	0.3181
BS00071183	3B	823762943	0.0808	0.0909	44	-0.0081	0.0729	0.3439
AX-94529943	6D	437805846	0.0843	0.1591	44	-0.0081	0.071	-0.2828
BS00104432	5A	636413981	0.0924	0.4545	44	-0.0081	0.0669	0.2169
BS00021704	6A	611851563	0.1015	0.4091	44	-0.0081	0.0628	-0.2783
BS00046264	6B	704974332	0.1069	0.4432	44	-0.0081	0.0605	-0.2383
BS00105878	3B	750361290	0.1845	0.2273	44	-0.0081	0.0379	-0.2119
BS00049370	2D	12977999	0.2257	0.0227	44	-0.0081	0.0301	-0.4389
BS00106043	5B	27459998	0.2541	0.1591	44	-0.0081	0.0257	-0.1887
AX-94545917	4B	25834775	0.2868	0.3068	44	-0.0081	0.0213	0.1402
BS00058591	5A	459003097	0.2892	0.25	44	-0.0081	0.021	0.1528
BS00049977	3A	688688670	0.3297	0.4091	44	-0.0081	0.0165	0.1425
BS00039211	2D	74981770	0.3483	0.1136	44	-0.0081	0.0146	0.1692
BS00060686	1B	675320277	0.3723	0.1023	44	-0.0081	0.0124	0.1652
BS00063425	5A	419868619	0.3815	0.2955	44	-0.0081	0.0116	0.1377
BS00035234	7B	711362365	0.3997	0.1364	44	-0.0081	0.0102	0.1934
AX-94527869	7A	191266404	0.4341	0.0455	44	-0.0081	0.0076	0.2255
BS00049818	6D	451020060	0.4389	0.0682	44	-0.0081	0.0073	0.1704
BS00050993	7B	36490052	0.4517	0.0455	44	-0.0081	0.0065	0.2011
BS00023673	7A	484221405	0.4791	0.25	44	-0.0081	0.0048	0.0989
AX-94446956	1D	457256590	0.5128	0.5	44	-0.0081	0.0029	0.0898
BS00038820	2B	64988240	0.531	0.1136	44	-0.0081	0.002	0.1167
AX-94401211	6B	46960454	0.5423	0.0795	44	-0.0081	0.0014	-0.1303
BS00018707	4B	95108797	0.5476	0.3295	44	-0.0081	0.0012	0.0741
BS00031140	2A	241087061	0.554	0.4545	44	-0.0081	0.0009	0.0833
AX-94406983	2B	752491121	0.5678	0.1818	44	-0.0081	0.0003	-0.0877
BS00076033	4B	609515971	0.5747	0.2727	44	-0.0081	0	0.0746
BS00107837	1B	674821632	0.6003	0.3068	44	-0.0081	-0.0011	0.0912
BS00031178	6A	51409030	0.6055	0.2727	44	-0.0081	-0.0013	0.07
BS00025017	5D	551059325	0.6304	0.1818	44	-0.0081	-0.0022	-0.1093
AX-94486277	4B	673203328	0.6534	0.2955	44	-0.0081	-0.0029	0.0552
BS00070903	5A	416170651	0.6637	0.375	44	-0.0081	-0.0033	0.0711
BS00073116	5D	546864119	0.6765	0.2727	44	-0.0081	-0.0036	0.0588
AX-94442305	2A	5637087	0.692	0.1818	44	-0.0081	-0.0041	-0.0607
BS00032039	1B	660528642	0.6987	0.0568	44	-0.0081	-0.0043	0.0936
BS00076248	3B	53567326	0.7064	0.0909	44	-0.0081	-0.0045	0.074

Table (13): Continue.

SNP	Chr	Position	P.value	MAF	nobs	R-without	R-with	Effect
SNP	Chr	Position	P.value	MAF	nobs	R-without	R-with	Effect
BS00044720	2D	78793739	0.7224	0.0682	44	-0.0081	-0.0049	-0.0779
BS00078124	6A	617182750	0.7376	0.2273	44	-0.0081	-0.0052	-0.0529
BS00022625	1B	163096405	0.7445	0.2727	44	-0.0081	-0.0054	0.0447
BS00074083	7B	62687249	0.7466	0.1136	44	-0.0081	-0.0054	-0.0588
BS00033795	6A	402473588	0.755	0.4091	44	-0.0081	-0.0056	0.0463
BS00084133	5D	550441920	0.7891	0.2727	44	-0.0081	-0.0063	0.0573
BS00082503	1D	412219194	0.7928	0.3182	44	-0.0081	-0.0063	0.044
BS00083630	6A	5604416	0.8038	0.2727	44	-0.0081	-0.0065	0.0332
BS00077716	4A	597693165	0.8166	0.0455	44	-0.0081	-0.0067	0.0602
BS00040798	3A	528555143	0.8259	0.4205	44	-0.0081	-0.0069	0.0295
BS00080749	2D	72171433	0.8287	0.1136	44	-0.0081	-0.0069	-0.0394
AX-94454241	1D	10717734	0.8332	0.2614	44	-0.0081	-0.007	0.03
BS00022653	4B	526928412	0.8426	0.3636	44	-0.0081	-0.0071	-0.0285
BS00030651	3B	764694076	0.8445	0.3409	44	-0.0081	-0.0071	-0.0247
BS00040283	7B	709256065	0.8525	0.2727	44	-0.0081	-0.0072	0.0254
AX-94392216	1D	11498675	0.8533	0.2045	44	-0.0081	-0.0072	-0.0288
BS00044237	6B	192349655	0.8538	0.0455	44	-0.0081	-0.0072	0.0471
AX-94488939	5A	664484771	0.8642	0.3636	44	-0.0081	-0.0074	-0.0243
BS00107766	4A	599846443	0.8831	0.0227	44	-0.0081	-0.0076	0.0531
BS00024921	2A	733091124	0.8846	0.2045	44	-0.0081	-0.0076	-0.0207
AX-94540417	1B	431456742	0.8945	0.2727	44	-0.0081	-0.0077	0.0206
BS00024786	7A	79542853	0.9116	0.4773	44	-0.0081	-0.0078	-0.0145
AX-94558874	5A	25838327	0.9441	0.0682	44	-0.0081	-0.008	-0.0152
BS00050057	5B	658370171	0.9636	0.0909	44	-0.0081	-0.008	-0.0085
BS00064691	5D	496067152	0.9781	0.0227	44	-0.0081	-0.0081	-0.0097
BS00066143	5A	533072163	0.9874	0.2273	44	-0.0081	-0.0081	-0.0024
BS00046963	6B	150665020	0.9902	0.4205	44	-0.0081	-0.0081	-0.0018
AX-86163814	1A	391256838	1	0	44	NA	NA	NA
AX-86167869	6A	90032650	1	0	44	NA	NA	NA
AX-94382081	7B	599423337	1	0	44	NA	NA	NA
AX-94457966	7B	11253456	1	0	44	NA	NA	NA
AX-94559367	6A	111530836	1	0	44	NA	NA	NA
BS00021745	7D	629830671	1	0	44	NA	NA	NA
BS00022411	1B	629159325	1	0	44	NA	NA	NA
BS00024548	3A	701852813	1	0	44	NA	NA	NA
BS00037020	4B	595271188	1	0	44	NA	NA	NA
BS00042105	4B	616274476	1	0	44	NA	NA	NA
BS00043169	7D	629449615	1	0	44	NA	NA	NA
BS00050109	3A	680749608	1	0	44	NA	NA	NA
BS00057851	5B	629930541	1	0	44	NA	NA	NA
BS00075815	5B	536046952	1	0	44	NA	NA	NA
BS00076192	1B	1779797	1	0	44	NA	NA	NA
BS00077891	7A	647308576	1	0	44	NA	NA	NA
BS00089403	4D	505433571	1	0	44	NA	NA	NA
BS00089597	5D	552040143	1	0	44	NA	NA	NA
BS00097126	2D	27651343	1	0	44	NA	NA	NA
BS00100939	2B	29991102	1	0	44	NA	NA	NA

Table (15): The statistical effect, physical position (Pos.) and correlation significances (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	effect	p.value	effect	p.value	effect
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

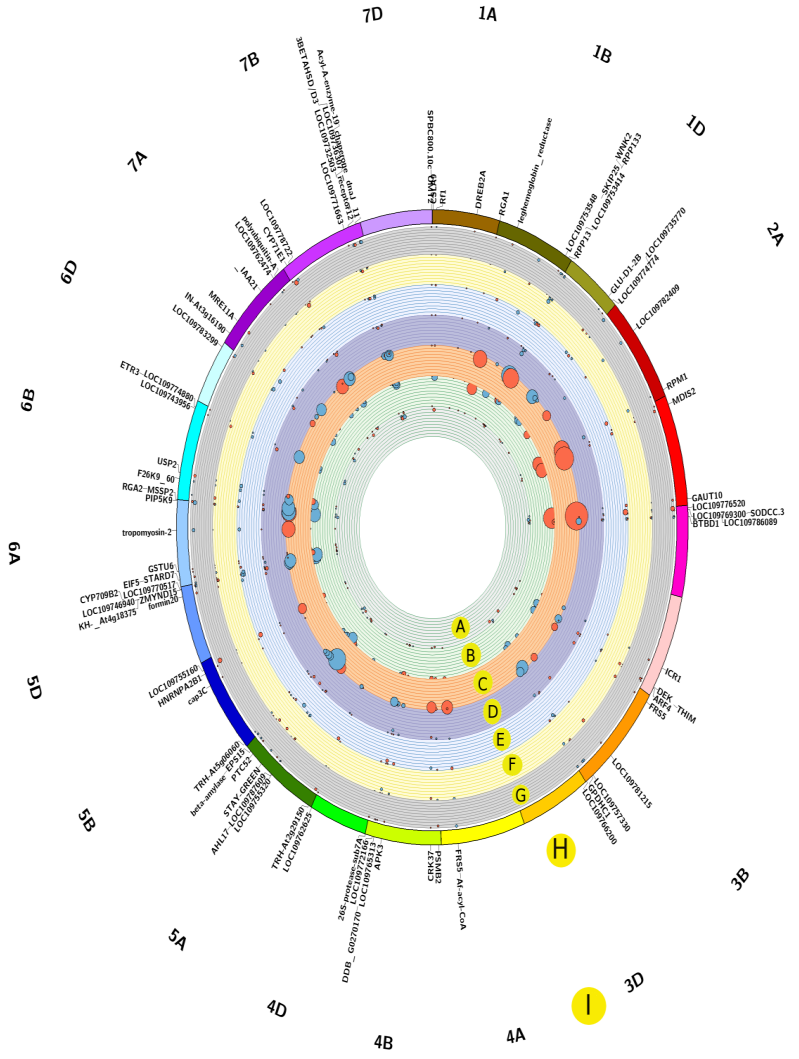

Fig. (7): The genomic distribution and GWAS analysis of the studied SNP markers. The significance value and statistical effect regarding SNP marker and their correlation with STR (A), DF (B), PH (C), NT (D), NSL (E), NS (F), SH (G) traits. The size and color of the circles indicate the p.value and effect markers. (H) Genes near these markers are shown by their chromosome location on the wheat genome (I).

Luo *et al.* (2020) mapped genetic markers that were associated with 15 agronomic traits under two levels of salt stress in a new constructed RIL wheat population using 55 thousand SNPs. Genotyping showed that 21,154 SNPs (39.9% of the total SNP markers) were different between parental lines. Among them, the most SNPs (1756) were belonged to chromosome 2A, while the least SNPs (just 245) were belonged to chromosome 4D. After analysis, 345 lines were retained, and a total of 16,011 SNPs in the first group were chosen to construct a genetic map. Marker-trait association analysis showed that 90 stable QTLs for 15 traits were detected, and they were distributed on all wheat chromosomes except 4D, 6B and 7D chromosomes. These QTLs individually explained about 2.34–32.43% of the phenotypic variation with LOD values ranged from 2.68 to 47.15. It was found that four QTL clusters were located on 2D, 3D, 4B and 6A chromosomes.

The marker-based and pedigree-based kinship analyses were used to study the genetic variations in QTLs associated with salt tolerance in Chinese wheat accessions **(Yu *et al.*, 2020)**. A panel of 307 wheat accessions, including local landraces, exotic cultivars used in Chinese breeding programs and Chinese cultivars was subjected to a genome-wide association study to dissect the genetic basis of salinity tolerance. A total of 402,176 SNPs with average SNP density of 0.49 Mb was used in GWAS analysis to detect QTLs for salt tolerance. One hundred and seventeen significant SNP trait-associations were detected with a suggestive threshold (P-value <1.0e-4). Of

these, 102 SNPs were clustered in three main genomic regions on 1A (72), 3B (10) and 6B (20) chromosomes. All of these QTLs were associated with Na+ exclusion, reduced Na+ uptake, regulation of K+ and/or Na+ transport, or Ca2+ and Mg2+ accumulation.

Hu *et al.* 2020 performed a GWAS analysis of yield and related traits in common wheat under salt-stress conditions. A total of 389 SNPs representing 11 QTLs were signicantly associated with traits under different salt treatments, with phenotypic explanation rate ranged from 9.14 to 50.45%. Of these, repetitive and pleiotropic loci on 4A, 5A, 5B and 7A chromosomes were significantly linked to yield and yield related traits under low salinity conditions. Spike length-related loci were mainly located on 1B, 3B, 5B and 7A chromosomes under different salt treatments. Two loci on 4D and 5A chromosomes were related with plant height trait in low and high salinity environment, respectively. Three salt-tolerant related loci were confirmed to be important in two bi-parental populations. Distribution of favorable haplotypes indicated that superior haplotypes of pleiotropic loci on group-5 chromosomes were strongly selected and had a potential for increasing wheat salt tolerance.

By studying genes located near the SNP markers associated with wheat agronomic traits under salinity, 13 different genes were identified (Table 16).These genes include *CYP709B2*, which was reported to be highly associated with salinity tolerance in *Arabidopsis thaliana*. It was concluded

that plants with mutant *CYP709b3* may be sensitive to ABA and salt stress during germination **(Mao *et al.,* 2013)**.

Table (16): List of genes located in the vicinity of trait-associated with 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	LOC109781215
BS00021704	phosphatidylinositol 4-phosphate 5-kinase 9	*PIP5K9*
BS00078124	monosaccharide-sensing 2	*MSSP2*
BS00064146	LOC109732503	LOC109732503
BS00101408	LOC109736307	LOC109736307
BS00050057	LOC109755160	LOC109755160
BS00107837	LOC109753414	LOC109753414
BS00000006	beta-amylase	-
BS00076622	3beta-hydroxysteroid-dehydrogenase/decarboxylase	*3BETAHSD/D3*
BS00024921	disease resistance RPM1	*RPM1*

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. 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 gene of *PIP5K9* showed a significant association with SH trait. 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 (16), *MSSP2* gene was genetically near to BS00078124 marker, which is correlated with DF trait (Table 13). A significant expression of *MSSP2* transport protein gene 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 16). 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.,* 2018b)**. GWAS analysis revealed a significant association with BS00024921 marker, which was associated with DF trait and 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)**.

In conclusion , the genetic diversity and population structure of a set of local and international wheat genotypes were investigated . These genotypes were successfully assigned to different groups depending on their

genetic background. This study revealed the genetic structure of adapted and imported wheat genotypes, which could be used to select potential wheat genotypes 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 used to select salinity-tolerant genotypes could be integrated into national genetic improvement programs. The SNP genotyping analysis is a very potential technology that could be efficiently applied to detect some genes that control wheat response to salinity stress.

SUMMARY

The objectives of this study are to investigate the population structure of 70 Egyptian, Syrain and Iranian wheat accessions and to identify some markers associated with salinity tolerance in bread wheat. In addition to identify some genes that control some important agronomic parameters of wheat under salinity stress.

The wheat germplasm panel was 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 green house of Agricultural genetic Engineering research institute (AGERI), Agricultural Research Institute (ARC), and the experimental filed of faculty of environmental agricultural sciences, Suez university in Arish , Sinai, Egypt. This subset was chosen from the International Center for Agricultural Research in the Dry Areas (ICARDA) and Agricultural Research Center gene banks, Giza, Egypt. The genome association analysis (GWAS) and population structure analysis was conducted using six SCoT, five SSR and 93 SNP markers.

A total number of 61 PCR-bands were revealed using SSR and SCoT primers, where SCoT analysis provided a higher number of bands (46 bands) compared to SSR analysis (15 bands). The maximum number of bands was obtained from SCoT-05 primer (10 bands). Additionally, the total number of polymorphic bands was 48 bands, where SCoT-10, and SCoT-01

primers revealed the maximum number of polymorphic bands (eight bands). The PCR primers of SCoT-02 and SSR-01 revealed the maximum percentage of polymorphism (100%). On the other hand, out of the 91 SNP primers used for SNP genotyping, only 17 makers were monomorphic.

Analysis of the structure using allele frequency of Egyptian, Syrian and Iranian wheat genotypes indicated that these genotypes belong to four different population groups. Where, for the most portion, Egyptian, Syrian and Iranian genotypes were clustered depending on their country of origin. On the other hand, some genotypes showed a type of genetic migration, which could be caused by varietal adaptation. Similar results were retrieved using the phylogenetic analysis. Most genotypes were almost clustered, depending on their geographical origin, although some Egyptian genotypes were clustered with the Iranian and Syrian genotypes, which could indicate their source of origin. Such analysis could indicated a number of potential foreign genotypes that could be successfully adapted in the Egyptian environment through local breeding programmes.

SNP genotyping was used to detect genes that are related to wheat response to salinity stress. GWAS analysis revealed 13 significant SNP markers that were. These markers are distributed across the chromosomes of 7B (3 markers), 6A (3 markers), 5A (2 markers), 2B (1 marker), 2A (1 marker), 5B (1 marker), 3B (1 marker), and 1B (1 marker) . The effect of these markers on STR trait was ranged from -0.56 (BS00076622 marker) to 0.469

(BS00064146 and BS00101408 markers). DF trait was correlated with four SNP markers (BS00024921, BS00083630, BS00078124, and BS00038820 markers), where its effect were ranged from -2.544 (BS00078124 marker) to 2.526 (BS00038820, and BS00024921 markers). Some SNP markers showed correlation with multiple traits such as BS00038820 (DF and PH traits), BS00107837 (NS and NT traits), and BS00089954 (NS and STR traits) markers.

By studying genes located near the SNP markers associated with wheat agronomic traits under salinity, 13 different genes were identified. Most of these genes included cytochrome P450 709B2, MALE DISCOVERER 2, STAY-GREEN, phosphatidylinositol 4-phosphate 5-kinase 9, monosaccharide-sensing 2, beta-amylase, 3beta-hydroxys- teroid-dehydrogenase /decarboxylase, and disease resistance RPM1. Most of these genes were reported to be highly associated with salinity tolerance in different plant species.

Our reported salinity-associated genes can provide a more comprehensive blueprint for salinity tolerance in wheat bread. These genes can be studied using gene expression technologies to identify possible protein-protein interaction networks that control wheat response to salinity stress. In addition, the SNP marker technology has shown its utility in supplying more detailed molecular markers can be used for marker-assisted selection in local and international breeding programs.

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نوعا من الهجرة الجينية ، والتي يمكن أن تكون ناجمة عن التكيف مع الأصناف. تم الحصول على نتائج مماثلة باستخدام تحليل النشوء والتطور. كانت معظم الأنماط الجينية متجمعة تقريبا، اعتمادا على أصلها الجغرافي ، على الرغم من أن بعض الطرز الجينية المصرية كانت متجمعة مع الأنماط الجينية الإيرانية والسورية ، مما قد يشير إلى مصدرها الأصلي. يمكن أن يشير هذا التحليل إلى عدد من الطرز الجينية الأجنبية المحتملة التي يمكن تكييفها بنجاح في البيئة المصرية من خلال برامج التربية المحلية.

خلال دراستنا ، استخدمنا التنميط الجيني SNP لاكتشاف الجينات المرتبطة باستجابة القمح لإجهاد الملوحة. كشف تحليل GWAS عن 13 علامة SNP. يتم توزيع هذه العلامات عبر الكروموسومات (3 SNPs) 7B ، (1 SNP) 6A ، (2 SNPs) 5A ، (1 SNP) 2B ، (1 SNP) 2A ، (1 SNP) 5B ، (1 SNP) 3B ، و (1 SNP) 1B. يتراوح تأثير هذه العلامات على صفة STR من 0.56- (BS00076622 بادئ) إلى 0.469 (BS00064146 و BS00101408 بادئات). ترتبط سمة DF مع 4 علامات SNP (BS00024921 ، BS00083630 ، BS00078124 ، و BS00038820) ، حيث يتراوح تأثيرها من 2.544- (BS00078124) إلى 2.52 (بادئات BS00038820 و BS00024921). أظهرت بعض علامات SNP ارتباطاًا بسمات متعددة مثل بادئات BS00038820 (سمات DF و PH) و BS00107837 (سمات NS و NT) و BS00089954 (سمات NS و STR).

من خلال دراسة الجينات الموجودة بالقرب من علامات SNP المرتبطة بالسمات الزراعية للقمح تحت الملوحة ، حددنا 13 جينا مختلفا. تشتمل معظم هذه الجينات على السيتوكروم P450 709B2 و MALE DISCOV- ERER 2 و STAY-GREEN و phosphatidylinositol 4-phosphate 5-kinase 9 و 2 monosaccharide-sensing و beta-amylase ، و beta-hydroxysteroid-dehydrogenase / decarboxylase 3 ، وجين مقاومة الأمراض RPM. تم الإبلاغ عن ارتباط معظم هذه الجينات بدرجة كبيرة بتحمل الملوحة في أنواع نباتية مختلفة.

الملخص العربي

اهداف هذه الدراسة هي دراسة التركيبة الجينية ل 70من أنواع القمح المصرية والسورية والإيرانية وتحديد العوامل الوراثية التي تتحكم في استجابة إجهاد الملوحة لقمح الخبز.

بالإضافة إلى التعرف علي بعض المعلمات الجزيئية المرتبطة بتحمل ضغط الملوحة في القمح. تتكون مجموعة الأصول الوراثية للقمح التي تمت دراستها من 70 نوع تم الحصول عليها من مصر وسوريا وإيران. تم إجراء تقييم تحمل الملوحة خلال عامي 2018 و 2019 في الدفيئة بمعهد بحوث الهندسة الوراثية الزراعية (AGERI) ، معهد البحوث الزراعية (ARC) ، والحقل التجريبي بكلية علوم الزراعة البيئية ، جامعة السويس بالعريش. ، سيناء، مصر. تم اختيار هذه المجموعة من أنواع القمح من المركز الدولي للبحوث الزراعية في المناطق الجافة (إيكاردا) وبنوك الجينات في مركز البحوث الزراعية ، الجيزة ، مصر. تم إجراء تحليل ارتباط الجينوم (GWAS) وتحليل التركيبة الجينية باستخدام ستة SCoT وخمسة SSR و 93 معلم جزئي بتكنولوجيا ال SNP.

تم الكشف عن إجمالي عدد 61 حزمة PCR باستخدام بادئات SSR و SCoT ، حيث قدمت مقايسة SCoT عددا أكبر من الحزم (46) حزما مقارنة بمقايسة SSR (15 حزما). تم الحصول على أقصى عدد من الحزم من بادئ SCoT-05 (10 حزم). بالإضافة إلى ذلك ، كان العدد الأجمالي للحزم متعددة الأشكال 48 حزما ، حيث كشف كل من بادئي SCoT-10 و SCoT-01 الحد الأقصى لعدد الحزم متعددة الأشكال (8 حزما) . أظهرت البادئات SCoT-02 و SSR-01 النسبة المئوية القصوى لتعدد الأشكال (100%). من ناحية أخرى ، من بين 91 بادئة SNP المستخدمة في التنميط الجيني للقمح ، 17 لم تظهر تعدد الأشكال.

يشير تحليل التركيب باستخدام تردد الأليل للأنماط الجينية للقمح المصري والسوري والايراني إلى أن هذه الطرز الجينية تنتمي إلى 4 مجموعات جينية مختلفة. حيث ، بالنسبة للجزء الأكبر ، يتم تجميع الأنماط الجينية المصرية والسورية والإيرانية اعتمادا على بلدهم الأصلي. من ناحية أخرى ، أظهرت بعض الأنماط الجينية

جامعة عين شمس
كلية الزراعة

رسالة دكتوراة

إسم الطالب : السمان محمود محمد علي
عنوان الرسالة : إنشاء خريطة وراثية عالية الدقة لقمح الخبز من خلال المسح الجينومي المترابط
إسم الدرجة : دكتور الفلسفة في العلوم الزراعية (وراثة)

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صفحة الموافقة على الرسالة

إنشاء خريطة وراثية عالية الدقة لقمح الخبز من خلال المسح الجينومي المترابط

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إنشاء خريطة وراثية عالية الدقة
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الجينومي المترابط

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