Genetic and Genomic Architecture of Salt Tolerance in Bread Wheat

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Genetic and Genomic Architecture of Salt Tolerance in Bread Wheat

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In loving memory of my father (late)

Dedicated to

My Loving Mother

who always dreamed of and fought for my excellence in education

&

Plant Breeders and Geneticists

striving to fight the hunger

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Abstract

Soil salinization is the consequence of climate change and soil salinity significantly reduces wheat yield. Therefore, development of salt tolerant wheat is a feasible option for 1 billion hectares of salt affected land and wheat breeding for this trait could be enhanced by marker assisted selection (MAS) and identification of major genes for salt tolerance. The Axiom Wheat Breeder's Genotyping Array was used to genotype 154 F_2 wheat lines developed from parents with contrasting salt tolerance. A high-density genetic linkage map consisting of 988 single nucleotide polymorphisms (SNPs) markers was constructed and 49 quantitative trait loci (QTL) were mapped for salt tolerance related traits and mineral nutrients concentrations under salt stress. Two Na^+ exclusion (NAX) QTLs located on chromosome 2A coincided with a major reported QTL (*Nax1* or *HKT1;4*) while two major NAX QTLs mapped on 7A contributed 18.79 and 11.23 % to salt tolerance. Another 13 QTLs including major QTLs were mapped for K^+, Ca^{+2} and Mg^{+2} concentrations while 27 novel QTLs were identified for tissue Boron, Copper, Iron, Manganese Phosphorus, Sulphur and Zinc concentrations under salinity. Several of these QTLs were validated in two mapping populations.

The segregating markers were annotated/located on 1257 genes for various ion channels, transcription factors (TFs), signaling pathways, genetic and epigenetic factors, tolerance mechanisms, metabolic pathways etc. The *in-silico* transcriptomics analysis found 258 of these genes to be differentially expressed under salinity, another 74 genes were found to be vital for plants under both normal and saline conditions. Another 156 genes showed the expression only under salt stress while 54 of them had significant number of alignments with salt-expressed transcriptome. The transcriptomics analysis for 478 NAC, WRKY, MADS-box, AP2-containing, MYB and MYB-related TF families revealed that 181 TFs were differentially expressed under salinity in wheat. Taken together, the SNPs, QTLs, genes, transcripts and TFs identified in this study will be a valuable source for wheat breeding for salt tolerance.

Keywords: Bread wheat, salt tolerance, genetic markers, genetic linkage map, genetic/QTL mapping, population genetics, quantitative genetics, transcriptomics analysis, transcription factors

ÖZET

İklim değişikliğinin bir sonucu toprak tuzlanmasıdır; toprak tuzluluğu buğday verimini önemli ölçüde azaltmaktadır. Bu nedenle, tuzlanmış 1 milyar hektar arazi için tuz toleranslı buğdayın geliştirilmesi mantıklı bir hedeftir. Buğdayda, bu özelliğe sağlayan önemli genlerin belirlendiğinde Markör Destekli Seleksiyon (MAS) yöntemiyle ıslah çalışmaları etkin olabilmektedir. Aksiyom Buğday Yetiştiricisinin Genotipleme Microçipi, tuz toleransı olarak farklı iki ebeveynlerden geliştirilen 154 buğday F2 hatlarını genotiplemek için kullanılmıştır. Bulgulardan 988 tek nükleotid polimorfizmi (SNP) marköründen oluşan yüksek yoğunluklu bir genetik bağlantı haritası oluşturulmuş, tuz toleransı ile ilgili ve tuz stresi altında mineral besin konsantrasyonu etkileyen 49 kantitatif özellik mevkii (QTL) haritalanmıştır. Kromozom 2A üzerinde yer alan iki Na^+ dışlama (NAX) QTL, önceden raporlanmış önemli bir QTL'e (Nax1 veya HKT1;4) denk gelmiştir; bu arada 7A üzerinde haritalanmış iki önemli NAX QTL, %18.79 ile %11.23 oranında tuz toleransına katkıda bulunmuştur. K^+, Ca^{2+} ve Mg^{2+} konsantrasyonlarını etkileyen önemli QTL içeren 13 QTL daha haritalanmış, oysaki tuz stresi koşullarında Bor, Bakır, Demir, Mangan, Fosfor, Kükürt ve Çinko konsantrasyonlarını etkileyen 27 yeni QTL belirlenmiştir. Bu QTL'lerin birkaçı, iki haritalama popülasyonunda doğrulandı

Hatlarını ayrılan SNP markörleri, çeşitli iyon kanalları, transkripsiyon faktörleri (TF'ler), sinyal yolları, gen ve epigenetik faktörler, tolerans mekanizmaları, metabolik yollar, ve benzer fonksiyonlu 1257 gen üzerinde konumlandırılmıştır. *In silico* transkriptom analizi aracılığıyla, bu genlerin 258'inin gen ifadeleri tuzluluk altında etkilendiğini belirlenmiştir. Bunların dışında 74 genin ifade edilmesi hem tuzlu hem de normal koşullarında kritik olduğunu gösterilmiştir. Ayrıca sadece tuz stres koşullarında ifade edilmiş 156 genin 54'ü, tuzluluktan etkilenmiş transkriptom'a önemli benzerliğe sahip olmuştur. NAC, WRKY, MADS-box, AP2 içeren, MYB ve MYB'le ilişkili tanskriptom faktör (TF) aileleri üye olan 478 genin 181'in gen ifadeleri, transkriptom analizi aracılığıyla buğdayda tuz stres koşullarında etkilendiğini tespit edilmiştir. Bu çalışmada tanımlanan SNP'ler, QTL'ler, genler, transkriptler ve TF'ler, tuz toleransı için buğday yetiştiriciliğinde değerli bir kaynak olacaktır.

Table of Contents

List of Figures

List of Tables

Chapter 1: Mapping QTLs for Salt Tolerance & Mineral Concentration

1.1. Introduction

More than one billion hectares of land is affected by salinity worldwide and this is on the rise due to climate change and subsequent soil degradation and salinization [1]. Early wheat growth and development and as a result the grain yield is significantly reduced by salinity due to Na^+ influx toxicity, which severely disturbs leaf function [2]. On the other hand, 100-110% extra food production is required to feed the growing human population by 2050 [3]. Therefore, development of salt tolerant wheat is the need of the hour, which could be used for sustainable production on this large area. This could help to cope with climate change and meet the growing food demand. In comparison to drought, genetic studies for salt tolerance in wheat are limited, which hinders the development of salt tolerance wheat [4]. Similarly, development of salt tolerant cultivars is also limited by the severity and complexity of salt stress, which occurs as osmotic stress at an earlier phase followed by ionic stress [5]. The first phase of stress, i.e. osmotic stress as consequence of higher salt concentrations in vicinity of plant roots, leads to reduced water uptake, and inhibits plant growth and development [4]. In the later ionic stress phase, Na^+ influx into plant roots and shoots results in leaf chlorosis and even plant mortality because of deleterious effects on the photosynthesis process [4,5].

The use of wheat yield data from saline fields as a salt tolerance index is debatable due to variation in Na^+ soil profiles, differences in salt tolerance at different growth stages and variability in soil pH and drought occurrence [4]. However, screening of wheat for salt tolerance in hydroponics and pot cultures in controlled greenhouse conditions has shown the presence of significant genetic variation in wheat for salt tolerance [6–8], which could be used for the development of salt tolerant wheat. However, the genetic and physiological complexity of multi-faceted and multi-genic salt tolerance traits are poorly understood due to the lack of genetic studies, which has greatly limited wheat breeding for salt tolerance.

Recent advances in next-generation sequencing (NGS) and genomic knowledge have opened new horizons and opportunities for improving multi-genic complex traits such as abiotic stress tolerance including salinity and drought. The use of sequencing data for identification of molecular markers linked to economic traits in plants provides opportunity of marker assisted selection (MAS) that helps to accelerate the identification and selection of targeted genes in breeding populations in a significantly shorter time than classical breeding [9–14]. Unlike morphological markers, these DNA sequence-based markers are not affected by environmental changes, and thus act as more reliable selection index or tool for complex crop traits like biotic and abiotic stresses [12,15]. Due to limited wheat sequencing/genomic data, progress in MAS in wheat had been limited, but fast improvement in NGS technologies in the last decade and advances in genotyping methods have produced large genomic datasets. These can be utilized for designing sequence-tagged markers such as single nucleotide polymorphisms (SNPs) and simple sequence repeats (SSRs) markers [16–18]. NGS enables the identification of large number of markers, e.g. more than 68,000 SNPs associated with *Ae. tauschii* genes on chromosome 5D were mapped using sequencing data [18].

SNPs are the most widely used markers for gene mapping and germplasm characterization because they are sequence tagged, co-dominant, rapid, cost-effective and highly abundant [18], which makes them suitable for the development of multiplexed SNP microarrays like Affymetrix GeneChip [19]. These can be used for high-throughput genotyping in wheat. For example, the recently developed Axiom Wheat Breeders' Genotyping array, that contains probes for 35,143 pre-validated SNPs for all wheat chromosomes, is a cost-effective system for screening wheat mapping populations. It can simultaneously genotype 384 wheat samples, thus providing an opportunity for high-throughput genotyping in wheat [20]. This array was used for constructing a high-density (HD) linkage map which was used to map genomic regions associated with yield and drought tolerance-related traits in wheat [21].

The high-throughput genotyping data from these multiplexed SNP arrays is routinely utilized for construction of high-density linkage maps, a prerequisite for quantitative trait loci (QTL) mapping for multi-genic complex traits such as drought and salt tolerance [21–25]. Besides QTL mapping, positional cloning of genes can also be performed by using high-density linkage maps. Additionally, high-density linkage maps can also be used as comparative genomics tools to study chromosomal organization and evolution, as they are constructed from sequence-based SNPs [22]. When analyzed with morphological data, the linkage map markers help to tag the genomic regions containing QTLs for studied traits e.g. several QTLs in bread wheat were mapped for salt tolerance and related traits using linkage maps and morpho-physiological data [24,26,27]. A total of 40 QTLs for shoot Na^+ and K^+ concentrations, seedling biomass and chlorophyll content at the seedling stage were mapped in wheat under salinity; and a sodium exclusion (NAX) QTL on a Chromosome 2A marker interval (wPt-3114-wmc170) was linked to a 10% enhancement in seedling biomass. Although two of total five QTLs for NAX were co-localized with QTLs for seedling biomass, the contribution of all NAX QTLs to seedling biomass was just 18% [26]. Therefore, mapping of major and novel QTLs in more mapping populations is required, which could then be used for MAS and breeding wheat for salt tolerance.

Based on the above discussion, it is concluded that several other factors besides NAX and K^+ could be involved in conferring salt tolerance to wheat; e.g. Mg^{2+} and Ca^{2+} accumulation has also been reported to influence salt tolerance in wheat. Several Mg^{2+}, Cl^{-1} and Ca^{2+} concentrations

QTLs under salt stress were mapped on 5A and several other wheat chromosomes [28]. Apart from Ca^{2+}, K^{+}, and Mg^{2+}, the genetics of micronutrients such as Boron, Cu, Fe, Mn, S, P and Zn is not known under salt stress, and was studied only under normal irrigation and drought stress condition [25]. Therefore, we studied the genetic bases of Boron, Cu, Fe, Mn, S, P and Zn micronutrient concentrations, in addition to NAX, Mg^{2+}, K^{+} and Ca^{2+} concentrations, in root and shoot tissues under salt stress in bread wheat.

This study was aimed to: (a) construct a high-density linkage map for an F_2 population depicting phenotypic variation for salt tolerance (b) map QTLs associated with salt tolerance related traits and mineral nutrient concentrations under salt stress

1.2. Review of Literature

Salt stress occurs in two phases in plants i.e. osmotic stress in which higher salt concentration in vicinity of plant roots hinders the water uptake by plant roots leading to reduced water uptake and plant growth [4]. It is followed by more severe ionic stress phase that is caused by Na^{+} influx into plant roots and shoots. The Na toxicity results in leaf chlorosis or mortality due to deleterious effects on the photosynthesis process [4,5]. Therefore, less sodium uptake or sodium exclusion is one of the main salt tolerance mechanism in wheat. For examples, salinity caused 82, 51 and 33% reduction in wheat grain yield, dry shoot weight and germination vigor. Unlike shoot K^{+}/Na^{+} ratio and shoot Na^{+}, the water loss from wheat root and shoot was negatively correlated with shoot K^{+}. The sodium exclusion i.e. low Na^{+} accumulation, high shoot K^{+} accumulation, higher photochemical efficiency and PSII activity; and reduced non-photochemical quenching (NPQ) in tolerant genotypes maintained stable osmotic potential at germination, seedling and adult plant growth stage. The genotypes exhibiting these traits produced significantly higher dry biomass under salt stress [8]. A novel QTL for sodium exclusion (*Nax1*) was mapped on chromosome 2AL in durum wheat which accounted for 38% of phenotypic variation for the trait [29]. The QTL mapping in wheat for salt tolerance has largely been focused on studying QTLs for Na exclusion and K^{+} concentration (1.2.3).

1.2.1. Conventional Vs High-throughput Genotyping

Before the advent of high-throughput NGS technologies, the genotyping was performed by limited number of molecular markers through polymerase chain reaction (PCR). For example, two AFLP markers in 144 combinations and 103 SSR markers were used to genotype the mapping population using PCR in durum wheat [29]. Similarly, 263 SSR markers were used for genotyping in an F_7 recombinant inbred line (RIL) population of bread wheat and 100 was them were found polymorphic [24]. In another study, 152 doubled haploid (DH) lines were genotyped using 1,150 SSR markers, and 233 of them were polymorphic [26]. However, NGS technology has helped to identify large number of genetic variations or molecular markers in plats e.g. 6,948

ISBP markers and 362 SSRs were located on wheat chromosome 1AL arm alone [30], and 68,500 SNPs linked to genes of on 5D chromosome in *Ae. tauschii* were identified and compared with 5D chromosome of bread wheat [18]. Such deep coverage of genome allows high-throughput genotyping which could be used for more precise QTLs mapping in plants.

Using the NGS sequencing, several genotyping arrays have been developed, which can genotype thousands to hundreds of thousand markers simultaneously as compared to few hundred markers in repeated PCRs genotyping. Thus, they provide high-throughput genotyping platforms to genotype large number of markers simultaneously. These high-density genotyping arrays are a powerful tool for characterizing genomic diversity and marker–trait associations in mapping populations. They also help in studying ancestral relationships among the parents and individuals in mapping populations [31]. For example, wheat 90K SNP iSelect array [31] which has probes for 90,000 gene-associated SNPs, and was used to characterize the genetic diversity in allotetraploid and allohexaploid wheat. The array includes the SNPs distributed across the whole wheat genome in mapping populations belonging to diverse geographical location/origin [31]. Another such high-density genotyping array has 660K gene-associated SNPs and has been utilized for genotyping the wheat mapping populations [32].

Another such high-density genotyping array is the Axiom Wheat Breeders' Genotyping Array contains probes for 35,143 gene-associated SNPs distributed on all wheat chromosomes; and has ability to genotype 384 samples simultaneously using the 384-microplate configuration. Thus, it provides fast, inexpensive and high-throughput genotyping in wheat. Following the genotyping, density-based spatial clustering algorithms are used for precise ad accurate SNP calling [20]. The 35 K array was used for genotyping in 100 durum lines and 9,113 of 3,5143 SNPs were found to be polymorphic. Some of these markers were discarded on the basis having minor allele frequency, and 9,484 polymorphic SNPs after inclusion of high variants or OTV SNPs were used for downstream analysis [21]. The appropriate array can be selected according to budget, desired coverage and needs of experiment.

1.2.2. High-density Linkage Maps

The PCR based genotyping is hectic, time consuming and expensive as it involves several individual PCRs for individual markers. Therefore, the genetic linkage map constructed from this kind of genotyping data were low-density i.e. higher distance between mapped markers [24–26]. However, the genotyping data from high-density arrays can be used for construction of high-density linkage maps which provide the opportunity to map more accurate QTLs. For example, using the genotyping data from wheat 90K array, 46,977 SNPs were mapped on whole wheat genome for eight DH populations [31]. Similarly, a genome-wide high-density linkage map consisting of around 47,000 SNPs including 8067 SNPs from wheat 90 K array and 38,894 SNPs from wheat 660 K array was constructed in wheat. This map was 4121 cM long and included 20,012, 22,142 SNPs and 4807 SNPs mapped on A, B and D genome, respectively [32]. In another study, Axiom Wheat HD genotyping array having 819,571 SNPs was used to construct high-density whole-genome genetic linkage maps in Savannah × Rialto, Avalon ×Cadenza, and Synthetic × Opata mapping populations that consisted of 16 039, 18 942 and 31 808 SNPs, respectively. Additionally, these three maps were used to construct a consensus linkage map consisting of 56,505 SNPs [19].

The Wheat Breeders' Genotyping Array or 35 K array-based SNP calling data was used to map 6303, 7328, 8820, 2997, and 9434 polymorphic SNPs in Savannah × Rialto, Avalon ×Cadenza, Synthetic × Opata, Apogee × Paragon, and Chinese Spring × Paragon DH and RIL mapping populations [20]. Wheat 35 K array was used for genotyping in durum wheat and from 9,484 polymorphic SNPs, 1345 were mapped to the genetic linkage map. The assignment of lower number of markers to linkage map is due to absence of D sub-genome in durum wheat and low segregation under drought. The SNPs mapped on the chromosomes showed co-linearity with previously mapped wheat maps. The genetic linkage map was used to map QTLs for coleoptile length, plant height, root osmotic stress ratio, lodging, root volume stress ratio and days to heading [21].

1.2.3. QTL mapping for salt tolerance; and mineral nutrients under normal, drought and saline conditions

The F2, RILs, DHs, and near isogenic lines (NILs) are suitable mapping populations for QTL mapping in wheat [15,21,24,28,33,34] and QTL mapping for various traits under salt stress is discussed here. Although millions of hectares worldwide are salt-affected [35], but QTL mapping and other genetic studies are limited for the topic due to complexity of salt tolerance and interaction of salinity with other stresses. The QTL mapping for salt tolerance is mostly focused on sodium exclusion (NAX), K^+ accumulation and grain yield under salt stress. For example, in a RIL population, total 98 QTLs including 24 grain yield QTLs with less than 10 % contribution to phenotypic variation were mapped. The loci on chromosome 1A, 2B, 3B, 6B, 1D, and 2D enhanced the yield in 10–12 ds m^{-1} salinity. Two QTL clusters on Chromosome 3B contained 27 QTLs, and *gmw33*, *gwm247*, *gwm282*, *gwm566* markers associated with yield QTLs contributed 20%, 43%, 17% and 43% to the trait phenotypes, respectively [36].

In another study, several minor QTLs for leaf NAX, K^+ concentration, plant height, thousand kernel weight, grain yield, days to maturity and kernels/m2 were mapped in wheat. The minor QTLs showed the complexity of salt tolerance, and previously mapped QTLs for NAX and seedling biomass hydroponics condition were also found in field condition; but these QTLs had very little contribution to grain yield. However, a stable QTLs with was were co-located with plant height and days to maturity genes was mapped [37] implying the importance mapping QTLs for agronomic traits to find stable QTLs for salt tolerance. Similarly, a genetic linkage map was constructed using the DArTs and SSR markers for a RIL population consisting of 319 individuals. Total 65 QTLs were mapped for 13 physiological and yield related traits. Among these QTLs, four additive and seven epistatic QTLs were involved in salt tolerance. The physiological traits showed greater effects on salt tolerance response as compared to the morphological traits. Two additive QTLs for leaf NAX were co-located with QTLs for shoot fresh and dry weight on chromosome 1B and 3B [24].

For shoot NAX and K^+ concentrations, seedling biomass and chlorophyll content, total 40 QTLs were mapped in wheat under salinity at the seedling stage. Among the QTLs, and leaf NAX QTL on a Chromosome 2A marker interval (wPt-3114-wmc170) was linked to 10% ıncrease in seedling biomass. Although two of total five QTLs for NAX were co-localized with QTLs for seedling biomass, the contribution of all NAX QTLs to seedling biomass was just 18% [26]. Therefore, mapping of major and novel QTLs in more mapping populations is the need of hour. In a wheat RIL population of 131 plants, total 34 QTL were mapped for dry weight and Na^+ in saline conditions. Among the 18 additive and 16 epistatic QTLs, five and 11 QTLs had significant QTL into treatment effects. Among them, leaf NAX and K^+/Na^+ QTL on chromosome 5A coincided with *Nax2,* and a previously reported *Xgwm6* marker on chromosome 4B was associated with dry weight under salinity [27] which could be useful for MAS. In another study, 150 wheat accessions were genotyped with wheat 90 K SNP array GWAS was performed using phenotypic data for NAX and K^+. The GWAS found 37 QTLs and 187 SNPs for leaf NAX and K^+ under saline conditions that included four QTLs on chromosome 2AL, 3AL, 1BS and novel QTLs were identified on chromosome 1BS and 1DL. The *AtABC8*, *ZIP7*, *6-SFT* and *KeFC* were found be the candidate associated with QTL-linked SNPs. The transcriptomics and qPCR analysis for these candidate genes fund missense mutations that were responsible for salt tolerance variations [38], which can be used for breeding of salt tolerant wheat.

The studies for QTL mapping for ions other than Na^+ and K^+ under salt stress are rare and only one study reported QTLs Cl^-, Mg^{2+} and Ca^{2+} under salt stress. For example, the QTLs for Cl^- in wheat differed under field and hydroponics conditions and a major QTL for Cl^- was mapped on chromosome 5A at *barc56-gwm186* marker interval. This QTL contributed 27–32% of Cl^- phenotypic variation in field condition. Additionally, six and 13 QTLs for Mg^{2+} and Ca^{2+} were also mapped on chromosome 2A, 3A, 4A, 2B, 3B, 4B, 5B, 6B, 1D, 4D and 7D. The most important Mg^{2+} and Ca^{2+} QTLs contributed 15 and 13% to phenotypic variation and were mapped on chromosome 3A and 1D, respectively. These QTLs were co-localized with QTLs for Cl^- [28]. In another study, four QTLs for grain Fe concentrations were mapped on chromosome 2A, 5A, 7A and 7B while five QTLs for grain Zn concentrations were mapped on chromosome 2A, 4A, 5A, 7A and 7B under normal or non-saline growth conditions [39]. However, QTLs for most of micro and macronutrients were mapped under drought condition and were mapped in clusters on chromosome 2A, 5A, 6B and 7A that were co-located with genes for grain protein content [25].

1.3. Materials and Methods

1.3.1. Plant material

Two contrasting wheat accessions (WTSD91 and WN-64) for salt tolerance were selected from a greenhouse hydroponics screening from a pool of 150 genotypes at Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan. WN-64 and WTSD91 were found to be highly susceptible and moderately salt tolerant respectively, under 300 mM NaCl salinity in hydroponic culture in 2011-12 [7]. Both the parents were crossed in the field during the same growing season; crossed seeds were harvested and grown during 2012-13 to raise F_1 hybrids. The wheat spikes were covered with butter paper bags at anthesis stage to ensure purity and F_2 seeds were obtained.

1.3.2. Growth conditions

The experiment was conducted at 40° 53′ 25″ N, 29° 22′ 47″ E in Sabanci University, Istanbul in a Venlo-type greenhouse capable of computerized control for evaporative cooling, supplemental lighting and heating. The temperatures were regulated to be 25 ± 4 °C and 20 ± 4 °C during day and night throughout the experimental period. A total of 250 F_2 lines (WTSD91 × WN64) were grown in inert perlite and 5 days after germination, 200 healthy and uniformly growing seedlings were transplanted to 2.7-L hydroponic pots containing aerated nutrient solution as explained in previous studies [40], after removal of residual endosperm from the seedling roots. NaCl amounting to 75 mM salinity was added to hydroponic pots on the following day. The nutrient solution was changed every four days and the salinity level was increased by 75 mM NaCl successively at every solution change until it reached 300 mM NaCl level on the 12th day after transplantation. Plants were kept under salt stress for 32 days, which included 20 days at 300 mM salinity.

1.3.3. Phenotyping

Based on phenotypic variation, plants were categorized into four groups: (i) tolerant (T) plants with 5 fully expanded healthy green leaves having no signs of salt injury; (ii) moderately tolerant (MT) plants having 4-5 fully expanded green leaves with minor salt injury signs on the leaf tips; (iii) susceptible (S) plants having reduced growth, i.e. 2-3 leaves with severe signs of salt injury signs and/or 1-2 dead leaves; and (iv) highly susceptible (HS) plants having 2-3 leaves showing severe injury and 60-100% leaf mortality. Mineral analysis was performed by using four pools from each group of plants. Wheat roots and shoots were washed thrice in dH_2O and were oven dried at 65 °C for 72 hours. Subsequently, dry root and shoot weights (DRW and DSW) were recorded and root and shoot tissues were analyzed to measure the mineral concentrations following the previously reported method [40].

For this purpose, dried root and shoot tissues were ground to fine powder in an agate vibrating cup mill (Fritsch GmbH; Germany). For each sample, between ~0.15-0.2 g tissue powder was added with 2 ml of 30% H_2O_2 and 5 ml of 65% HNO_3, and tissues were digested in a closed-vessel microwave system (Mars Express; CEM Corp; NC, USA). Milli-Q water was added to digested solutions to make a final volume of 20 ml and Zn, S, P, Mn, Mg, K, Fe, Cu, Ca and Boron concentrations in both root and shoot tissues were measured by inductively coupled plasma optical emission spectrometry (ICP-OES; Vista-Pro Axial; Varian Pty Ltd; Mulgrave, Australia). Further information about the working principle and measurement of mineral by ICP-OES can be found here [41]. The 20 ml diluted digested solution was further diluted 50 times (1:50) to measure Na^+ concentration.

To exclude any unexpected variation, ICP-OES data for minerals was also measured for standard values using standard durum wheat flour (SRM 8436, NIST, Gaithersburg, MD). The concentration values for all 24 traits (Table 1.1) was obtained by multiplication of ICP-OES values by the dilution factor and dividing the result by the dry weight of tissue used for digestion. As sodium exclusion means less uptake of Na^+ by root and shoot, Na^+ concentration values were multiplied by -1 to obtain values for shoot Na exclusion (SNAX) and root Na exclusion (RNAX). Finally, calculation of linear correlation coefficients between different traits was performed by Statistix 8.1 software.

1.3.4. DNA extraction and genotyping

The youngest plant leaf/leaves were used for DNA extraction from parents and 164 F_2 lines by using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). DNA extraction from plants with complete leaf mortality was performed using root tissue. The DNA concentrations for all samples were quantified with the Quant-iT PicoGreen dsDNA Assay Kit (ThermoFisher Scientific, Waltham, MA, USA), and a total of 1.5 µg of gDNA for each line and parent was dissolved in 10 mM Tris-HCl pH 8.0 to make a final volume of 30 µl for genotyping. The Axiom Wheat Breeder's Genotyping Array (Affymetrix, Santa Clara, CA, USA) or “wheat 35K array” was used for genotyping of each sample for 35,143 SNPs. Genotyping was carried out using the Affymetrix GeneTitan MT system at Bristol Genomics Facility (Bristol University, UK) as per Affymetrix procedure (Axiom 2.0 Assay Manual).

1.3.5. Analysis of genotyping data

Axiom Analysis Suite 1.1.0.616 program was used for SNP calling, which uses cluster separation, deviation from expected cluster positions and call rate to classify the SNPs into six different categories [19]. For this purpose, the Axiom Best Practices Genotyping Workflow was utilized with default DQC cut-off = 0.82, QC call rate cut-off = 92% and wheat SNP call rate cut-off = 97% for classifying SNPs. However, 10 F_2 lines failed to pass the DQC and QC cut-offs, so the downstream analysis was performed by using the SNP call codes of 154 F_2 lines.

1.3.6. Genetic linkage map construction

The call codes for segregating or "poly high resolution" markers were extracted by using Axiom Analysis Suite for genetic linkage map construction. However, a sequential Bonferroni correction based chi-square test [42] was applied to remove markers showing significant segregation distortion ($P < 0.05$). The SNP linkage map construction was done through MapDisto 2.0 b93 [23] by grouping the markers with logarithm of the odds ratio (LOD) score= 6, recombination fraction= 0.3 and Kosambi mapping. The linkage groups were ordered by using the Seriation algorithm and were assigned to chromosomes by comparison of shared markers with a published consensus wheat linkage map [19]. The comparison indicated the division of chromosomes into multiple linkage groups, which were combined and re-ordered in MapDisto. To improve the marker order and for producing shorter individual chromosome maps, rippling of marker order with window size= 5 and checking for inversions was also done.

1.3.7. QTL mapping

Single salinity treatment phenotypic data was utilized for mapping additive QTLs for all traits, by the composite interval mapping (CIM) method. For this purpose, LOD threshold= 2.5 and walking speed= 1-cM was used in the QTL IciMapping V4.1.0 program [43]. The graphical drawing of the mapped QTLs and linkage maps was done using MapChart 2.30 program [44]. The individual QTL contribution to phenotypic variation of salt tolerance and mineral concentrations was quantified following the method defined by Zhang and colleagues [45]. The dry root and shoot weights are thought to be reliable and direct measurements of salt tolerance [15,26]. Therefore, for calculating the individual QTL contributions to salt tolerance, data for DRW and DSW was used.

1.4. Results

1.4.1. Phenotypic Variation in mapping population

Significant phenotypic variation in terms of salt injury was detected in the F_2 population and 33, 31, 49 and 51 plants were found to be T, MT, S and HS respectively. Similarly, phenotypic variation in macro/micronutrient levels was detected across population groups defined for their salt tolerance level. The root Fe conc. (RFeC), root Mg conc. (RMgC), root P conc. (RPC), shoot Ca conc. (SCalC), shoot Cu conc. (SCuC) and shoot Fe conc. (SFeC) were higher in highly susceptible plants as compared to tolerant plants. Meanwhile root K conc. (RKC), root Mn conc. (RMnC), root Zn conc. (RZnC), shoot K conc. (SKC) and shoot Zn conc. (SZnC) were higher in tolerant plants compared to the highly susceptible ones, hinting that retention of these nutrients could be involved in conferring salt tolerance. Similarly, RNAX, SNAX, DRW and DSW were largely reduced in HS plants compared to T plants; thus, better performance for these traits is also vital for salt tolerance (**Table 1.1**). The correlation coefficient between concentration values for root and shoot for some nutrients were found to significantly higher e.g. NAX in both tissue types correlated strongly with increased K and Ca concentration in shoots (**Table 1.2**).

Table 1.1. Phenotypic variation for salt tolerance and nutrient concentrations traits across different salt tolerance groups in wheat F_2 population

Plant Traits (mg/g)	Tolerant	Moderately Tolerant	Susceptible	Highly Susceptible
RBC	11.11	12.95	11.39	10.92
RCalC (ppm)	1454.72	1628.32	1597.81	1566.14
RCuC	14.01	13.97	14.24	13.04
RFeC (ppm)	2213.19	2413.70	2507.53	2671.15
RKC	2.12	1.79	1.31	1.13
RMgC	0.06	0.07	0.07	0.12
RMnC	39.47	33.26	31.01	31.58
RNAX (%)	-3.01	-4.45	-5.24	-5.93
RPC	0.43	0.49	0.47	0.52
RSC	0.21	0.24	0.23	0.23
RZnC	56.08	53.33	51.54	50.53
SBC	6.72	17.67	11.63	14.54
SCalC (ppm)	2240.04	2580.24	2636.08	2748.05
SCuC	7.22	7.90	8.19	8.74
SFeC	181.20	173.00	208.95	266.08
SKC	3.88	3.04	2.49	2.11
SMgC	0.11	0.12	0.13	0.12
SMnC	63.85	64.79	67.71	63.25
SNAX (%)	-2.85	-3.89	-6.03	-7.73
SPC	0.53	0.54	0.56	0.50
SSC	0.29	0.30	0.33	0.31
SZnC	62.95	58.37	60.13	55.36
DSW (g)	0.28	0.19	0.10	0.06
DRW (g)	0.15	0.12	0.07	0.03

Table 1.2. Correlation coefficients for salt tolerance and nutrient concentrations traits measured in wheat F$_2$ population

	BR	BS	CaR	CaS	CuR	FeR	KR	KS	MgR	MgS	MnR	MnS	NaExR	NaExS	PR
BS	0.70														
CaR	0.55	0.78													
CaS	-0.07	0.57	0.75												
CuR	0.40	-0.27	0.09	-0.48											
FeR	-0.30	0.42	0.55	0.97	-0.64										
KR	0.87	0.81	0.38	-0.01	-0.06	-0.16									
KS	0.26	-0.39	-0.63	-0.98	0.49	-0.98	0.22								
MgR	-0.50	0.25	0.09	0.70	-0.94	0.85	-0.14	-0.74							
MgS	0.03	0.31	0.82	0.80	0.13	0.68	-0.23	-0.80	0.19						
MnR	-0.02	-0.50	-0.85	-0.94	0.16	-0.85	0.09	0.93	-0.45	-0.95					
MnS	0.17	-0.14	0.46	0.10	0.80	-0.06	-0.32	-0.12	-0.55	0.68	-0.42				
NaExR	-0.25	0.42	0.63	0.98	0.51	0.97	0.19	0.98	0.75	0.79	0.92	-0.10			
NaExS	-0.51	0.21	0.40	0.90	0.62	0.97	0.37	0.96	0.85	0.66	-0.80	-0.02	0.96		
PR	-0.08	0.65	0.51	0.88	-0.80	0.91	0.19	-0.84	0.89	0.42	-0.68	0.37	0.86	0.82	
PS	0.30	-0.23	0.28	-0.21	0.95	-0.38	-0.21	0.20	-0.79	0.41	-0.12	0.95	0.23	0.35	-0.63
SR	0.49	0.89	0.96	0.84	-0.19	0.68	0.47	-0.71	0.33	0.71	-0.84	0.19	0.72	0.51	0.72
SS	-0.21	-0.16	0.49	0.46	0.40	0.38	-0.58	-0.54	-0.07	0.87	-0.70	0.86	0.52	0.46	0.01
ZnR	0.24	-0.39	-0.65	-0.98	0.46	0.97	0.22	0.98	-0.71	-0.82	0.94	-0.16	0.98	0.96	-0.82
ZnS	0.09	-0.64	-0.47	-0.85	0.84	0.89	-0.22	0.81	-0.90	-0.36	0.63	0.43	0.83	0.80	-1.00

1.4.2. SNPs calling categories

Genotyping data from the wheat 35K array for 154 lines was used for SNP calling and clustering. On the basis of the Axiom Best Practices Genotyping Workflow and default thresh-holds, the SNPs were grouped into into six categories: (a) Poly high resolution (PHR) were co-dominant polymorphic SNPs having a minor allele for at least two samples for each SNP; (b) Monomorphic or mono high resolution (MHR) SNPs had only a single allele or allele cluster; (c) No minor homozygote (NMH) were dominant polymorphic SNPs having two allelic clusters including one heterozygote; (d) Off-Target Variants (OTV) SNPs had four allelic clusters i.e. dominant, heterozygous, recessive and null alleles; (e) Call Rate Below Threshold (CRBT) SNPs passed all threshold cluster properties except the call rate cut-off i.e. more than 3% plants did not give signals for these SNPS, thus being not suitable for further analysis; and finally (f) Other type SNPs which failed one or multiple quality thresholds for cluster properties (**Figure 1.1**). Among all 35,143 SNPs of wheat 35K array, the largest group of 16,210 (46.1%) were MHR, followed by 8,141 (23.2%) 'other' SNPs, while only 51 (0.15%) SNPs were found to be OTVs (**Table 1.3**). For genetic linkage map construction, call codes for polymorphic or PHR SNPs, which accounted for 3,381 or 9.6% of all 35,143 SNPs, and OTVs were utilized.

Table 1.3: SNP calling distribution for 154 bread wheat F_2 lines identified using the wheat 35K Array

SNPs calling categories	No. of Markers	Percent SNPs calling (%)
Mono High Resolution	16210	46.1
Poly High Resolution	3381	9.6
Other	8141	23.2
No Minor Homozygote	3017	8.6
Call Rate Below Threshold	4343	12.35
OTV	51	0.15
Total	**35143**	**100**

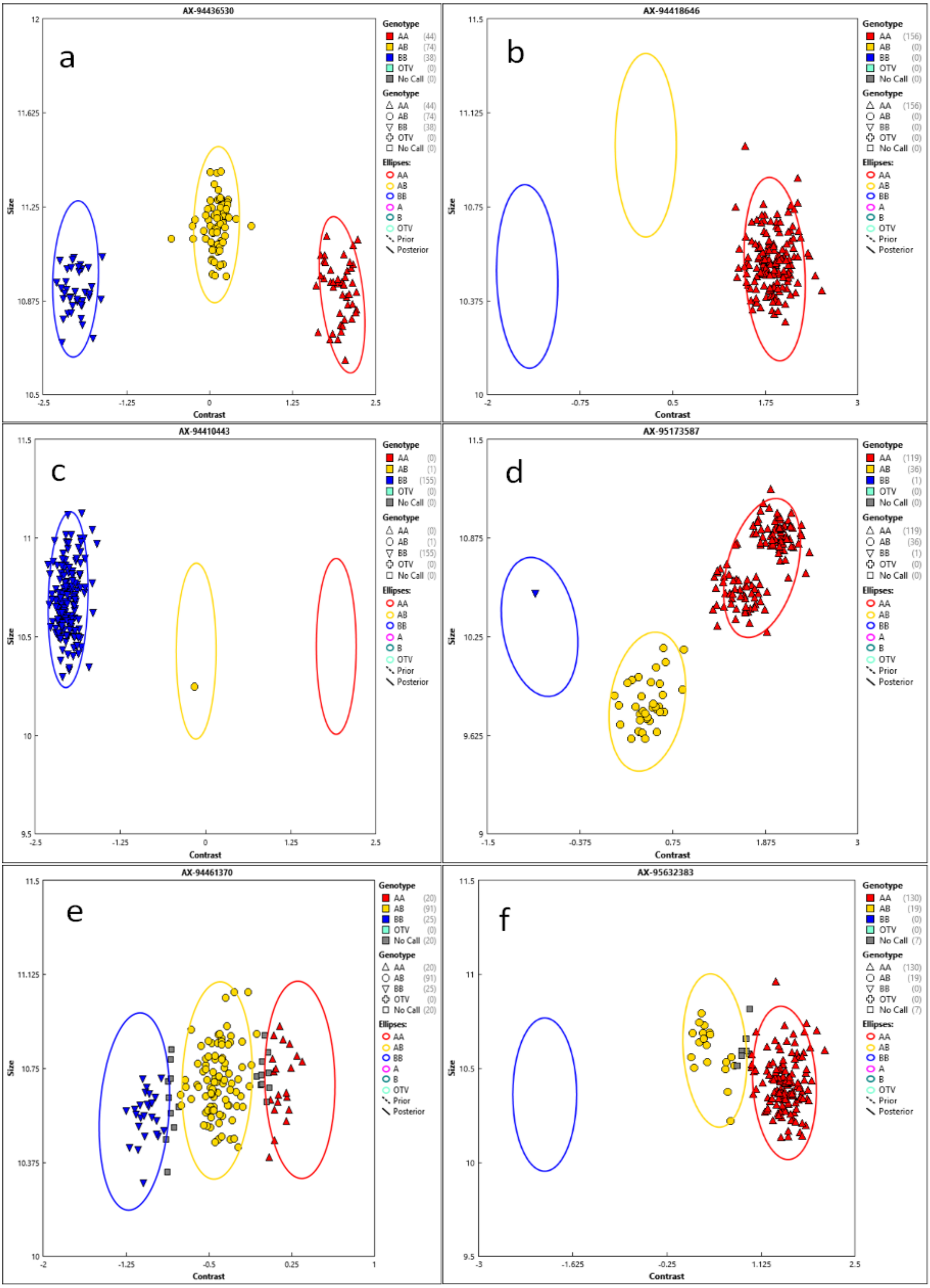

Figure 1.1: Representative allelic clusters for SNPs categories: (a) PHR; (b) MHR; (c) NMH; (d) OTVs (e) CRBT and (f) Other SNPs

1.4.3. Whole genome wheat genetic linkage map

A total of 1,032 PHR or polymorphic markers passed the sequential Bonferroni correction-based chi-square test for segregation distortion and were used for construction of a high-density genetic linkage map. Among them, 988 SNP markers were assigned across all the 21 wheat chromosomes; the remaining SNPs were not linked. The highest number of SNP markers (562) were mapped on wheat B genome chromosomes, while the lowest number of SNPs (84) were assigned to the D genome. The number of SNPs mapped on the A genome stood at 342. Among the B sub-genome chromosomes, the highest (183) and lowest (31) number of SNP markers were assigned to chromosomes 1B and 4B, respectively. The lowest (6) and highest (100) number of SNPs were mapped on chromosomes 6A and 3A, respectively for the A sub-genome. For the D sub-genome, chromosome 1D and 4D harbored 51 and 2 SNP markers, respectively. The whole genome linkage map had total length of 2317.88 cM while A, B and D sub-genomes had length of 975.56, 1133.16 and 209.16 cM, respectively. Average chromosomal length per marker was 3.71 cM for whole genome while for A, B and D sub-genomes, it was recorded to be 4.43, 2.68 and 4.03 cM, respectively. The maximum and minimum chromosomal map lengths were recorded for 2A (201.34 cM) and 6A (60.57 cM) for the A genome, and for 2B (221.26 cM) and 4B (100.04 cM) in the B genome, respectively. For the D genome, the maximum and minimum chromosomal lengths were recorded for chromosomes 1D (83.93 cM) and 4D (0.03 cM), respectively (**Table 1.4; Figure 1.2, 1.3, 1.4**).

1.4.4. Comparison of linkage and consensus maps

We also compared our high-density linkage map with a published consensus linkage map [19]. Most of the mapped SNPs, i.e. 511 (51.7%) of 988 SNPs were found to be mapped on the same chromosome as in the published consensus map. Additionally, 40.28% or 398 SNPs were mapped in wheat for the first time. The highest number of the newly assigned SNPs (247) was mapped on the B genome followed by A (132 markers) and D (19 markers) genomes. Among the chromosomes, the highest number of these SNPs were mapped on chromosomes 1B and 2A, which harbored 133 and 45 SNPs, respectively. In the D sub-genome, a maximum of 11 of these novel SNPs were mapped on chromosome 1D while 3, 1, 1, 2, and 1 of these SNPs were assigned to chromosome 2D, 4D, 5D, 6D and 7D (**Table 1.5**). Complete information about the novel SNPs is available in our paper [46]. The rest of the mapped markers, i.e. 79 SNPs, were mapped to different chromosomes compared to the published consensus linkage map. The largest number of such SNPs in our linkage map, i.e. 32 markers, were located on chromosome 2B followed by 17 SNPs being mapped to chromosome 2A. Interestingly, over one third i.e. 29 of these SNPs were mapped to respective homoeologous chromosomes (**Table 1.6**).

Table 1.4: Distribution of mapped PHR SNPs and comparison of chromosomal lengths in high-density genetic linkage map for an F_2 wheat lines

Chromosome	No. of Markers	Chromosome length (cM)	Length/ marker (cM)	Consensus map lengths (cM)[19]
1A	32	166.58	5.21	182.07
2A	88	201.34	2.29	203.99
3A	100	175.24	1.75	136.11
4A	66	110.58	1.48	75.68
5A	19	85.66	4.51	221.39
6A	6	60.57	10.1	189.4
7A	31	175.59	5.66	231.64
A Genome	**342**	**975.56**	**4.43**	**1240.28**
1B	183	173.30	0.95	182.35
2B	151	221.26	1.47	216.96
3B	59	187.64	3.18	234.56
4B	31	100.04	3.23	76.67
5B	66	201.77	3.06	208.75
6B	33	101.58	3.08	165.99
7B	39	147.57	3.78	279.28
B Genome	**562**	**1133.16**	**2.68**	**1364.56**
1D	51	83.93	1.65	151.29
2D	5	40.78	8.16	177.47
3D	14	15.01	1.07	234.87
4D	2	0.03	0.014	162.07
5D	4	53.28	13.32	167.57
6D	4	2.61	0.65	167.78
7D	4	13.52	3.38	73.34
D Genome	**84**	**209.16**	**4.03**	**1134.39**
Total	**988**	**2317.88**	**3.71**	**3739.23**

Table 1.5: Distribution of SNPs mapped for the first time in high-density linkage map

Chromosome	Number of Mapped SNPs	Chromosome	Number of Mapped SNPs
1A	13	4B	12
2A	45	5B	25
3A	36	6B	9
4A	22	7B	9
5A	7	1D	11
6A	1	2D	3
7A	8	4D	1
1B	133	5D	1
2B	47	6D	2
3B	12	7D	1

Table 1.6. SNPs mapped on different chromosomes in current and consensus linkage map

Marker	Chr	cM	C Map*	Marker	Chr	cM	C map*
AX-94577588	2A	38.86	2B	AX-94464444	2B	208.9	2A
AX-94761767	2A	50.83	4A	AX-94522700	2B	211.56	2A
AX-94503294	2A	56.43	4A	AX-94550066	2B	211.89	2A
AX-95186881	2A	123.35	6B	AX-94933710	2B	211.89	2A
AX-94842940	2A	174.96	1D	AX-94772515	2B	211.89	2A
AX-94730299	3A	115.32	3D	AX-94781925	2B	212.23	2A
AX-95174829	4A	4.54	2A	AX-95201020	2B	212.89	2A
AX-94522762	4A	50.48	5A	AX-94501432	2B	216.58	2A
AX-94625273	4A	52.12	5A	AX-94485356	2B	218.26	2A
AX-94858312	4A	52.45	5A	AX-94955614	2B	219.57	2A
AX-94425631	4A	53.11	5A	AX-94487841	2B	219.9	2A
AX-94424373	4A	53.77	5A	AX-94651736	2B	220.23	2A
AX-94779282	4A	74.48	2B	AX-94467784	5B	83.94	7B
AX-95235132	4A	81.22	2B	AX-95180386	5B	151.16	2B
AX-94865451	4A	84.23	2B	AX-94691166	5B	191.81	2B
AX-94781123	4A	91.56	7A	AX-94833876	6B	2.41	2A
AX-94787647	1B	44.45	1D	AX-94755547	6B	10.33	2B
AX-94935020	1B	49.85	1A	AX-94844172	6B	22.51	2A
AX-94432182	1B	54.84	1A	AX-94391725	6B	22.84	2A
AX-94629244	1B	77.55	1A	AX-94668676	6B	51.44	2A
AX-95087336	2B	0	6B	AX-94883829	6B	54.07	2B
AX-94461046	2B	0.66	6B	AX-94425612	6B	54.4	2B
AX-94725996	2B	4.99	6B	AX-95109622	6B	58.07	2B
AX-94789435	2B	7.95	6B	AX-95147766	7B	71.13	4A
AX-94416076	2B	11.62	6B	AX-95074259	7B	103.31	2B
AX-94783697	2B	16.97	5B	AX-94735540	7B	109.58	2B
AX-94463530	2B	85.36	2A	AX-94962080	7B	114.43	2B
AX-95071189	2B	106.01	5A	AX-94921162	7B	120.06	2B
AX-94795824	2B	166.91	4A	AX-94664270	7B	133.33	2B
AX-95009583	2B	167.24	4A	AX-94538131	7B	144.46	5A
AX-94426619	2B	171	6B	AX-94660701	7B	147.57	2A
AX-94505646	2B	174.83	6B	AX-94970894	1D	10.29	1B
AX-94435221	2B	177.42	6B	AX-95253982	1D	10.61	1A
AX-94592204	2B	181.17	6B	AX-94426211	1D	29.56	5B
AX-94489861	2B	186.58	6B	AX-94962653	1D	65.76	1B
AX-94562544	2B	196.33	6B	AX-94530345	1D	72.79	1B
AX-95019187	2B	204.3	2A	AX-94490405	3D	14.68	3A
AX-94570263	2B	204.96	2A	AX-94840398	5D	53.28	6A
AX-94689332	2B	206.28	2A	AX-95094605	6D	2.61	6A
AX-94449738	2B	206.93	2A	*Published map			

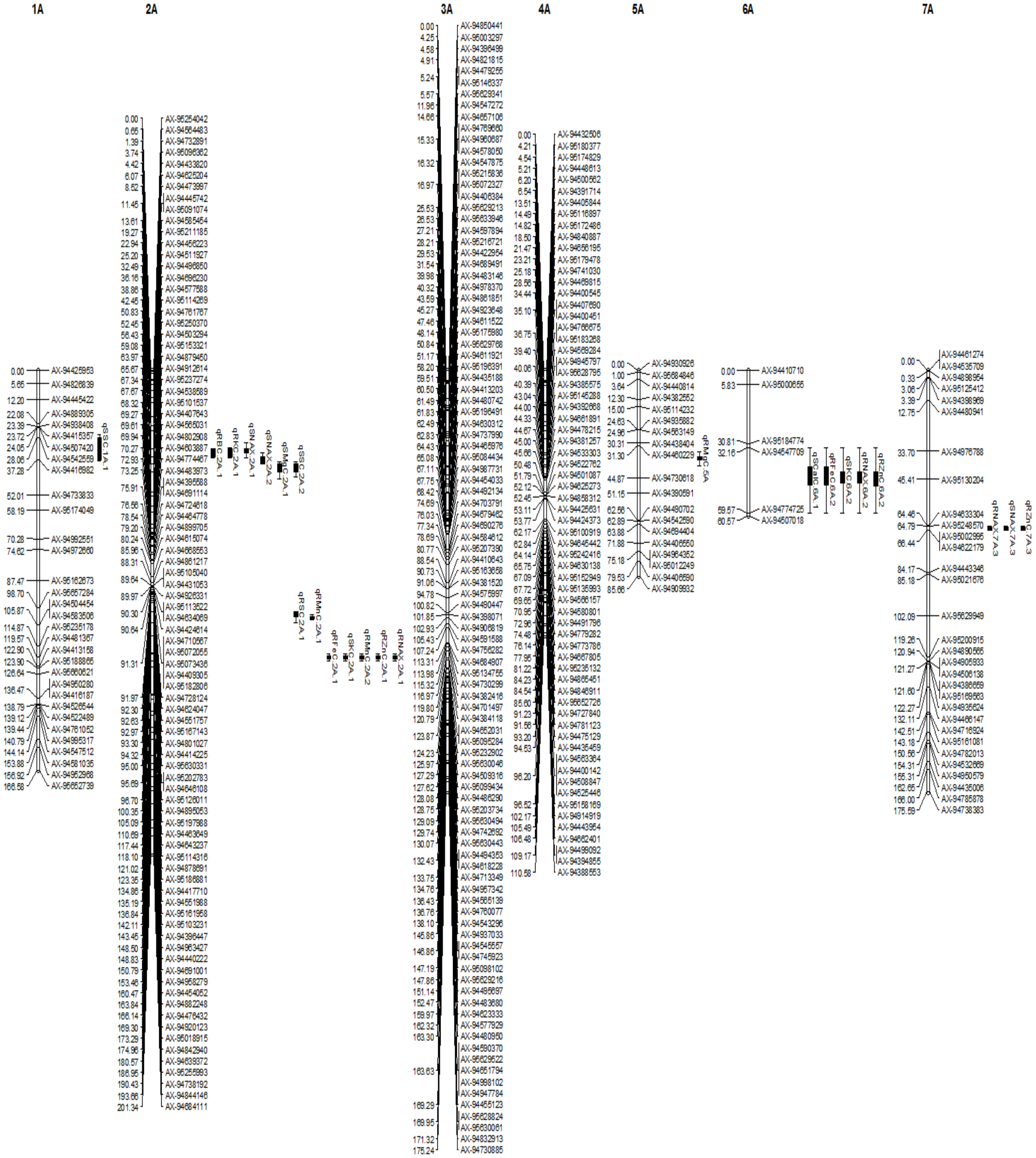

Figure 1.2: Genetic linkage map and additive QTLs located on A sub-genome of bread wheat for salt tolerance and nutrient concentrations under salt stress

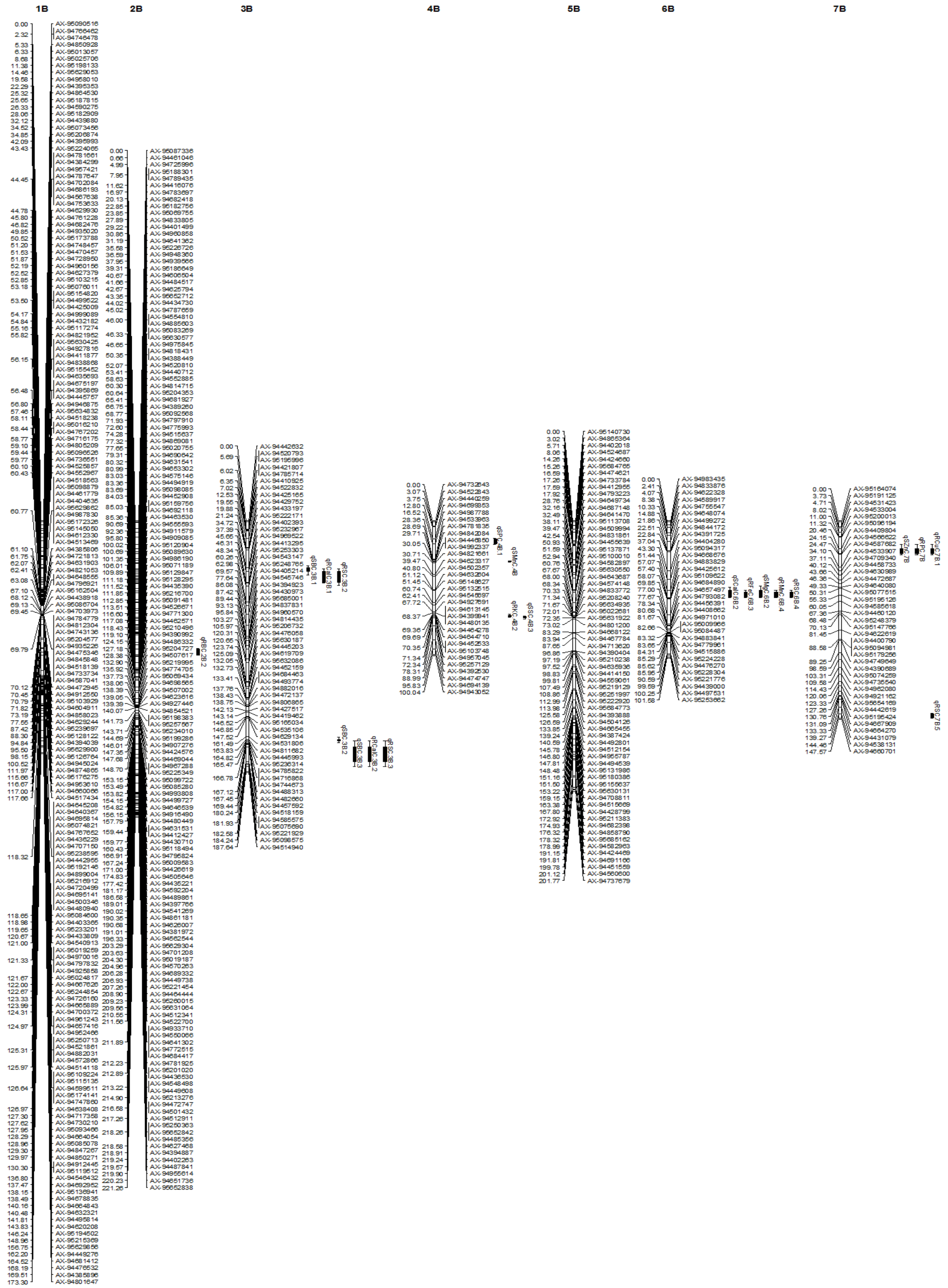

Figure 1.3: Genetic linkage map and additive QTLs located on B sub-genome of bread wheat for salt tolerance and nutrient concentrations under salt stress

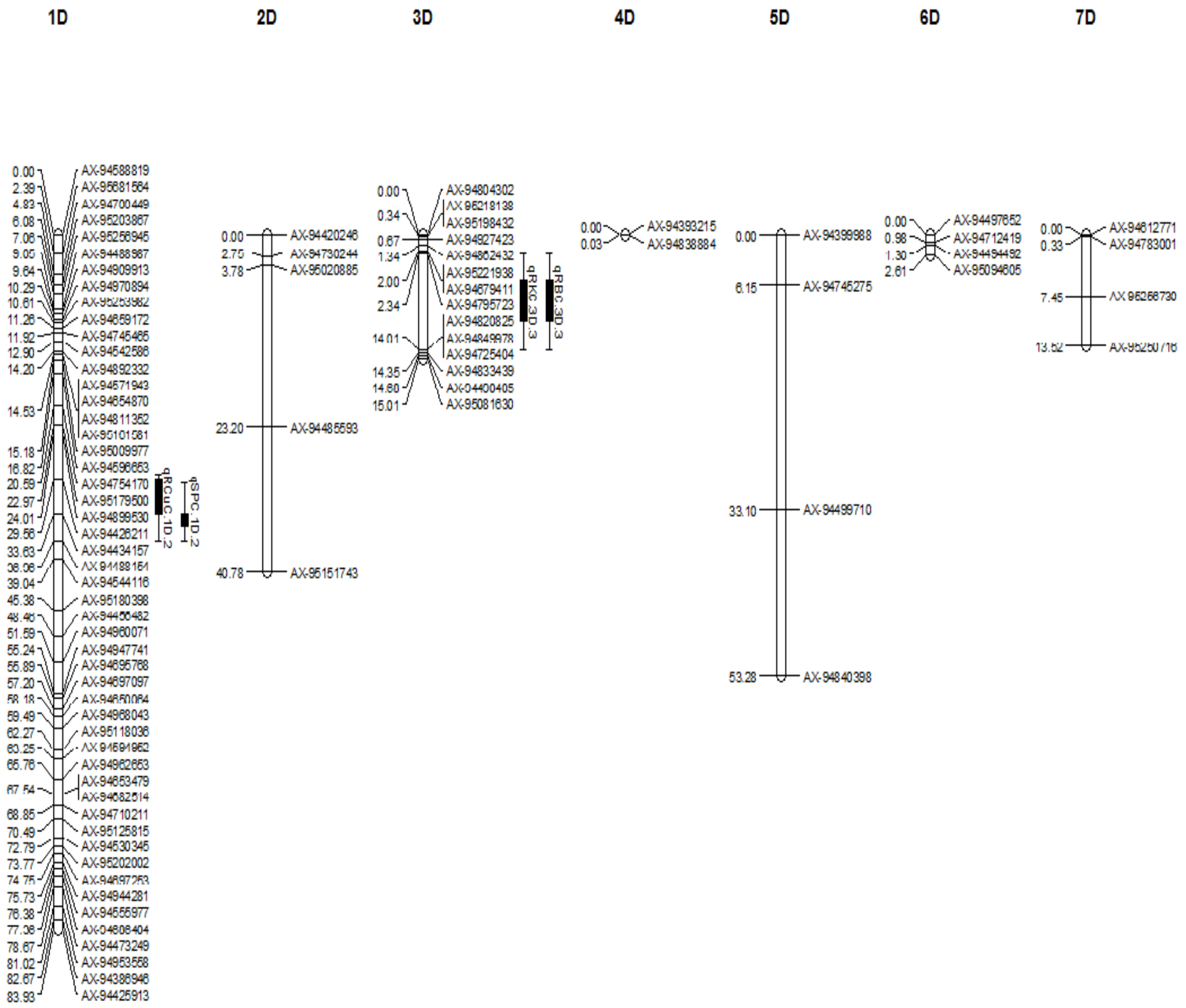

Figure 1.4: Genetic linkage map and additive QTLs located on D sub-genome of bread wheat for salt tolerance and nutrient concentrations under salt stress

1.4.5. QTL mapping for salt tolerance related traits and micronutrient concentrations

The composite interval mapping function of the QTL IciMapping program identified 49 additive QTLs for single-treatment salt tolerance related traits and micronutrient concentrations on 12 out of 21 wheat chromosomes. These QTLs were located on five chromosomes each of the A and B sub-genomes and two chromosomes belonging to the D genome (**Table 1.7; Figure 1.2, 1.3, 1.4**). In total six QTLs were mapped for NAX from root and shoot, while the major RNAX and SNAX QTLs mapped on chromosome 7A, i.e. qSNAX.7A.3 and qRNAX.7A.3, contributed 15.35 and 13.69% to the phenotypic variation of shoot and root NAX respectively. The contribution of qSNAX.7A.3 and qRNAX.7A.3 QTLs to DSW and DRW, i.e. salt tolerance, was recorded to be 19.79 and 11.23 %, respectively. Three minor root and shoot NAX QTLs were mapped on chromosome 2A while one was located on 6A.

The QTLs for root K^+ concentration (RKC) and shoot K^+ concentration (SKC) were located on chromosome 3D, 2A, 4B and 6A. These QTLs had minor contributions to phenotypic variation for K and salt tolerance and only the shoot QTL, qSKC.6A.2, had a notable contribution of 7.46 and 9.76% to both traits, respectively. Similarly, four QTLs for root and shoot Zinc concentrations (RZnC, SZnC) were located on chromosome 2A, 6A, 7A and 7B and the most important QTL, qRZnC.7A.3, made 11.23 and 12.08% contributions to phenotypic variation for Zn concentration and salinity tolerance, respectively. Among the five QTLs mapped for root and shoot Ca^{2+} concentrations (RCalC and SCalC), qSCalC.6B.2 and qRCalC.6B.3 QTLs contributed 6.52 and 10.91% to the phenotypic variation of CalC while their contribution to salt tolerance was 11.87 and 5.92%. Another three QTLs were mapped for shoot and root Mg^{2+} concentration (SMgC, RMgC). Among them, qSMgC.2A.1 and qSMgC.6B.2 QTLs had maximum contribution to SMgC (6.37%) and salt tolerance (8.36%) (**Table 1.7**).

For shoot Fe and Cu conc. (SFeC and SCuC), no QTLs were mapped, however, two RCuC QTLs on 7B and 1D were detected that made minor contributions to ST while qRCuC.1D.2 accounted for 6.06% of phenotypic variation in RCuC. Among the three RFeC QTLs, qRFeC.6A.2 and qRFeC.6B.3 contributed 12.96 and 5.92% to RFeC and salt tolerance respectively, while qRFeC.2A.1 made <4 contribution to both traits. Among the four QTLs mapped for RMnC and SMnC, qRMnC.2A.1 and qRMnC.2A.2 contributed 8.13 and 5.17% to the RMnC while qRMnC.6B.3, which was co-localized with qRFeC.6B.3, made 14.16 and 5.92% contributions to RMnC and salt tolerance. Eight QTLs were mapped for RSC and SSC, the maximum for any measured trait and were mapped on 1A, 2A, 3B, 4B, 6B and 7B. However, the QTLs for S, P and Boron made only minor contributions to salt tolerance despite accounting for up to 10.04% of the variation in nutrient concentrations. Finally, five QTL clusters with several co-localized QTLs were found on chromosomes 2A, 3B, 6A, 6B and 7A (**Table 1.7; Figure 1.2, 1.3, 1.4)**.

Table 1.7: The location of mapped additive QTLs on wheat chromosomes and their contribution to salt tolerance and mineral concentrations in 300 mM salinity

Trait	QTL	Marker Interval	Position (cM)	LOD	PQCMC	PQCST
RBC	qRBC.2A.1	AX-94496850--AX-94696230	32.49-36.16	3.59	1.29	0.14
	qRBC.2B.2	AX-94909085--AX-95120904	95.80-100.20	2.50	0.16	0.10
	qRBC.3D.3	AX-94795723--AX-94820825	2.34-14.01	5.68	2.29	0.21
RCalC	qRCalC.3B.1	AX-95232967--AX-94969522	37.39-45.65	2.86	5.27	0.68
	qRCalC.3B.2	AX-94457592--AX-94518159	169.44-180.2	2.84	5.38	0.98
	qRCalC.6B.3	AX-94668676--AX-94883829	51.44-57.07	7.09	10.91	5.92
RCuC	qRCuC.7B.1	AX-94409804--AX-94566622	20.46-24.15	2.75	2.83	0.08
	qRCuC.1D.2	AX-94426211--AX-94434157	29.56-33.63	2.53	6.06	0.39
RFeC	qRFeC.2A.1	AX-95114316--AX-94878691	118.10-121.02	2.51	3.98	3.81
	qRFeC.6A.2	AX-94547709--AX-94774725	32.16-59.57	26.7	12.96	3.15

	qRFeC.6B.3	AX-94668676--AX-94883829	51.44-57.07	18.10	8.73	5.92
RKC	qRKC.2A.1	AX-94496850--AX-94696230	32.49-36.16	3.70	4.79	0.14
	qRKC.4B.2	AX-95103748--AX-94957045	70.35-71.34	21.4	11.31	1.40
	qRKC.3D.3	AX-94795723--AX-94820825	2.34-14.01	4.01	7.96	0.21
RMgC	qRMgC.5A	AX-94460229--AX-94730618	31.30-44.87	6.20	4.96	5.58
RMnC	qRMnC.2A.1	AX-94895053--AX-95197988	100.35-105.10	5.96	8.13	0.37
	qRMnC.2A.2	AX-95114316--AX-94878691	118.10-121.02	3.01	5.17	3.81
	qRMnC.6B.3	AX-94668676--AX-94883829	51.44-57.07	19.20	14.16	5.92
RNAX	qRNAX.2A.1	AX-95114316--AX-94878691	118.10-121.02	2.53	4.85	3.81
	qRNAX.6A.2	AX-94547709--AX-94774725	32.16-59.57	9.35	6.46	3.15
	qRNAX.7A.3	AX-95248570--AX-95002995	64.79-66.44	2.51	13.69	11.23
RPC	qRPC.7B	AX-94409804--AX-94566622	20.46-24.15	2.59	2.73	0.08
RSC	qRSC.2A.1	AX-94895053--AX-95197988	100.35-105.10	3.50	5.65	0.37
	qRSC.3B.2	AX-95232967--AX-94969522	37.39-45.65	2.96	4.61	0.68
	qRSC.3B.3	AX-94457592--AX-94518159	169.44-180.24	2.92	6.42	0.98
	qRSC.6B.4	AX-94668676--AX-94883829	51.44-57.07	16.18	10.04	5.92
	qRSC.7B.5	AX-94538131--AX-94660701	144.46-147.70	2.52	2.89	1.97
RZnC	qRZnC.2A.1	AX-95114316--AX-94878691	118.1-121.02	2.83	5.25	3.81
	qRZnC.6A.2	AX-94547709--AX-94774725	32.16-59.57	11.22	7.45	3.15
	qRZnC.7A.3	AX-95248570--AX-95002995	64.79-66.44	2.52	12.08	11.23
SBC	qSBC.3B.1	AX-94402393--AX-95232967	34.72-37.39	2.70	5.86	0.59
	qSBC.3B.2	AX-94811682--AX-94445993	163.80-164.82	2.63	5.53	0.46
	qSBC.3B.3	AX-94457592--AX-94518159	169.5-180.24	2.82	4.91	1.01
SCalC	qSCalC.6A.1	AX-94547709--AX-94774725	32.16-59.57	29.1	8.98	3.08
	qSCalC.6B.2	AX-94668676--AX-94883829	51.44-57.07	11.41	6.52	11.87
SKC	qSKC.2A.1	AX-95114316--AX-94878691	118.1-121.02	2.51	4.34	5.18
	qSKC.6A.2	AX-94547709--AX-94774725	32.16-59.57	9.15	7.46	9.76
SMgC	qSMgC.2A.1	AX-94577588--AX-95114269	38.86-42.45	2.79	6.37	1.23
	qSMgC.6B.2	AX-94668676--AX-94883829	51.44-57.07	2.58	5.90	8.36
SMnC	qSMnC.4B	AX-94842084--AX-94446850	29.71-30.05	4.75	3.12	1.03
SNAX	qSNAX.2A.1	AX-94496850--AX-94696230	32.49-36.16	2.89	5.14	0.95
	qSNAX.2A.2	AX-94696230--AX-94577588	36.16-38.86	3.10	7.10	1.45
	qSNAX.7A.3	AX-95248570--AX-95002995	64.79-66.44	2.92	15.35	18.79
SPC	qSPC.4B.1	AX-94699353--AX-94987788	12.80-16.52	3.38	2.10	1.62
	qSPC.1D.2	AX-94434157--AX-94488154	33.63-3696	2.58	4.73	0.20
SSC	qSSC.1A.1	AX-94542559--AX-94416982	28.06-37.28	2.55	1.38	0.96
	qSSC.2A.2	AX-94577588--AX-95114269	38.86-42.45	4.13	2.13	0.91
	qSSC.4B.3	AX-94957045--AX-95257129	71.34-72.34	4.55	2.46	0.01
SZnC	qSZnC.7B	AX-94409804--AX-94566622	20.46-24.15	2.78	3.28	0.13

QTL: quantitative trait loci**, LOD:** logarithm of the odds ratio; **PQCMC:** percent QTL contribution for mineral concentration; **PQCST:** percent QTL contribution for salt tolerance

1.5. Discussion

A wheat 35K SNP array containing probes for 35,143 exome-captured SNPs was used for genotyping of 154 F_2 lines derived from hybridization of salt tolerant and sensitive wheat accessions. Analysis of the genotyping data by Axiom software revealed that majority of the SNPs, i.e. 16,210 (46.1%) of 35,143 SNPs, were monomorphic. We used only the 3,381 'PHR' or polymorphic SNPs, which accounted for 9.6% of whole array SNPs, for construction of genetic linkage map, in contrast to a recent study [19] which utilized 'PHR', 'OTV' and 'NMH' SNPs for the purpose. This was because of fact that instead of a pool of accessions, our material was an F_2 population, and only polymorphic SNPs exhibited the typical F_2 population segregation pattern. We removed the SNPs that showed segregation distortion using the Chi-square test coupled with sequential Bonferroni correction [42] because this is vital for obtaining a high-quality genetic linkage map, and 1,032 'PHR' SNPs passed the test. The MapDisto program, which is suitable for analyzing high throughput genotyping data was used for construction of genetic linkage map as classic programs e.g. JoinMap, MapMaker etc. cannot handle the high-throughput genotyping data. A whole-genome high density genetic linkage map of 21 wheat chromosomes consisting of 988 SNPs was constructed.

The lowest number of these markers (84) were mapped to the D genome, while A and B genome maps were populated with 342 and 562 SNPs, respectively. The least number of segregating markers being assigned to D genome is associated with its relatively recent evolutionary history/origin, resulting in lower nucleotide diversity in this sub-genome [47,48]. Therefore, the total length of our linkage map, i.e. 2317.88 cM, was shorter than the 3739.23 cM length of the reported consensus linkage map [19] because of lower segregation rate in the D genome under salt stress. Among all the markers assigned to wheat chromosomes, around 40% or 398 were mapped for the first time while 79 SNPs were mapped on different chromosomes as compared to the consensus map. The assignment of novel and conflicting SNPs indicated the presence of genetic diversity/variation between the wheat genotypes from Pakistan used in this study, compared with those (predominantly from Europe) used for the consensus map. Moreover, we genotyped an F_2 population in comparison to the homozygous wheat accessions in said study, which may have resulted in different segregation patterns. Even so, more than 86% or 511 out of 590 SNP markers found in both maps were mapped on the same chromosomes [19,46]. Similarly, a considerable number of conflicting markers were located on their homeologous chromosomes when compared to the consensus map, indicating exchange of orthologous sequences among the wheat sub-genomes during course of evolution.

Using the high-density genetic linkage map, a total of 49 QTLs for salt tolerance related traits and micronutrients concentration under salinity were mapped on 12 wheat chromosomes, which included four QTLs on two D chromosomes while the rest of the QTLs were identified on five chromosomes each from the A and B sub-genomes. Reduced Na^+ uptake or sodium exclusion (NAX) is considered one of the most important salt tolerance mechanisms in wheat because unchecked Na^+ influx into wheat leaves results in reduced photosynthesis and severe salt injury to leaves, which leads to stunted leaf growth or complete mortality, thus reducing yield significantly [2,4,8]. After the identification of a major NAX locus on chromosome 2A in wheat [29], QTL mapping for salt tolerance in wheat has largely been focused on mapping NAX QTLs [24,26,27]. We mapped a total of six QTLs for RNAX and SNAX, and two closely linked QTLs on chromosome 2A (qSNAX.2A.1, qSNAX.2A.2) and another 2A QTL, qRNAX.2A.1, coincided with three previously reported NAX QTLs on chromosome 2A in bread wheat [26] and a major NAX QTL *Nax1* (*HKT1;4*) in durum wheat [29]. Another NAX QTL mapped on chromosome 6A, qRNAX.6A.2, also coincided with a reported QTL [26,27]. We also identified two novel and major NAX QTLs on chromosome 7A i.e. qSNAX.7A.3 and qRNAX.7A.3, which accounted for 15.35 and 13.69 % of the SNAX and RNAX phenotypic variation respectively. These QTLs contributed 19.79 and 11.23 % to the salt tolerance phenotypes, i.e. DSW and DRW, respectively.

The HKT transporter genes are well known for regulating K^+ and/or Na^+ transport in plants, and they code for proteins responsible for reducing Na^+ transport to wheat leaf/shoot, thereby conferring salt tolerance [2]. Therefore, QTLs have also been mapped for K^+ concentration under salt stress in past studies [24,26–28]. In the present study, a major QTL for SKC, qSKC.6A.2, was identified on chromosome 6A, which contributed 7.46 and 9.76% to the SKC phenotypic variation and salt tolerance, respectively. Another novel/major QTL on chromosome 4B, qRKC.4B.2, contributed 11.31% of RKC phenotypic variation while a chromosome 3D QTL, qRKC.3D.3, coincided with a reported QTL [26]. Three RKC and SKC QTLs were co-localized with the RNAX and SNAX QTLs, which was consistent with higher correlation coefficients between phenotypic data of these traits.

Despite being important macronutrients for plant growth and development, the genetics of Ca^{2+} and Mg^{2+} accumulation under salt stress was unknown until recently [28]. We identified two major QTLs for SCalC and RCalC on chromosome 6B, i.e. qSCalC.6B.2 and qRCalC.6B.3, which accounted for 11.87 and 5.92% of salt tolerance, respectively. These QTLs contributed 6.52 and 10.91% to the phenotypic variation for SCalC and RCalC, respectively. These QTLs and two RCalC QTLs on chromosome 3B, qRCalC.3B.1 and qRCalC.3B.2, coincided with previously reported QTLs on the same chromosome [28]. However, a novel QTL on chromosome 6A, qSCalC.6A.1, was detected that accounted for 8.98% of the phenotypic variance for SCalC. Among the four QTLs for SMgC and RMgC, a chromosome 2A QTL, qSMgC.2A.1, contributed

6.37% to the phenotypic variation for SMgC and a chromosome 6B QTL, qSMgC.6B.2, accounted for 8.36% of the observed salt tolerance. A novel chromosome 5A QTL, qRMgC.5A, contributed 5.58% to salt tolerance.

Similarly, 27 novel QTLs for Boron, Cu, Fe, Mn, S, P and Zn concentrations under salt stress were identified in wheat shoot and root for the first time because QTLs for these macro- and micronutrients were previously described only under water stress conditions [25]. Some of these QTLs made major contributions to mineral concentrations and salt tolerance. A major QTL for RZnC on 7A, qRZnC.7A.3, accounted for 12.08 and 11.23% of the phenotypic variation for RZnC and salt tolerance. A FeC QTL mapped on chromosome 6B accounted for 5.92% for salt tolerance and another chromosome 6B QTL, qRMnC.6B.3, made a 14.16% contribution to phenotypic variation for RMnC. Finally, RFeC QTL on 6A, qRFeC.6A.2, presented 12.96% of the phenotypic variation for RFeC. Although QTLs for S, P and Boron made minor contributions to the variation for salt tolerance, they made major contributions to phenotypic variation for these mineral nutrients under salt stress, and this useful information can be used for breeding of biofortified or nutrient-rich wheat.

The QTL clusters containing co-localized QTLs for several salt tolerance related traits and mineral nutrient concentrations were located on chromosomes 2A, 3A, 3B, 6A, 6B and 7A. Such clusters are expected to appear as cellular Na^+ influx is expected to alter the concentrations of several other ions as well; indeed, such QTL clusters for these closely associated salt tolerance traits were also found in previous studies [24,25,27]. Such QTLs either represent a single gene or multiple tightly linked genes; e.g. the mapping of two closely linked chromosome 2A QTLs for NAX and the co-localized QTLs for ZnC and NAX. The QTL clustering or co-localization is also explained by significantly higher correlation coefficients among these traits.

1.6. Conclusions and Prospects

We developed a high-density genetic linkage map of 988 SNPs for an F_2 wheat population consisting of 154 lines, by using genotyping data obtained from the Axiom Wheat Breeder's Genotyping Array. A total of 49 QTLs were mapped for salt tolerance related traits and mineral nutrients' concentrations under salt stress. Two Na^+ exclusion (NAX) QTLs located on chromosome 2A coincided with a major reported QTL (*Nax1* or *HKT1;4*) while two other novel major NAX QTLs on 7A contributed 18.79 and 11.23% to the phenotypic variation in salt tolerance. Another 13 QTLs including major QTLs were mapped for K^+, Ca^{+2} and Mg^{+2} concentrations, while 27 novel QTLs were identified for tissue Boron, Copper, Iron, Manganese Phosphorus, Sulphur and Zinc concentrations under salt stress. Among them, a major ZnC QTL co-localized with a NAX QTL on 7A, contributed 11.23% to salt tolerance and 12.08% to Zn concentration. Therefore, several major and novel QTLs for salt tolerance were mapped. These

QTLs and their associated SNPs are a valuable resource for MAS and accelerated wheat breeding for salt tolerance in wheat. Similarly, major and novel QTLs for K, Ca, Mg, Fe, Cu, Mn and Zn concentrations can also be used for development of nutrient-enriched or biofortified wheat, by incorporating them in MAS and molecular breeding.

Chapter 2: Gene Annotations and Transcriptomics Analysis for SNPs

2.1. Introduction

The complexity of the wheat genome, arising from polyploidy and the presence of repetitive sequences on chromosomes and across sub-genomes makes it harder to identify the salt tolerance genes. Transcriptome analysis could be an effective way to identify gene but only one such study has been carried out to date that is based on a single genotype [49]. The genetic information is further limited by the fact that it reported the differential expression of genes in only the root tissue and transcripts expressed in leaf could have been ignored. Therefore, there is a dire need for performing more transcriptomic analyses in wheat to understand the mechanisms conferring salt tolerance. Keeping in view the limited information about the salt tolerance genes in wheat, a novel method presented here used the flanking sequences of segregating sequence-tagged-SNP markers from an F_2 population, showing variation for salt tolerance, for functional annotation of associated genes. Furthermore, *in silico* transcriptomics analysis of the annotated genes was performed to validate the possible involvement of these genes in conferring salt tolerance to wheat. Objective was the functional annotation of segregating F_2 SNPs and validation of annotated SNPs by in-silico transcriptomics analysis.

2.2. Methods

2.2.1. Sequences and annotation of segregating SNP markers

The flanking sequences of 3,381 polymorphic or 'PHR' SNPs were extracted from the genotyping data (see chapter 1) using Axiom Analysis Suite 1.1.0.616 software. These sequences were mapped to gene coding sequences (CDS) of the International Wheat Genome Sequencing Consortium (IWGSC) [50] utilizing the BLAST+ 2.2.30 program, which yielded 1,448 hits. From this mapping, basic local alignment search tool (BLAST) hits with less than 95% identity and/or alignment length of <60bp were discarded. The resultant 1,323 SNPs were located within/on 1,257 IWGSC CDS. The functional annotation of these CDS was performed with the Blast2GO V4.0 program [51]. The functional annotation was performed by utilizing the default parameters of NCBI blast followed by mapping and Gene Ontology (GO) annotation functions in Blast2GO. Finally, the annotations that had an E-value $\geq 1 \times 10^{-30}$ were removed.

2.2.2. *In silico* transcriptomics analysis

Testing for differential expression of the annotated genes was performed by alignment of the 1,257 IWGSC CDS with transcriptome reads expressed under saline and normal conditions in wheat [49] using the BLASTN 2.6.1+ program. The alignments with alignment scores >200 were recorded, and differential expression values were obtained by dividing salt/normal alignment counts ratio by 3.25 (347,200/106,600 spots expressed under salinity/control). The genes which showed 2-fold increase or decrease in expression were taken as differentially expressed under salinity.

2.3. Results

2.3.1. Top BLAST hit distribution of annotated genes

A total of 1,306 from 3,381 polymorphic SNPs, associated with 1,257 genes, were functionally annotated by BLAST alignment runs for each SNP with Blast2GO program and Blast2Go was run to find the 20 best hits in other species. The best BLAST alignments for the genes harboring these SNPs were found to be with sequences from 22 different species. The maximum number of top BLAST hits (480 hits) for these SNP-linked CDS were found with sequences of *Aegilops tauschii*, and a minimum of one top BLAST hit each for nine species were recorded. Notably 290, 280, 136 and 77 IWGSC coding sequences harboring segregating SNPs were found to be homologous to the sequences belonging to *Triticum urartu*, barley, bread wheat and *Brachypodium distachyon*, respectively (**Table 2.1**). The annotated genes harboring polymorphic SNPs had a wide range of functions. The information about sequence length, BLAST similarity mean, top BLAST hit species, top 20 BLAST hit species, top hit coverage ratio, annotation GO IDs, annotation GO counts, annotation GO terms and functions of the annotated genes are made available in our paper [46]. The functions of the annotated genes are briefly discussed below:

Table 2.1: Top BLAST hit distribution of 1306 wheat SNPs-linked CDS in different species by BLAST, mapping and annotation function of Blast2GO

Species	**BLAST top hits**	**Species**	**BLAST top hits**
Aegilops tauschii	480	*O. brachyantha*	3
Triticum Urartu	290	*Zea mays*	2
Hordeum vulgare	280	*B. sylvaticum*	1
T. aestivum	136	*Gossypium hirsutum*	1
Brachypodium distachyon	77	*Phyllostachys edulis*	1
T. durum	9	*P. praecox*	1
Sorghum bicolor	6	*Secale cereal*	1
Oryza sativa Japonica	5	*Zootermopsis nevadensis*	1
Setaria italic	3	*Agropyron mongolicum*	1
T. monococcum	3	*Avena longiglumis*	1
Dichanthelium oligosanthes	3	*A. sativa*	1
Total Species	**22**	**Total Annotations**	**1306**

2.3.2. Functionally annotated ion channels

A total of 44 SNP-carrying CDS were annotated to be ion transporters/channels, including seven potassium channels (HKT, K^+/H^+ exchanger, Jacalin-related lectin 3 (TaJRL3), K^+ outward-rectifying channel (KORC), K transporter 12, K (+) efflux antiporter and voltage-gated K channel), two chloride channels (CLC-e and CLC-g), one pyrophosphate-energized proton pump, four proton transporters, four magnesium channels including NIPA3 and NIPA4 transporter, five Zinc transporters including Zn transporter 6 and 7, four calcium, three nitrate and two sulfate transporters/channels, anionic, cationic, and metal channels. These ion channels are thought to be involved in conferring salt tolerance (**Table 2.2**).

2.3.3. Functionally annotated biomolecule transporters

In addition to ion channels, 50 SNP-carrying genes were predicted to be responsible for transport of important biomolecules such as protein, malate, glucose, hexose, fatty acid, drug and proline. Other genes were responsible for transmembrane transport, intracellular protein transport, protein transport to nucleus and vacuole, ATP transport, endocytosis and exocytosis or waste excretion at the cellular level. This group of genes could be involved in cellular homeostasis processes under salt stress conditions (**Table 2.3**).

2.3.4. Functionally annotated genes for hormonal signaling

Notably, 43 SNPs were found on genes involved in hormonal signaling pathways such as auxin, ethylene and ABA-activated signaling pathways, sugar, gibberellic acid and Jasmonic acid-mediated signaling pathways, auxin efflux and influx transport, basipetal auxin transport, auxin polar transport, auxin biosynthesis and response to auxin (**Table 2.4**).

2.3.5. Functionally annotated genes for cellular signaling

In addition to hormonal signaling, 45 SNP-carrying genes were annotated to be involved in cellular signaling processes such as the cell defense response, apoptotic signaling, G-protein signaling, ER-nucleus signaling, cell surface receptor signaling, Wnt signaling, intracellular signaling, cell to cell signaling, signal recognition, signal transduction and regulation of signal transduction (**Table 2.5**).

Table 2.2: Ion transporters/channels annotated to PHR SNPs in an F2 population

SNP ID	IWGSC Sequence Hit	Annotated Channel /Transporter	Transports
AX-94778362	lcl|Traes_4BL_5A58CACB2.1	HKT transporter	K
AX-95224228	lcl|Traes_6BS_E420DDD6D.1	K+-H+ exchange	K
AX-94995317	lcl|Traes_1DS_07F02E427.1	K transporter 12	K
AX-94388980	lcl|Traes_5DS_49CF8A4C4.1	K (+) efflux antiporter	K
AX-95215612	lcl|Traes_XX_D8915FD17.1	Voltage-gated K channel	K
AX-95654644	lcl|Traes_1BL_611DF0433.1	Jacalin-related lectin 3	K
AX-94699167	lcl|Traes_5AL_51E31BF07.1	Out-rectifying K channel	K
AX-94484138	lcl|Traes_3B_81DB429AC.1	Chloride channel CLC-e	Cl
AX-94546397	lcl|Traes_2DL_A591AC867.1	Chloride channel CLC-g	Cl
AX-95126745	lcl|Traes_4BL_E0ABA8471.1	Cation Ca exchanger 4	Ca
AX-95069958	lcl|Traes_1AS_429D67C42.1	Ca stress-gated channel 1	Ca

AX-94849975	lcl|Traes_7BL_13D715DB0.1	Ca homeostasis ER	Ca
AX-94662401	lcl|Traes_4BS_06DC8C269.1	Ca-transporting ATPase	Ca
AX-94635693	lcl|Traes_XX_0593C741B.1	Zn transporter 6	Zn
AX-94414919	lcl|Traes_1DS_C2EFEFBB9.1	Zn transporter 7	Zn
AX-95172326	lcl|Traes_1DS_D28FA6FF2.1	Zn transporter At3g08650	Zn
AX-94495517	lcl|Traes_XX_79F99051D.1	Metal tolerance C2	Zn
AX-95634832	lcl|Traes_1AS_0B179C27B.1	IQM1	Zn
AX-94755145	lcl|Traes_2DL_5C445EE47.1	Mg transporter NIPA3	Mg
AX-95159756	lcl|Traes_2AL_065DBAB56.1	Mg transporter NIPA4	Mg
AX-94692118	lcl|Traes_2BL_D5156A4A5.1	Mg transporter NIPA4	Mg
AX-94624155	lcl|Traes_4AL_7541D0C33.1	ER membrane body 2-X2	Mg,Fe
AX-95142803	lcl|Traes_2DL_CCAE7B431.1	Sulfate transporter	Sulfate
AX-94518655	lcl|Traes_3AL_224FB10D3.1	Sulfate transporter	Sulfate
AX-94852973	lcl|Traes_XX_7D456E213.1	Nitrate transporter	Nitrate
AX-94991110	lcl|Traes_2BL_0E87D8729.1	NRT1 PTR FAMILY	Nitrate
AX-95216700	lcl|Traes_2BS_88803DFE6.1	NRT1 PTR FAMILY	Nitrate
AX-94863332	lcl|Traes_3B_91715BB56.1	Anion transporter 7	Anions
AX-94775993	lcl|Traes_2BL_007AADDF1.1	G-3-Phosphate transporter1	Anions
AX-94550729	lcl|Traes_4BL_32F50466D.1	Mo-anion transporter	Anions
AX-94583481	lcl|Traes_3DS_50B54D1FC.1	WPP domain-associated	Cations
AX-94752371	lcl|Traes_2DL_51FF05F66.1	Cu-transporting HMA5	Copper
AX-94936984	lcl|Traes_7DS_D439AB891.1	Pyrophosphate H+ pump	Proton Pump
AX-94384299	lcl|Traes_XX_796D903AA.1	ATP synthase Mitochon.	Proton
AX-95118708	lcl|Traes_7DL_41A6D7A34.1	Cytochrome-c oxidase	Proton
AX-94486290	lcl|Traes_3AL_06CDB999D.1	ATP synthase Mitochon.	Proton
AX-94982994	lcl|Traes_2DL_B4C9A5695.1	H+-exporting ATPase	Proton
AX-94713620	lcl|Traes_XX_DFFB37624.1	Anthranilate BTase 1	Non-selective
AX-94909932	lcl|Traes_5AS_D7A8B1D1B.1	Mechanosensitive channel	Ions
AX-95257567	lcl|Traes_2DL_C065A5C4A.1	Solute carrier 22-15	Metal ions
AX-94985111	lcl|Traes_7AS_705BE4B61.1	Solute carrier fam 35-F1	Metal ions
AX-94757270	lcl|Traes_1AL_CEA78C84D.1	S deficiency-induced 1	S
AX-94560970	lcl|Traes_1BL_6741F0C8B.1	S deficiency-induced 1	S
AX-94869513	lcl|Traes_1DL_36CEA53FD.1	S deficiency-induced 1	S

Table 2.3: SNPs carrying genes annotated for transport of biomolecules in an F2 population

SNP ID	IWGSC Sequence Hit	Annotated Channel/Transporter	Transport
AX-94454146	lcl|Traes_XX_28E195696.1	MAPK activating-like	Protein
AX-94418123	lcl|Traes_3DL_8C0D8B750.1	IST1 isoform X2	Protein
AX-94560810	lcl|Traes_5BS_F8C1EF344.1	SEC1 family transport SLY1	Protein
AX-94542375	lcl|Traes_3B_ED8407FA7.1	transport Sec61 subunit alpha-like	Protein
AX-94960858	lcl|Traes_2BL_BBE53E272.1	transport SEC13 homolog B-like	Protein; mRNA
AX-94641362	lcl|Traes_XX_6F7C869CE.1	transport SEC13 homolog B-like	Protein; mRNA
AX-95210470	lcl|Traes_1BL_0CB993ADF.1	TOM1 2	Intracellular protein
AX-95103748	lcl|Traes_4BS_1FB8DFEA9.1	TOM1 2	Intracellular protein
AX-95172159	lcl|Traes_6BL_2546C9F2A.1	TOM1 2	Intracellular protein

AX-94434730	lcl|Traes_2DL_99A21BFBF.1	gamma-soluble NSF attachment	Intracellular protein
AX-95230703	lcl|Traes_XX_546E775F0.1	growth hormone-regulated TBC1-like	Intracellular protein
AX-95087248	lcl|Traes_5BL_AE259FBB7.1	GTPase-activating gyp7-like	Intracellular protein
AX-94937975	lcl|Traes_6DL_CFD8E7EFB.1	nuclear-pore anchor isoform X2	Protein to nucleus
AX-94506088	lcl|Traes_XX_03FEE38BD.1	importin-5 isoform X1	Protein to nucleus
AX-94388408	lcl|Traes_1DL_7F48CB11B.1	AP-3 complex subunit isoform X1	Protein to vacuole
AX-94515411	lcl|Traes_5AS_EA7EF5450.1	Retrovirus-related Pol poly LINE-1	Protein to vacuole
AX-94850928	lcl|Traes_1BL_C94CBC376.1	ADP, ATP carrier At5g56450	ATP transporter
AX-95075429	lcl|Traes_1BL_B6112C79D.1	Ran-specific GTPase-activating 2	Protein from nucleus
AX-95000586	lcl|Traes_1DL_102D07AB7.1	Ran-specific GTPase-activating 2	Protein from nucleus
AX-95235622	lcl|Traes_1DL_102D07AB7.1	Ran-specific GTPase-activating 2	Protein from nucleus
AX-94598441	lcl|Traes_2DL_B7D08C386.1	1 domain containing expressed	Protein import to nucleus
AX-94556198	lcl|Traes_5AL_F49663738.1	Hexose carrier HEX6	Proton; Glucose
AX-95090777	lcl|Traes_2BL_710FDE032.1	probable polyol transporter 4	Proton; Glucose
AX-95233137	lcl|Traes_4BL_057150686.1	monosaccharide-sensing 2-like	Hexose
AX-94581785	lcl|Traes_6AL_EAF53EB94.1	Bidirectional sugar transporter SWEET13	Sugar
AX-94604589	lcl|Traes_4DL_341830EFF.1	ALMT1	Malate
AX-94762683	lcl|Traes_4DL_8E805248E.1	ALMT1	Malate
AX-94766675	lcl|Traes_4AL_59D5504B1.1	NRT1 PTR FAMILY -like	Oligonucleotide
AX-94871419	lcl|Traes_5BL_5A648D29D.1	transparent testa 12	Drug
AX-94613383	lcl|Traes_XX_4AA6C2A2D.1	TRANSPARENT TESTA 12	Drug
AX-95085345	lcl|Traes_XX_4AA6C2A2D.1	TRANSPARENT TESTA 12	Drug
AX-94943274	lcl|Traes_3B_74BE823B11.1	TRANSPARENT TESTA 12-like	Drug
AX-94509316	lcl|Traes_3B_0CD91DCF2.1	ABC transporter D family member 1	Fatty acid
AX-94953610	lcl|Traes_1BS_A4FD92A761.1	ABC transporter G family member 15	Transmembrane
AX-95197645	lcl|Traes_6DL_F9179CEF0.1	Hippocampus abundant transcript 1	Transmembrane
AX-94844172	lcl|Traes_2DS_6477FCF5F.1	Nucleobase-ascorbate transporter 11	Transmembrane
AX-95653566	lcl|Traes_5DL_F657BF165.1	Nucleobase-ascorbate transporter 6	Transmembrane
AX-94445422	lcl|Traes_1AL_9C9EE44BB.1	Structural maintenance of chromosomes 4	Transmembrane
AX-94782013	lcl|Traes_7BL_BCA66B639.1	Ion channel chloroplastic isoform X1	Transmembrane
AX-95110067	lcl|Traes_5AL_67421246B.1	mitochondrial carrier	Transmembrane
AX-94686993	lcl|Traes_XX_081248D2E.1	ADP, ATP carrier mitochondrial	Transmembrane
AX-94667805	lcl|Traes_7AS_2240F6F53.1	WAT1-related At3g30340-like	Transmembrane
AX-94635936	lcl|Traes_5DS_5DB217D0C.1	amino acid permease family expressed	Polyamine
AX-94649598	lcl|Traes_6BL_205A0D282.1	equilibrative nucleotide transporter 1	Nucleoside
AX-94852257	lcl|Traes_XX_B472C1CD8.1	equilibrative nucleotide transporter 1	Nucleoside
AX-94638771	lcl|Traes_6AS_F8BA0011D.1	nuclear pore complex NUP160	Nucleocytoplasmic
AX-94975261	lcl|Traes_2DL_6AA91D6ED.1	exocyst complex EXO70A1-like	Waste excretion
AX-94970500	lcl|Traes_2AL_08BBCE84E.1	exocyst complex EXO70A1-like X1	Waste excretion
AX-95195424	lcl|Traes_7BL_EC4E05301.1	laminin subunit beta-1 isoform X2	Endocytosis
AX-95098004	lcl|Traes_4BS_83DF7B31C.1	proline transporter 2 isoform X1	Proline

Table 2.4: Annotated genes associated PHR SNPs for hormonal signaling in F2 lines

SNP ID	IWGSC Sequence Hit	Annotated Gene	Annotated Function
AX-94406752	lcl|Traes_6BL_8BE7AE308.1	histone demethylase 1	auxin biosynthesis
AX-95200381	lcl|Traes_XX_2EB5F009A.1	Mitochondrial	Auxin polar transport
AX-94905933	lcl|Traes_7AS_34AF6845D1.1	F-box LRR-repeat MAX2	Auxin polar transport
AX-95115269	lcl|Traes_5BL_C9FD62D61.1	auxin transport BIG	Auxin Signaling/transport
AX-95079372	lcl|Traes_3AL_F777559B7.1	auxin Efflux Carrier family	Auxin efflux carrier
AX-94464561	lcl|Traes_1DL_F7B852321.1	ABC transporter B 11	Auxin efflux transport
AX-95014459	lcl|Traes_3B_80E2E63D9.1	ABC transporter B 11	Auxin efflux transport
AX-95204844	lcl|Traes_3B_CED217F21.1	ABC transporter B 11	Auxin efflux transport
AX-94635873	lcl|Traes_3B_CED217F21.1	ABC transporter B 11	Auxin efflux transport
AX-94645208	lcl|Traes_1BS_6C40DD844.1	ABC transporter B 21	Basipetal auxin transport
AX-94845848	lcl|Traes_1BS_6C40DD844.1	ABC transporter B 21	Basipetal auxin transport
AX-94433809	lcl|Traes_1DS_9C041C718.1	ABC transporter B 21	Basipetal auxin transport
AX-94667626	lcl|Traes_1DS_9C041C718.1	ABC transporter B 21	Basipetal auxin transport
AX-94707625	lcl|Traes_3DL_C426849EA.1	ABC transporter B 4	Auxin signaling/transport
AX-94613078	lcl|Traes_7AL_354EEE44E.1	auxin-responsive IAA21	Auxin signaling pathway
AX-95236314	lcl|Traes_XX_872A5B7DF1.1	F-box only 6	Auxin signaling pathway
AX-94899004	lcl|Traes_1BS_4BA296C26.1	Auxininhibitor	Auxin signaling pathway
AX-94701908	lcl|Traes_7AS_9FAAF308F.1	Peroxisomal signal 1 receptor	Response to Auxin
AX-94706037	lcl|Traes_2BL_C834427B5.1	Molybdopterin biosyn CNX1	Auxin, JA, ABA signaling
AX-94956073	lcl|Traes_1BL_ACAA87960.1	IAA-amido synthetase	Auxin, JA signaling
AX-94556600	lcl|Traes_XX_36CE0AE62.1	chloroplast stem-loop 1 kDa	JA signaling pathway
AX-94537482	lcl|Traes_XX_4EEEFFFB0.1	phospholipase D delta	ABA Signaling pathway
AX-94934089	lcl|Traes_2BL_B657F7F3A.1	Pathogenesis-related 1	ABA Signaling pathway
AX-94652395	lcl|Traes_4BL_46AF42528.1	Ca-dependent kinase isoform 11	ABA, Ca signaling
AX-94917451	lcl|Traes_6AL_E1137B391.1	Ca-dependent kinase 26	ABA, Ca signaling
AX-94414225	lcl|Traes_2DL_2AE90059F.1	endochitinase 2-like	Ca-calmodulin signaling
AX-94832924	lcl|Traes_2BL_3647661C2.1	IQ-DOMAIN 32	Response to ABA
AX-94658755	lcl|Traes_6DL_8AA89780B.1	GDSL esterase lipase At5g33370	Response to Salicylic acid
AX-95660165	lcl|Traes_6AS_AC231C610.1	UDP-N-AGS-peptide N-AGST	GA signaling pathway
AX-94970016	lcl|Traes_1AS_C41D89877.1	ethylene receptor	Ethylene signal pathway
AX-94747860	lcl|Traes_1BS_ADCD5C43B.1	ethylene receptor	Ethylene signal pathway
AX-94480940	lcl|Traes_1DS_35954E987.1	Ethylene receptor 1	Ethylene signal pathway
AX-95012678	lcl|Traes_7DS_3673F1C73.1	Ethylene Insensitive 3	Ethylene signal pathway
AX-95025377	lcl|Traes_4DS_2706FACC1.1	Ethylene-insensitive 2	Ethylene signal pathway
AX-94414949	lcl|Traes_5BL_852C4D56F.1	E3 ubiquitin- ligase listerin	Sugar mediated signaling
AX-94634070	lcl|Traes_4DL_BA5A6A3DF.1	Ubiquitin c-t hydrolase 26	Sugar mediated signaling
AX-94961611	lcl|Traes_4BL_4332984FC.1	Ubiquitin c-t hydrolase 26	Sugar mediated signaling
AX-94686353	lcl|Traes_5BL_2748468D7.1	ETIF3 subunit E	Sugar mediated signaling
AX-94797912	lcl|Traes_2BS_7CEBE03A7.1	exportin-T-like isoform X1	Sugar mediated signaling

Table 2.5: Annotated genes associated with PHR SNPs for cellular signaling in F2 lines

SNP ID	IWGSC Sequence Hit	Annotated Gene	Annotated Function
AX-95651903	lcl|Traes_5AL_6F771C72A.1	Cell division cycle 5	Defence response signal
AX-94731801	lcl|Traes_5DS_A721EE226.1	large-G nucleotide-binding 1	G-Protein signaling
AX-95629522	lcl|Traes_3AL_91BE453AD.1	G -coupled receptor	G-Protein signalling
AX-94489010	lcl|Traes_1BS_CE84ED791.1	Diacylglycerol kinase 1	G-Protein signalling
AX-94760192	lcl|Traes_6DS_E6CCD9F0C.1	large proline-rich BAG6 X2	Apoptotic Signaling
AX-94525765	lcl|Traes_3DS_237382675.1	large proline-rich bag6-A X1	Apoptotic Signaling
AX-94900436	lcl|Traes_3B_46A0FDA8D.1	tunicamycin induced partial	ER-nucleus signalling
AX-94434519	lcl|Traes_XX_F4BACCBA9.1	tunicamycin induced partial	ER-nucleus signalling
AX-94987830	lcl|Traes_1DS_5852D4AB6.1	dnaJ ERDJ3B-like	ER-nucleus signalling
AX-94983435	lcl|Traes_2AS_54C0F6864.1	Wall-assoc. receptor kinase 2	Cell surf. Recept. signal
AX-94402263	lcl|Traes_2AS_82E3A46B8.1	Ser-threonine- kinase svkA	Recept. Signaling protein
AX-94885603	lcl|Traes_2DL_36F021DAA.1	Wall-assoc. receptor kinase 3	Cell surf. Recept. Signal
AX-94597114	lcl|Traes_4AL_E42620042.1	Wall-assoc. receptor kinase 5	Cell surf. Recept. Signal
AX-94514671	lcl|Traes_2DS_554CD5259.1	Wall-assoc. receptor kinase 2	Cell surf. Recept. Signal
AX-94966165	lcl|Traes_5AL_FFA3A0686.1	Wall-assoc. receptor kinase 3	Cell surf. Recept. Signal
AX-94610041	lcl|Traes_6AS_BF9259EB6.1	wall-associated kinase 4	Cell surf. Recept. Signal
AX-94445537	lcl|Traes_6DL_119F2A402.1	casein kinase family	Wnt signaling pathway
AX-94384227	lcl|Traes_XX_55FF231D31.1	casein kinase I	Wnt signaling pathway
AX-94682514	lcl|Traes_1BL_0BFA34BAC.1	casein kinase I elta-like X1	Wnt signaling pathway
AX-95255810	lcl|Traes_2BL_E8D89B38E.1	ETO1 1	Cell-cell signalling
AX-95020755	lcl|Traes_2BL_E8D89B38E.1	ETO1 1	Cell-cell signalling
AX-94783438	lcl|Traes_5DL_6DA0BEF01.1	ser-threonine- kinase WNK9	Intracellular signal
AX-94821053	lcl|Traes_1BS_90AEC8678.1	tyrosine-phosphatase MKA1	Intracellular signal
AX-94728950	lcl|Traes_1DS_4C0964710.1	Protein-tyrosine-phosphatase	Intracellular signal
AX-94847267	lcl|Traes_1DS_4C0964710.1	Protein-tyrosine-phosphatase	Intracellular signal
AX-94797832	lcl|Traes_1DS_36D3EE932.1	CBL-interacting kinase 17	Intracellular signal
AX-94774467	lcl|Traes_2AS_2B84A0A98.1	ser-threonine- kinase WNK9	Intracellular signal
AX-94628613	lcl|Traes_5AL_2A40F0298.1	ser-threonine- kinase D6PK	Intracellular signal
AX-95179502	lcl|Traes_6DS_8C9544AC8.1	Signal recognition receptor α	Signal recognition
AX-94651794	lcl|Traes_3AL_C3C4EA828.1	signal recognition subunit SRP72	Signal recognition
AX-94842052	lcl|Traes_XX_7303F0600.1	signal recognition subunit SRP72	Signal recognition
AX-94852292	lcl|Traes_6BS_63350D7F6.1	adagio 2	Signal transduction
AX-94665765	lcl|Traes_2DL_207E2CB39.1	rho GTPase-activating 5-like	Signal transduction
AX-95115431	lcl|Traes_XX_AE15DF105.1	ser-threonine phosphatase 2A	Signal transduction
AX-95631372	lcl|Traes_XX_AE15DF105.1	ser-threonine phosphatase 2A	Signal transduction
AX-95250713	lcl|Traes_XX_0DD1E6265.1	ser-threonine phosphatase 2A	Signal transduction
AX-94567442	lcl|Traes_5DL_DB93C2CFF.1	ser-threonine phosphatase 2A	Signal transduction
AX-95106335	lcl|Traes_1BL_036AD7CCA.1	ankyrin repeat At5g02620	Signal transduction
AX-94585083	lcl|Traes_1DL_39B0D97A0.1	ankyrin repeat At5g02620	Signal transduction
AX-94610182	lcl|Traes_4AS_B2CDBFB54.1	Response regulator PRR73	Signal transduction
AX-95143961	lcl|Traes_3B_4CF90C501.1	ultraviolet-B receptor UVR8	Signal transduction
AX-94527643	lcl|Traes_2DS_69E67704C.1	pyruvate dehydrogenase	Signal Transduction
AX-94521861	lcl|Traes_1DS_97DDD3DBB.1	E3 ubiquitin- ligase RF298	signal transd. regulation
AX-94519391	lcl|Traes_XX_1744B5472.1	ubiquitin- ligase-like	signal transd. regulation

2.3.6. Functionally annotated genes as transcription factors

Interestingly, 63 SNPs were linked to genes from 35 different classes of transcription factors (TFs). These TFs included VIN3-like 1, ARF21, NAC78, MYB44, bHLH91, BLH7, SCR1, EIN2, PPR, PHD finger At1g33420, ZNFX1-NFXL1, CAMTA2, ABI5, NAC17, bZIP17, WRKY16, WRKY70, salt tolerance 1, Rice SLEEPERS 2, GATA26, MADS box VRT-2, MADS25, HSFA2, HSFA3, HSFA5, bHLH13, bHLH36, bHLH140 etc. The first 12 TFs in the above list harbored 2-4 segregating SNPs. These TFs were annotated for functions such as transcription factor activity, transcription regulation, DNA binding, iron homeostasis, Zn ion binding, protein dimerization, ABA synthesis, response to ABA, translation initiation, photoperiodism, transcription activation, auxin signaling, vernalization and salt tolerance response (**Table 2.6**).

2.3.7. Functionally annotated genes for cellular biosynthesis processes

Total 41 SNPs were located on genes coding for cell growth and development processes such as cell morphogenesis, cell division, mitotic nuclear division, cell cycle, growth regulation, cell growth and proliferation, regulation of meristem growth, monopolar cell growth, autophagy, pre-autophagosome, apoptosis, programed cell death, HR, cell wall organization and biogenesis, secondary cell wall, pectin and cellulose biogenesis (**Table 2.7**).

2.3.8. Functionally annotated genes for plant growth and development

Furthermore, 51 SNPs were present on the genes for plant growth-related processes such as seed germination, gravitropism, cotyledon and lateral root development, leaf and shoot morphogenesis, vegetative to reproductive growth transition, xylem development, flowering time, regulation of flowering time, pollen recognition, germination and development; mega-geametogenesis, pollen tube growth regulation, ovule and embryo sac development; and embryonic pattern specification. These SNPs could be linked to early and reproductive growth mechanisms conferring evasion of salt stress by fast growth (**Table 2.8**).

2.3.9. Functionally annotated genes for tolerance mechanisms

Another 48 SNPs were found on genes for resistance to biotic and abiotic stresses like drought, freezing, toxicity, wounding, systemic acquired resistance, hypersensitive response, fungus resistance, defense response; leaf rust, stripe rust and downy mildew resistance (**Table 2.9**).

2.3.10. Functionally annotated genes for genic and epigenetic functions

Another 198 SNPs were located on genes for genic and epigenetic functions such as chromatin modification/silencing, helicases, hydrolases, DNA repair mechanisms, DNA, tRNA, rRNA and histone-lysine methylation, genes silencing, RNA splicing, nucleotide binding, DNA replication, transcription and translation (**Table 2.10**).

Table 2.6: Annotated genes associated with transcription factors in mapping population

SNP ID	IWGSC Sequence Hit	Annotated TFs	Annotated Function
AX-94465976	lcl|Traes_XX_E150BB1BE.1	bHLH13	Response to ABA; TF activity
AX-94554793	lcl|Traes_4DL_A15D3AA00.1	bHLH140	ABA synthesis; DNA binding
AX-94830380	lcl|Traes_4BL_BEF6AF501.1	bHLH36	Transcription regulation
AX-95229999	lcl|Traes_2AL_FAB4B4A20.1	bHLH91	DNA binding; protein dimerization
AX-95156431	lcl|Traes_2BL_D3FAA4D64.1	bHLH91	Protein dimerization
AX-94665638	lcl|Traes_4AS_A79A68739.1	MYB44	DNA binding
AX-95202516	lcl|Traes_4AS_A79A68739.1	MYB44	DNA binding
AX-94577588	lcl|Traes_2BL_00608F8D6.1	ORG2	Transcription regul.; iron homeostasis
AX-95000138	lcl|Traes_5BL_A848F629F.1	RAX3	DNA binding
AX-94413608	lcl|Traes_5AL_69A441A78.1	SPT20	Mitochondrion based reaction
AX-94927446	lcl|Traes_2DL_9D82DB947.1	GTE7	Transcription regulation
AX-95629862	lcl|Traes_1DS_18F13A3DD.1	VIP2	Zn ion binding
AX-94936573	lcl|Traes_7BL_6EAAD1A2B.1	WRKY16	Transcription regulation
AX-94640775	lcl|Traes_7DL_A9EF00572.1	WRKY70	TF activity; transcription regulation
AX-94787613	lcl|Traes_XX_C631D846E.1	bZIP17	TF activity; transcription regulation
AX-94935882	lcl|Traes_5AS_B2606FAD3.1	BLH7	TF activity; transcription regulation
AX-95012249	lcl|Traes_XX_7FEA63ED8.1	BLH7	TF activity; transcription regulation
AX-94625273	lcl|Traes_5DL_2408DACC6.1	NAC17	TF activity; transcription regulation
AX-94757955	lcl|Traes_6AL_8BA1FF8B2.1	NAC78	Transcription regulation
AX-94653461	lcl|Traes_6DL_73054EBDC.1	NAC78	Transcription regulation
AX-94744507	lcl|Traes_2DL_640A09678.1	GATA26	transcriptional activator; TF complex
AX-94724618	lcl|Traes_XX_D1B4BBFA0.1	HSFA3	TF activity; transcription regulation
AX-94520583	lcl|Traes_6DS_C59B6322F.1	HSFA5	Transcription regulation
AX-95168091	lcl|Traes_5DL_B1D24781B1.1	HSFA2	TF activity; transcription regulation
AX-94922434	lcl|Traes_5AL_31F3633C11.1	GTF3C3	photoperiodism, flowering
AX-94622179	lcl|Traes_7BL_65294D713.1	TAF1	Translation initiation; trans. regulation
AX-94690681	lcl|Traes_5DL_4F9BD63F0.1	GT4	Vesicle transport
AX-95072327	lcl|Traes_3AL_6E16C8167.1	SCR1	TF activity; transcription regulation
AX-94947784	lcl|Traes_3DL_BF7D83705.1	SCR1	TF activity; transcription regulation
AX-95155637	lcl|Traes_5DL_A39210547.1	SCR21	TF activity; transcription regulation
AX-94755547	lcl|Traes_XX_16C919FDB.1	CAMTA2	Transcription activation; Ca signaling
AX-95629304	lcl|Traes_XX_16C919FDB.1	CAMTA2	Transcription activation; Ca signaling
AX-94526913	lcl|Traes_5BL_92902F993.1	CAMTA3	Transcription activation; Ca signaling
AX-94569284	lcl|Traes_4DS_990D31BB1.1	Rice Sleepers 2	TF activity; Transcription regulation
AX-94767467	lcl|Traes_2BL_1EBB348CB.1	MYB DIV	TF activity; transcription regulation
AX-95132994	lcl|Traes_2DS_1C0609A0E.1	DYT1	TF activity; transcription regulation
AX-94925230	lcl|Traes_2BS_4600D4B54.1	CIGR1	TF activity; transcription regulation
AX-94641302	lcl|Traes_2BL_F480B8D1F.1	BBP	RNA spliceosome; RNA PM II TF
AX-94596410	lcl|Traes_XX_E0B2E5623.1	ZNFX1-NFXL1	TF activity, Response to salinity/SA
AX-94504393	lcl|Traes_3B_245ED3424.1	ZNFX1-NFXL2	Transcription repression; RNA PM II TF
AX-94694404	lcl|Traes_6DL_B03D4CD04.1	MADS25	TF activity; transcription regulation
AX-95104113	lcl|Traes_7DS_90668ED2B.1	MADS box VRT-2	TF activity; transcription regulation
AX-94892332	lcl|Traes_1BL_00B75394B.1	FXBL-2	TF activity; transcription initiation/Reg
AX-95105440	lcl|Traes_1BL_3E662FC82.1	RISC	TF activity; transcription initiation/Reg
AX-94974381	lcl|Traes_3DL_6065765A2.1	PPR	TF activity; transcription regulation
AX-95629481	lcl|Traes_XX_7341490D9.1	PPR	TF activity; transcription regulation

AX-94796715	lcl|Traes_1BL_C25B5DDB4.1	MYBL1	Transcription regulation
AX-94926509	lcl|Traes_XX_232DC04EB.1	CEK	TF activity; transcription regulation
AX-95088362	lcl|Traes_3B_BE85B533C.1	PHD finger At1g33420	TF activity; transcription regulation
AX-95117955	lcl|Traes_3DL_6D0122C51.1	PHD finger At1g33420	Transcription regulation; Zn^{+2} binding
AX-94781835	lcl|Traes_4DS_77170C64A.1	PHD finger At1g33420	Transcription regulation; Zn^{+2} binding
AX-94518563	lcl|Traes_XX_52727D83C.1	PHD finger	Histone/Zn^{+2} binding, transcription reg
AX-95652714	lcl|Traes_5AL_0CFF03836.1	Salt tolerance1	Salinity response; TF activity; transcription reg
AX-94995865	lcl|Traes_7AL_C7CF7087B.1	LOC100282457 X1	TF activity; transcription regulation
AX-94620208	lcl|Traes_1BL_DE2CF9613.1	ABI5	TF activity; transcription regulation
AX-94957342	lcl|Traes_3AL_AE2469D5A.1	VIN3 1	Auxin signal; DNA binding; transcript reg
AX-95256931	lcl|Traes_6AS_967D58FB4.1	VIN3 1	Auxin signal; DNA binding; transcript reg
AX-95128343	lcl|Traes_7BS_F44A273F8.1	ARF21	Auxin signal; DNA binding; transcript reg
AX-94613078	lcl|Traes_7AL_354EEE44E.1	ARF21	Auxin signal; DNA binding; transcript reg
AX-95012678	lcl|Traes_7DS_3673F1C73.1	EIN2	Salinity response; TF; hormone signaling
AX-95025377	lcl|Traes_4DS_2706FACC1.1	EIN2	Ethylene signaling; transporter activity
AX-94861978	lcl|Traes_1BS_BEF75A847.1	VIN3 1	Vernalization response; flowering
AX-94475346	lcl|Traes_2AL_37EF1A21C.1	VIN3 1	Vernalization response; flowering

Table 2.7: Annotated genes associated with cell division, growth and development processes

SNP ID	IWGSC Sequence Hit	Annotated Growth Gene	Annotated Function
AX-94965212	lcl|Traes_XX_92E827A90.1	CAP-Gly domain linker 1	Cell morphogenesis
AX-94881482	lcl|Traes_2BL_2F1728CDC.1	UDP-galactose transporter 1	Regulation of cell cycle
AX-94940203	lcl|Traes_5BL_716390097.1	Cell division protease ftsH	Cell division
AX-94958010	lcl|Traes_1DL_E64D7EE6A.1	Zinc finger 830	Mitotic nuclear division
AX-95150132	lcl|Traes_6BS_3177B0E2D.1	ternary complex factor MIP1	cell growth and proliferation
AX-94933359	lcl|Traes_6DS_9B3DF8820.1	ternary complex factor MIP1	cell growth and proliferation
AX-95104040	lcl|Traes_2BS_79A4D889F.1	LONGIFOLIA 2-like	Monopolar cell growth
AX-94981854	lcl|Traes_6DL_5DE35B12C.1	nucleo TPR-like	Regulation of Meristem growth/RMG
AX-95233201	lcl|Traes_1DS_5026B1001.1	protease Do-like chloroplast	Regulation of Meristem growth
AX-95200381	lcl|Traes_XX_2EB5F009A.1	Mitochondrial	RMG; megagametogenesis
AX-95010896	lcl|Traes_7DL_3632B8F7B.1	Endo-plasmic homolog	Regulation of Meristem structure
AX-95209190	lcl|Traes_XX_49BFFD772.1	LAZY1	Growth regulations
AX-95162504	lcl|Traes_1AS_9716EE0D3.1	auxin-indep growth promoter	Growth Promotor
AX-94711916	lcl|Traes_7DS_923940091.1	eyes absent homolog 4	Multicellular organization
AX-94512154	lcl|Traes_5AL_DA29118ED.1	Trafficking particle complex 8	Pre-autophagosome
AX-94433182	lcl|Traes_5DL_5A9F9A3C5.1	Activating BECN1- autophagy	Autophagy
AX-94385658	lcl|Traes_3DL_171CAEEDB.1	Receptor kinase At3g55450	Autophagy
AX-94829027	lcl|Traes_7BL_1C6415BB2.1	Rp1	Apoptosis
AX-94560729	lcl|Traes_3B_E792EFE61.1	Metacaspase 1	Programmed cell death
AX-94497096	lcl|Traes_XX_3C69724A2.1	LRR receptor ser-thre-kinase	Programmed cell death; HR
AX-94806968	lcl|Traes_2DL_42C74A0D6.1	Xyloglucan ETG hydrolase 26	Cell wall biogenesis
AX-94490173	lcl|Traes_6AL_E967F4C5D.1	Xyloglucan ETG hydrolase 30	Cell wall biogenesis
AX-95171223	lcl|Traes_1BS_DB9A576D4.1	RNA-binding 34	Cell wall biogenesis
AX-94518139	lcl|Traes_1DS_3B91A8AF9.1	RNA-binding 34	Cell wall biogenesis

AX-95085078	lcl|Traes_1DS_3B91A8AF9.1	RNA-binding 34	Cell wall biogenesis
AX-94567843	lcl|Traes_5DS_B58E43114.1	GDP-mannosyltransferase	Cell wall biogenesis
AX-94882016	lcl|Traes_3AL_9EFDF544B.1	Glucuronosyltransferase	Secondary Cell wall biogenesis
AX-94472137	lcl|Traes_3B_29EA1B8AF.1	cellulose synthase A catalytic	Secondary Cell wall biogenesis
AX-95221938	lcl|Traes_3B_953DE91A0.1	Xyloglucan glycosyltransferase3	cell wall organization
AX-95154188	lcl|Traes_5BL_71D5394B1.1	xyloglucan glycosyltransferase 9	cell wall organization
AX-94906933	lcl|Traes_5BL_71D5394B1.1	xyloglucan glycosyltransferase 9	cell wall organization
AX-94655564	lcl|Traes_7BS_7712933C1.1	casparian strip membrane 3	cell wall organization (CWO)
AX-94987788	lcl|Traes_4DS_6D60BF8CD.1	Polygalacturonate 4-alpha-GT	Pectin biosynthesis; CWO
AX-94906737	lcl|Traes_5BL_F393A3875.1	Polygalacturonate 4-alpha-GT	Pectin biosynthesis; CWO
AX-94962557	lcl|Traes_2BS_80CC51D52.1	Cellulose synthase	Cellulose biosynthesis; CWO
AX-95209232	lcl|Traes_2BS_F0846FCC2.1	Cellulose synthase	Cellulose biosynthesis; CWO
AX-94773579	lcl|Traes_2DS_114C3E73E.1	Cellulose synthase	Cellulose biosynthesis; CWO
AX-95174829	lcl|Traes_2DS_114C3E73E.1	Cellulose synthase	Cellulose biosynthesis; CWO
AX-95181534	lcl|Traes_4AL_B5DA1307A.1	CASP	Cellulose biosynthesis; CWO
AX-95079927	lcl|Traes_6BL_38857B770.1	Cellulose synthase E2	Cellulose biosynthesis; CWO

Table 2.8: Annotated genes associated with plant growth and development processes

SNP ID	IWGSC Sequence Hit	Annotated Growth Gene	Annotated Function
AX-94596445	lcl|Traes_2DS_5FC457D47.1	lactoylglutathione lyase	Seed germination
AX-95631688	lcl|Traes_1AS_2D57359FC.1	Zinc finger CCCH-G patch domain	Gravitropism
AX-95211457	lcl|Traes_1AS_838E31709.1	D111 G-patch domain-family	Gravitropism
AX-94406752	lcl|Traes_6BL_8BE7AE308.1	lysine histone demethylase1-3	Cotyledon development
AX-94715813	lcl|Traes_6BL_C9B82742C.1	lysine histone demethylase1-1	Root development
AX-94509994	lcl|Traes_5DL_89190A61A.1	Long chain acyl- synthetase 2	Lateral root; shoot wax development
AX-95223107	lcl|Traes_XX_8B2605152.1	Penta3peptide mitochondrial	Photomorphogenesis
AX-94487713	lcl|Traes_5AL_B0DA241FA.1	calmodulin binding	Leaf morphogenesis
AX-94905933	lcl|Traes_7AS_34AF6845D1	F-box LRR-repeat MAX2 homolog	Shoot morphogenesis
AX-94502695	lcl|Traes_6AL_8412DC639.1	receptor-like ser-threo ALE2 X1	Shoot and cuticle development
AX-95088095	lcl|Traes_3DL_348F9FA7E.1	stomata closure actin-binding 1	stomatal closure
AX-94549735	lcl|Traes_4AS_768089976.1	Retinoblastoma-binding 5	Veg/reproductive transition
AX-94764856	lcl|Traes_6DL_6149F16FF.1	SNW SKI-interacting	Veg/Reproductive transition
AX-94753558	lcl|Traes_5AL_E32D685B5.1	BAG chaperone regulator 6-X2	Veg/reproductive transition
AX-94480941	lcl|Traes_7AS_07B4F6D56.1	Myb O	Veg/reproductive transition
AX-95223925	lcl|Traes_3AS_647411E39.1	Histone-lysine N-CH3transferas ATX2	Veg/Rep transition; SiRNA in RNAi
AX-95204727	lcl|Traes_2DS_7A7CEA0F6.1	tyrosyl-DNA phosphodiesterase 1-X1	Veg/reproductive transition
AX-95219146	lcl|Traes_5DL_E82D6D246.1	cellulose synthase A catalytic 9	Xylem development; V/R transition
AX-94400545	lcl|Traes_4AL_27EA1105D.1	1-PPDL-4-phosphate 5-kinase	Flower development
AX-94572866	lcl|Traes_1DS_989E741CF.1	Gamma-tubulin complex sub-4	Reg of flower development
AX-94514118	lcl|Traes_1DS_78C10527F.1	Penta3peptide mitochondrial	Reg of flower development
AX-94880760	lcl|Traes_2BS_8506C57C5.1	serine threonine- kinase TIO	Reg of flower development
AX-94866715	lcl|Traes_5DL_AFE0A4571.1	Flowering time control FPA	Flowering Time
AX-95223960	lcl|Traes_5DL_0C788D94B.1	flowering time control FY	Flowering Time
AX-95118036	lcl|Traes_1DL_96D83DE2D.1	early flowering 3-B1	Flowering Time
AX-94476686	lcl|Traes_5DS_4EF52E6D5.1	nucleolar complex 2 homolog	Floral meristem determination

AX-95215369	lcl|Traes_XX_99715DADF.1	Aldose reductase	Flowering; photoperiod; Vernalization
AX-95016265	lcl|Traes_3B_99B90170D.1	meiosis 5	Meiotic division
AX-94452159	lcl|Traes_3B_2EF6B03AC.1	Ornithine carbamoyltransferase	Meiosis Processes
AX-94895903	lcl|Traes_XX_842E6419D.1	RAFTIN 1	Pollen Development
AX-94858790	lcl|Traes_XX_180E57BE9.1	guanine nucleotide exchange F1	Pollen Tube growth regulation
AX-94858312	lcl|Traes_5BL_BB3E54A34.1	4ketide alphapyrone reductase 1	Sporopollenin formation
AX-94936563	lcl|Traes_5DL_E491F9C9B.1	4ketide alphapyrone reductase 1	Sporopollenin formation
AX-94636419	lcl|Traes_5AL_F4A33E39D.1	Defective in Exine Formation 1	Pollen Exine formation
AX-94736177	lcl|Traes_3B_6D1E9BFCD.1	pollen Ole e1 allergen partial	Pollen allergen protein
AX-94804508	lcl|Traes_2BL_919DD0DC3.1	Ser-threonine- kinase receptor	Pollen recognition
AX-94839262	lcl|Traes_7DS_80059151A.1	G lectin S-receptor ser-threo At2g19130	Pollen recognition
AX-95096480	lcl|Traes_2BS_40572FD0B.1	cellulose synthase D4	Pollen germination
AX-94400181	lcl|Traes_2AS_0F4EA930D.1	Asparagine--tRNA ligase	Ovule development
AX-95018779	lcl|Traes_7DL_FCD7F6E8A.1	SLOW WALKER 1	Megagametogenesis
AX-94802908	lcl|Traes_2AS_FA3F483D6.1	SUMO-activating enzyme sub 2	Embryo Development
AX-95143232	lcl|Traes_XX_CBABC5E213.1	eukaryotic translation initiation F6-2	Embryo Development
AX-95147560	lcl|Traes_5AL_CD19FF15F.1	monogalactosyldiacylglycerol synthase	Embryo Development
AX-94399553	lcl|Traes_4BL_4C6830129.1	OBERON 4-like	Embryonic pattern specification
AX-95122517	lcl|Traes_5DL_A24584DE2.1	OBERON 2	Embryonic pattern specification
AX-94583506	lcl|Traes_1AL_8E1027E44.1	periodic tryptophan 2 homolog	Embryo Sac Development
AX-94787647	lcl|Traes_1BS_51AEF862E.1	DEAD-box ATP-RNA helicase 17	Embryo sac egg cell develop

Table 2.9: Annotated genes associated with biotic and abiotic tolerance mechanisms

SNP ID	IWGSC Sequence Hit	Annotated Tolerance Gene	Annotated Function
AX-95020885	lcl|Traes_2BS_821123BD4.1	E3 ubiquitin- ligase BAH1- 1	SAR; HR response to bacteria
AX-94640027	lcl|Traes_2AS_0C40A33F9.1	importin subunit alpha-1a-X1	HR response; programmed cell death
AX-94804196	lcl|Traes_6BL_DF9519C97.1	alpha-1,3-m-g 2-beta-N-acetyl glucosaminyltransferase X1	Hyperosmic response
AX-95180377	lcl|Traes_XX_D8C919B5D.1	USP family	General stress response
AX-94905933	lcl|Traes_7AS_34AF6845D1.1	F-box LRR-repeat MAX2	Drought stress
AX-95010896	lcl|Traes_7DL_3632B8F7B.1	endoplasmin homolog	Drought and salt stress
AX-94592974	lcl|Traes_1BL_EC48F290A.1	folate-biopterin transporter 7	Drought and cold
AX-94596445	lcl|Traes_2DS_5FC457D47.1	lactoylglutathione lyase	Response to freezing
AX-95017610	lcl|Traes_6AL_AC87E1B0B.1	alcohol dehydrogenase-like 6	Response to wounding
AX-94395797	lcl|Traes_5DL_CB157FB12.1	glutathione S-transferase T3	Response to toxicity
AX-94489717	lcl|Traes_3AS_85329195A.1	MLO 1	Defence Response to biotic stimuli
AX-94457592	lcl|Traes_3B_0B4A5999D.1	Cinnamyl-alcohol dehydrogenase	Systemic Aquired Resistance
AX-94395993	lcl|Traes_1BS_8A297503E.1	Universal stress A	Response to Fungus
AX-94733734	lcl|Traes_1DS_9927F6B6E.1	Universal stress A	Response to Fungus
AX-94520810	lcl|Traes_2BL_BD8DBA189.1	low-molecular-weight cysteine-rich LCR69 precursor	Response to Fungus
AX-94509994	lcl|Traes_5DL_89190A61A.1	Long chain acyl- synthetase 2	Response to Fungus and insects
AX-94687148	lcl|Traes_5BL_DC6216475.1	Enhanced Downy Mildew 2	Downy Mildew
AX-94849392	lcl|Traes_1DS_5FF8D9E2D.1	stripe rust resistance YR10	Stripe Rust YR10 gene
AX-95078562	lcl|Traes_5DL_B623328B1.1	Leaf Rust 10 Disease-Resistance locus receptor Protein Kinase	Leaf Rust resistance
AX-94646539	lcl|Traes_2BS_37A5902AE.1	Disease resistance RPM1	Defence response; Apoptosis

AX-94927423	lcl|Traes_3B_1B523D215.1	Disease resistance RPM1	Defence response; Apoptosis
AX-95255993	lcl|Traes_2AS_534751FCA.1	disease resistance RPM1	Defence response; Apoptosis
AX-95232967	lcl|Traes_3B_B2DF123991.1	Disease resistance RPM1	Defence response; Apoptosis
AX-94521803	lcl|Traes_3B_EACD6AB3F.1	Disease resistance RPM1	Defence response; Apoptosis
AX-94950579	lcl|Traes_7DL_E82F3FAE6.1	Disease resistance RPM1	Defence response; Apoptosis
AX-94587682	lcl|Traes_XX_1287F0C30.1	Disease resistance RPM1	Defence response; Apoptosis
AX-95654169	lcl|Traes_2DL_04714FD93.1	disease resistance RPM1-like	Defence response; Apoptosis
AX-94539094	lcl|Traes_6BS_F47E1B8D6.1	Disease resistance RPP13	Defence response
AX-94929285	lcl|Traes_1AL_11CA0CD021.1	disease resistance RPP13 1	Defence response
AX-94771300	lcl|Traes_2BS_AF733E06F.1	disease resistance RPP13 1	Defence response
AX-94714504	lcl|Traes_5AL_0C6FD67B4.1	disease resistance RPP13 1	Defence response
AX-94739487	lcl|Traes_5BL_45FCF6181.1	disease resistance RPP13 1	Defence response
AX-95185160	lcl|Traes_5DL_184F40820.1	disease resistance RPP13 1	Defence response
AX-95119868	lcl|Traes_7BL_C02327782.1	disease resistance RPP13 1	Defence response
AX-95077961	lcl|Traes_5AL_887144AB5.1	disease resistance RPP13-like	Defence response
AX-95227700	lcl|Traes_5BL_502C0C0E7.1	disease resistance RPP13-like	Defence response
AX-94624138	lcl|Traes_6BL_CA144B632.1	disease resistance RPP13-like	Defence response
AX-94538909	lcl|Traes_1DS_EFA1BC727.1	disease resistance RPP13 3	Defence response
AX-94749619	lcl|Traes_7AS_3E7C4250A.1	disease resistance RPP13 3	Defence response
AX-94807936	lcl|Traes_5DL_EFD502314.1	disease resistance RPP13 2	Defence response
AX-94768083	lcl|Traes_5BL_964089395.1	disease resistance RPP13 3	Defence response
AX-94999037	lcl|Traes_5DL_B6AAC8E52.1	disease resistance RPP13 3	Defence response
AX-94532962	lcl|Traes_3B_D434789FF.1	disease resistance At4g19050 X1	Defence response
AX-94724718	lcl|Traes_5DS_3589CEE81.1	disease resistance RGA1	Defence response
AX-94878145	lcl|Traes_6BL_190F6DF901.1	disease resistance RGA3 X1	Defence response
AX-95164323	lcl|Traes_6BL_190F6DF901.1	disease resistance RGA3 X1	Defence response
AX-94442619	lcl|Traes_7AL_5117186E1.1	Enhanced Disease Resistance 2	Defence response
AX-94905502	lcl|Traes_5BL_5A815D611.1	F-box CPR30-like isoform X1	Defence response

Table 2.10: Annotated genes associated with genetic and epigenetic processes

S. No.	Annotated Genetic and Epigenetic Processes	Annotated SNPs
1	Chromatin remodeling, modification, silencing	6
2	DNA and RNA helicases	14
3	Nucleotide excision and mismatch repair	11
4	DNA replication and DNA biosynthesis	9
5	Transcription activation, regulation and modification	34
6	Translation initiation, elongation and termination	31
7	DNA, tRNA and rRNA methylation	13
8	Histone-lysine methylation	4
9	Spliceosomal complexes, RNA splicing & modification	31
10	RNA based gene silencing	6
11	Nucleic acid metabolism and nucleotide transfer	6
12	hydrolase activity, nucleic acid, DNA and RNA binding	33
	Total	198

2.3.11. Functionally annotated genes for metal ion binding and cellular components

Another 160 SNPs were located within genes responsible for Ca, Fe, Mg Zn and metal ion binding activities, and structural molecules that were part of different cellular organelles such as the plasma membrane, vesicles, endosomes, Golgi bodies, endoplasmic reticulum, mitochondrion, plastids, nucleus and cell wall (**Table 2.11**).

2.3.12. Functionally annotated genes for functional genes

In total, 170 SNPs were found within genes having enzymatic activity (transferases, ligases, hydrolases), in particular; genes for kinases carried 77 SNPs. Meanwhile, other SNPs were closely related to post-translational modifications, such as phosphorylation, protein localization, phosphorylation and glycosylation (**Table 2.12**).

2.3.13. Functionally annotated genes for metabolic pathways

The largest group of 275 SNPs were located on genes for various metabolic processes like photosynthesis, protein, lipid and sugar biosynthesis; oxidation-reduction processes, carbohydrate metabolism, respiration processes, the Krebs cycle, protein metabolism and catabolism, ROS scavenging and antioxidant activities (**Table 2.13**).

Table 2.11: Annotated genes associated with ion biding and cell organelles

S. No.	Annotated Ion Binding and Cellular Components	Annotated SNPs
1	Zn ion binding	18
2	Ca ion binding	9
3	Mg ion binding and metabolism	5
4	Iron and metal ion binding	19
5	Membrane component	37
6	Extracellular matrix	2
7	Vesicle	17
8	Microtubule motor and cytoskeleton	9
9	Vacuole organization; Endosome; Trans-Golgi Network	4
10	Endoplasmic Reticulum	6
11	Mitochondrion	6
12	Plastid	20
13	Nucleus	3
14	Cell Wall	5
	Total	160

Table 2.12: Annotated genes associated with functional proteins and enzymes

S. No.	Annotated Functional Proteins	Annotated SNPs
1	Protein serine/threonine kinase; Phosphorylation	40
2	Kinase activity; Phosphorylation	35
3	Hydrolase activity	16
4	Actin/Protein binding	11
5	Transferase activity	10
6	Protein dephosphorylation	9
7	Peptide signal processing	4
8	Heat shock proteins	8
9	Catalytic activity	8
10	Post-translational protein modification	7
11	Protein glycosylation	7
12	ADP/ATP binding	5
13	Ligase activity; metal ion binding	4
14	Isomerase activity	2
15	Protein tyrosine kinase; Phosphorylation	2
16	Protein localization	2
	Total	170

Table 2.13: Annotated genes associated with cellular metabolic pathways

S. No.	Annotated Metablic Pathway	Annotated SNPs
1	Chloroplast fission, Chloroplast avoidance movement	9
2	Chloroplast envelope, thylakoid membrane and stroma	11
3	Photosystem reaction centre	2
4	Chlorophyll biosynthesis, catabolism and energy pathway	10
5	Electron transport chain; cell redox homeostasis	13
6	Chloroplastic DNA synthesis, replication and translation	3
7	Synaptonemal complex	3
8	NADP biosynthesis; Removal of superoxide radicals	4
9	Sucrose, Glactose, Glucose, Mannose & Malate metabolism	12
10	Nucleotide-sugar and D-glucose metabolism	6
11	Carbohydrate metabolism	25
12	Monooxigenase activity; Oxidation-reduction process	46
13	Kreb cycle; Mg ion binding	5
14	Respiratory chain complexes, photorespiration & glycolysis	9
15	Aromatic compound and amino acid biosynthesis	13
16	Proteosome based protein catabolism	8
17	Ubiquitin-dependent protein catabolism	29
18	Proteolysis based protein catabolism	12
19	Amino acid catabolism	7
20	Fatty acid/lipid biosynthesis and catabolism	22
21	Glutathione, Flavonoid, Carboxylic acid, Lactate, cytokinin, Vitamin B and E based antioxidant response	19
22	Metabolism Enzymes	7
	Total	275

2.3.14. *In silico* expression analysis of annotated genes

Gene expression analysis of 1257 annotated genes showed that 122 genes were up-regulated, 136 genes showed down-regulation during salt stress, while 156 genes were expressed under salt stress only.

2.3.14.1. Differentially expressed ion channels, transporters and signaling genes

In total 46 genes for transporters and signaling molecules were expressed. The genes for K, nitrate, sulphate, drug, ABA, mRNA, proteins, anions, cations, ions, auxin and transmembrane transport were differentially expressed under salt stress. Similarly, genes for auxin, ABA, G-protein, ER-nucleus, ethylene signaling, signal initiation and transduction were also expressed under salinity (**Table 2.14**).

2.3.14.2. Differentially expressed ion channels, transporters and signaling genes

A total of 42 genes including NAC78, SPT20, GT4 and BBP TFs and functional proteins including several protein serine/threonine kinases, protein kinases, protein glycosylation, phosphorylation, catalytic enzymes, phospholipid binding, hydrolase and transferases were differentially expressed under salt stress (**Table 2.15**).

2.3.14.3. Differentially expressed genic and epigenetic factors under salinity

The 37 genic and epigenetic-related genes differentially expressed under salt stress were for RNA binding, chromatin modification and re-modeling, DNA replication/biosynthesis, helicases, transcription regulation, translation initiation and termination, splicosomal complex and splicing, Glycine--tRNA and DNA ligases, DNA repair and RNA-based gene silencing (**Table 2.16**).

2.3.14.4. Differentially expressed metal ion binders and cellular components

A total of 27 genes for these groups were differentially expressed under salt stress that coded for Ca, Mg, Zn and general metal ion binding, Fe S cluster binding during aerobic respiration, membrane components and protein targeting microtubule motor, plastid, vesicles, extracellular matrix, mitochondrion etc. (**Table 2.17**).

2.3.14.5. Differentially expressed growth and stress related genes and hypothetical proteins

In total 46 genes for these groups were differentially expressed under salt stress that coded for embryo development, pollen recognition, hypersomic response, xylem and pollen development, gravitropism, vegetative to reproductive transition, embryonic pattern specification, systemic acquired resistance, response to fungus, secondary cell wall biogenesis, response to cold and drought, flower development, regulation of cell cycle, defense response, cellulose biosynthesis, stripe rust resistance, multicellular organization development, cell wall organization and hypothetical, uncharacterized and predicted proteins (**Table 2.18**).

Table 2.14: Differentially expressed genes and their associated SNPs for ion channels, transporters, and signaling molecules under salt stress in wheat

Annotated Gene	Linked SNP	Annotated Function	FEC
transparent testa 12	AX-94871419	Drug transport	4.92
TRANSPARENT TESTA 12-like	AX-94943274	Drug transport	4.46
transport SEC13 homolog B-like	AX-94960858	Protein; mRNA transport	4.31
transport SEC13 homolog B-like	AX-94641362	Protein; mRNA transport	4.31
phospholipase D delta	AX-94537482	ABA activated Signalling pathway	3.69
Ran-specific GTPase-activating 2	AX-95075429	Protein transport from nucleus	3.38
Ran-specific GTPase-activating 2	AX-95000586	Protein transport from nucleus	3.38
Ran-specific GTPase-activating 2	AX-95235622	Protein transport from nucleus	3.38
Pathogenesis-related 1	AX-94934089	ABA activated Signalling pathway	3.38
casein kinase family	AX-94445537	Wnt Signalling pathway	3.38
Proton-exporting ATPase	AX-94982994	Proton transport	2.77
mechanosensitive ion channel X1	AX-94909932	Ions transport	2.77
auxin transport BIG	AX-95115269	Auxin Signaling and polar transport	2.77
Nitrate transporter	AX-94852973	Nitrate transport	2.46
WPP domain-associated	AX-94583481	Cations transport	2.46
Two-component PRR73	AX-94610182	Signal transduction	2.46
Cell division cycle 5	AX-95651903	Defence response signalling pathway	2.31
Structural maintenance of chr 4	AX-94445422	Transmembrane transport	2.15
ABC transporter B 11	AX-95204844	Auxin efflux transport	2.15
ABC transporter B 11	AX-94635873	Auxin efflux transport	2.15
auxin response factor 5-like	AX-95256931	Auxin-activated signaling pathway	2.15
F-box only 6	AX-95236314	Auxin-activated signaling pathway	2.15
Peroxisomal targeting signal 1 receptor	AX-94701908	Response to Auxin	2.15
Diacylglycerol kinase 1	AX-94489010	G-Protein coupled signalling	2.15
TOM1 2	AX-95103748	Intracellular protein transport	2.00
ABC transporter B 11	AX-95014459	Auxin efflux transport	2.00
dnaJ ERDJ3B-like	AX-94987830	ER-nucleus signaling Pathway	2.00
serine threonine- kinase D6PK	AX-94628613	Intracellular signal	2.00
Serine threonine- kinase svkA	AX-94402263	Receptor/Phosphorylation signaling	0.46
CBL-interacting kinase 17	AX-94797832	Intracellular signal	0.46
ABC transporter B 4	AX-94707625	Auxin signaling/efflux/influx; Salt tolerance	0.45
molybdate-anion transporter-like	AX-94550729	Anions transport	0.43
Retrovirus-related Pol poly line-1	AX-94515411	Protein to vacuole transport	0.31
probable polyol transporter 4	AX-95090777	Proton/Glucose transport	0.31
ethylene receptor	AX-94970016	Ethylene-activated signalling	0.31
large G nucleotide-binding 1	AX-94731801	G-Protein coupled signalling	0.31
signal recognition particle SRP72	AX-94842052	Signal recognition	0.31
ser/threo phosphatase 2A 57 kDa	AX-95115431	Signal transduction	0.25
ser/threo phosphatase 2A 57 kDa	AX-95631372	Signal transduction	0.25
equilibrative nucleotide transporter 1	AX-94852257	Nucleoside transporter	0.22
ABC transporter B 11	AX-94464561	Auxin efflux transport	0.21
outward-rectifying K channel	AX-94699167	K transport	0.21
Sulfate transporter	AX-95142803	Sulphate transport	0.15
equilibrative nucleotide transporter 1	AX-94649598	Nucleoside transporter	0.13
laminin subunit beta-1 isoform X2	AX-95195424	Endocytosis	0.10

Table 2.15: Differentially expressed genes and their associated SNPs for functional proteins, enzymes, and transcription factors under salt stress in wheat

Annotated Gene	Linked SNP	Annotated Function	FEC
Trehalose-phosphatase	AX-94727470	Trehalose biosynthesis; dephosphorylation	5.08
serine threonine- kinase	AX-94837095	Protein Kinase; Phosphorylation	3.69
GDSL esterase lipase At5g45910	AX-95015996	Hydrolase activity	2.92
PTI1-like tyrosine- kinase 3	AX-94408063	Protein tyrosine kinase; Phosphorylation	2.77
LRR receptor Ser/Threo RPK2	AX-95002541	Protein Ser/Thre kinase; Phosphorylation	2.77
serine threonine- kinase CDL1like	AX-94771710	Protein Ser/Thre kinase; Phosphorylation	2.77
LRR receptor-like Ser/Thre-PK2	AX-94519749	Protein Ser/Thre kinase; Phosphorylation	2.77
GDSL esterase lipase	AX-94718756	Hydrolase activity	2.77
NAC78 TF	AX-94757955	Transcription regulation	2.62
phosphatidylinositol 4-kinase gamma7	AX-94848821	Kinase activity; Phosphorylation	2.46
sphingosine kinase 1-like	AX-94448613	Kinase activity; Phosphorylation	2.46
PTI1-like tyrosine- kinase 3	AX-94491964	Protein tyrosine kinase; Phosphorylation	2.46
Tubby-like F-box 7	AX-94485190	Protein localization	2.46
Beach domain-contain Protein C2	AX-95219995	Phospholipid binding	2.26
Purple acid phosphatase 3	AX-95129444	Dephosphorylation	2.23
Heat shock 70 kDa mitochondrial	AX-94806865	HSP; unfolded protein binding	2.19
ARF5-like TF	AX-95256931	TF activity, Auxin signalling	2.15
NAC78 TF	AX-94653461	Transcription regulation	2.15
receptor kinase At2g42960	AX-95214734	Protein Ser/Thre kinase; Phosphorylation	2.15
receptor kinase At2g42960	AX-95161581	Protein Ser/Thre kinase; Phosphorylation	2.15
receptor kinase At2g42960	AX-94664571	Protein Ser/Thre kinase; Phosphorylation	2.15
Zn finger CCCH domain-19-X2	AX-95082229	Protein glycosylation	2.05
ADP-ribosylation GTPase-activating AGD3	AX-94637605	Protein Kinase; Phosphorylation	2.00
pantothenate kinase 2	AX-94412585	Pantothenate kinase; Phosphorylation	0.48
Salt tolerance 1	AX-95652714	Response to salinity, TF activity, Zn^{+2} binding	0.46
Molybdenum cofactor sulfurase	AX-94831861	Catalytic activity	0.46
Molybdenum cofactor sulfurase	AX-95229410	Catalytic activity	0.46
Translocase of chloroplast	AX-94767652	Hydrolase activity; GTP binding	0.46
Heat shock 70 kDa 4L	AX-94660066	ATP binding; Heat shock protein	0.41
ABC1-like partial	AX-94425541	Protein Kinase; Phosphorylation	0.31
receptor-Ser/Thre kinase ALE2	AX-94507617	Protein Ser/Thre kinase; Phosphorylation	0.31
LRR receptor Ser/Thre-At1g67720	AX-94521484	Protein Ser/Thre kinase; Phosphorylation	0.31
proline-rich receptor kinase PERK8	AX-95081630	Protein Ser/Thre kinase; Phosphorylation	0.31
Ankyrin repeat & FYVE domain1	AX-94412955	Protein glycosylation; metal ion binding	0.31
isoform X2	AX-94421807	Catalytic activity	0.31
BBP TF	AX-94641302	mRNA splicing; RNA polymerase II TF	0.21
receptor kinase	AX-94766336	Protein Ser/Thre kinase; Phosphorylation	0.21
serine threonine- kinase HT1-like	AX-94996868	Protein Ser/Thre kinase; Phosphorylation	0.21
SPT20 TF	AX-94413608	Mitochondrion based reaction	0.15
GT4 TF	AX-94690681	Vesicle transport	0.15
pentatricopeptide repeat-At1g10270	AX-94683572	Protein glycosylation	0.15
Sulfotransferase 16	AX-94785611	Sulfotransferase activity	0.15

Table 2.16: Differentially expressed genes and their associated SNPs for genic and epigenetic factors under salt stress in wheat

Annotated Gene	Linked SNP	Annotated Function	FEC
pumilio homolog 1-like	AX-94892657	RNA binding	4.31
MODIFIER OF SNC1 1	AX-94731008	Chromatin modification	3.18
chromatin-remodeling complex ATPase	AX-94438918	Chromatin remodeling by NURF complex	2.92
STICHEL-like isoform X1	AX-94644710	DNA replication factor C; DNA biosynthesis	2.77
U5 nuclear ribonucleo 200 kDa helicase	AX-94679404	Helicase activity	2.46
Squamosa promoter-binding 6	AX-94767736	Transcription regulation	2.46
translational activator GCN1	AX-94914919	Translation activation	2.36
Fanconi anemia group J	AX-95630232	DNA duplex unwinding; DNA helicase	2.15
eukaryote translation initiation factor3D	AX-94972660	Regulation of translation initiation	2.15
Leucine-rich repeat receptor kinase At5g49770	AX-94975845	rRNA processing	2.15
tRNA pseudouridine synthase	AX-94411823	RNA binding; pseudouridine synthesis	2.15
PLATZ transcription partial	AX-95069755	Transcription regulation	2.00
DNA polymerase V	AX-95252437	Transcription; DNA biosynthesis	2.00
splicing factor 3B subunit 1-like	AX-94623475	Splicosomal complexes	0.48
calcium binding partial	AX-94880269	Helicase; Nucleotide, ATP and Ca binding	0.46
mediator complex sub 10 CG5057-PA	AX-94419780	Transcription regulation	0.46
glycine--tRNA ligase mitochondrial	AX-94668553	Glycine--tRNA ligase	0.46
splicing factor 3B subunit 3-X1	AX-94485356	mRNA splicing	0.46
DNA ligase 1-like	AX-94390275	DNA ligase (ATP)	0.41
argonaute 2-like	AX-94463649	RNA based gene silencing	0.41
PRKR-interacting 1	AX-94643687	dsRNA binding	0.38
titin homolog	AX-95209627	Chromatin silencing by sRNA	0.36
tyrosyl-DNA phosphodiesterase 1-X1	AX-95204727	DNA repair; translation initiation; Veg/Rep transition	0.31
RNA polymeraseII transcription coactivator KIWI	AX-95073250	Transcription coactivator; transcription regulation	0.31
mTERF family	AX-95074821	Transcription regulation	0.31
growth-regulating factor 11-like	AX-95126011	Transcription regulation; development	0.31
Eukaryote translation initiation factor 3K	AX-95014588	Translation initiation factor and regulator	0.31
30S ribosomal S1	AX-95230097	Translation; ribosomal structural component	0.31
Peptide chain release factor 1	AX-94552967	Translation release or termination factor	0.31
U1 small nuclear ribonucleo 70 kDa	AX-94925858	Precatalytic spliceosome; mRNA splicing;	0.31
argonaute 1D	AX-94765773	RNA based gene silencing	0.31
argonaute 1D	AX-95230022	RNA based gene silencing	0.31
U4 U6 nuclear ribonucleo Prp31	AX-94408000	precatalytic spliceosomal complex assembly	0.18
ATP-depend RNA helicase DHX8	AX-94661891	Helicase activity	0.15
calcium binding partial	AX-95026221	Helicase; Nucleic acid, ATP and Ca binding	0.15
pumilio homolog 1-like	AX-94834230	RNA binding	0.15
STICHEL-like 3 isoform X1	AX-95166641	DNA replication factor C; DNA biosynthesis	0.12

Table 2.17: Differentially expressed genes and their associated SNPs for metal and ion binders; and cellular components under salt stress in wheat

Annotated Gene	Linked SNP	Annotated Function	FEC
epidermal growth factor receptor substrate 15	AX-95206919	Ca ion binding	4.62
erythroid differentiation-related factor 1	AX-94590370	Membrane component	3.69
Kinesin KIF22	AX-95010121	Microtubule motor	3.69
quinolinate chloroplastic isoform X3	AX-95629102	Fe S cluster binding; aerobic respiration	3.38
Ser/Threo-phosphatase 6 regulatory subunit-3	AX-94625794	Vesicle	2.92
hydroxyproline-rich glyco -like	AX-95109768	Membrane component	2.77
calnexin homolog	AX-94449290	Ca ion binding	2.67
transmembrane 64	AX-95199834	Membrane component	2.15
UV-B-induced chloroplastic-like	AX-95183268	Plastid	2.15
enhancer of mRNA-decapping 4-like	AX-95630712	Plastid	2.00
calcium binding partial	AX-94880269	Nucleic acid, ATP & Ca binding	0.46
auxilin-related 2-like	AX-95653825	Membrane component	0.46
70 kDa peptidyl-prolyl isomerase	AX-95202783	ER membrane; Protein folding	0.41
E3 ubiquitin- ligase RFWD3-like	AX-95167772	Zn ion binding	0.31
E3 ubiquitin- ligase RFWD3-like	AX-94793259	Zn ion binding	0.31
squamosa promoter-binding 12	AX-94977405	Metal ion binding	0.31
tesmin TSO1 CXC domain containing	AX-95657302	Membrane component	0.31
WW domain-containing oxidoreductase	AX-94968296	Membrane component	0.31
Coatomer subunit beta -1	AX-94679692	Membrane component	0.31
dentin matrix acidic phospho 1-X2	AX-95085280	extracellular matrix	0.31
dentin matrix acidic phospho 1-X2	AX-94966306	extracellular matrix	0.31
endochitinase A-like	AX-95179399	Vesicle	0.31
AP-5 complex subunit beta- partial	AX-95106206	Vesicle; membrane coat adaptor	0.31
acyl-binding domain-containing 5-like	AX-94893329	Mitochondrion	0.31
calcium binding partial	AX-95026221	Nucleic acid, ATP & Ca binding	0.15
pentatricopeptide repeat-cont mitochondrial	AX-94437015	Plastid	0.15
Bifunctional riboflavin kinase FMN phosphatase	AX-94383420	Mg+2 binding; Vita B biosynthesis	0.10

Table 2.18: Differentially expressed genes and their associated SNPs for growth and stress responses under salt stress in wheat

Annotated Gene	Linked SNP	Annotated Function	FEC
eukaryote translation initiate factor 6-2	AX-95143232	Embryo development	5.54
alpha-1,3-m-g 2-beta-N-acetylglucosaminyltransferaseX1	AX-94804196	Hyperosmic response	4.00
stomatal closure-related actin-binding 1	AX-95088095	stomatal closure-actin-binding1	2.77
cellulose synthase A catalytic sub 9	AX-95219146	Xylem development, Veg/Rep transition	2.69
RAFTIN 1	AX-94895903	Pollen Development	2.56
Zinc finger CCCH-type with G patch domain-contain	AX-95631688	Gravitropism	2.46
SNW SKI-interacting	AX-94764856	Vegetative to reproductive transition	2.46
hypothetical protein F775_09600	AX-94484517	mRNA processing regulation; stress granule assembly	2.46
hypothetical protein TRIUR3_31150	AX-95167695	Plastid	2.46
OBERON 4-like	AX-94399553	Embryonic pattern specification	2.36
cellulose synthase A catalytic subunit 4	AX-94472137	Secondary Cell wall biogenesis	2.35
1-phosphatidylinositol-4-phosphate 5-kinase	AX-94400545	Flower development	2.15

hypothetical protein F775_04744	AX-94531833	plastid; integral membrane component	2.15
UDP-galactose transporter 1	AX-94881482	Regulation of cell cycle	2.00
disease resistance RPP13 1	AX-94771300	Defence response	2.00
Uncharacterized protein ycf45	AX-94682476	chloroplast; ATP binding; mitochondrion	2.00
Uncharacterized protein ycf45	AX-95631185	chloroplast; ATP binding; mitochondrion	2.00
eyes absent homolog 4	AX-94711916	Multicellular organization development	0.46
CASP	AX-95181534	Cellulose biosynthesis; cell wall organization	0.46
Disease resistance RPM1	AX-94950579	Defence response; Apoptosis	0.46
Universal stress A	AX-94395993	Response to Fungus	0.38
Universal stress A	AX-94733734	Response to Fungus	0.38
disease resistance RPP13 1	AX-95119868	Defence response	0.38
tyrosyl-DNA phosphodiesterase 1-X1	AX-95204727	DNA repair; translation initiation; Veg/Rep transition	0.31
Subtilisin-like protease	AX-94715752	Cell wall	0.31
serine threonine- kinase TIO	AX-94880760	Regulations of flower development	0.31
serine threonine- kinase receptor	AX-94804508	Pollen recognition	0.31
E3 ubiquitin- ligase BAH1-like 1	AX-95020885	SAR; HR; Response to bacteria	0.31
folate-biopterin transporter 7	AX-94592974	Drought and cold response	0.31
stripe rust resistance YR10	AX-94849392	Stripe Rust YR10 gene	0.31
disease resistance RPP13 3	AX-94538909	Defence response	0.31
predicted protein	AX-94655060	glucose catabolism; integral membrane component	0.31
UPSTREAM OF FLC isoform X1	AX-94889934		0.31
F-box kelch-repeat At1g30090	AX-94996973		0.31
xyloglucan glycosyltransferase 9	AX-95154188	cell wall organization	0.22
xyloglucan glycosyltransferase 9	AX-94906933	cell wall organization	0.22
hypothetical protein TRIUR3_09865	AX-94946190	integral plasma membrane component	0.18
hypothetical protein TRIUR3_09865	AX-94861240	integral plasma membrane component	0.18
LRR receptor Ser/Threo- kinase At5g15730	AX-94497096	integral plasma membrane component	0.15
Armadillo repeat-containing 6	AX-95123732		0.15

2.3.14.6. Differentially expressed metabolic pathways related genes

The largest group of 65 metabolic genes that were differentially expressed under salt stress coded for protein desumoylation, nucleotide-sugar metabolism, oxidation reduction process, cell redox homeostasis, aerobic respiration, antioxidant molecule biosynthesis such as carboxylic acid, glutathione, Flavonoid, Vitamin B etc, long chain and unsaturated fatty acid metabolism, Sugar phosphorylation, alcohol dehydrogenase, aromatic AA metabolism, Alcohol dehydrogenase, ubiquitin – proteolysis – preotesome dependant protein catabolism, Synaptonemal complex, Kreb cycle, polysaccharide metabolism, chloroplast fission and avoidance movement, ATPase activity and electron transport chain (**Table 2.19**).

2.3.14.7. Other important but non-differentially expressed genes

A total of 74 genes including very important transporter, signaling, TFs, ion channels, genetic and metabolic processes were found to have large number of significant alignments with both the control and salt-expressed transcriptome, thus implying that they are vital for plant performance under both conditions (**Table 2.20**). Similarly, another 56 genes showed a high number of significant alignments with the salt-expressed transcriptome and need further investigation (**Table 2.21**).

Table 2.19: Differentially expressed genes and their associated SNPs for metabolic processes under salt stress in wheat

Annotated Gene	**Linked SNP**	**Annotated Function**	**FEC**
Midasin	AX-94851996	Protein desumoylation, ubiquitin release	5.23
trifunctional UDP-glucose 4,6-dehydratase UDP-4-keto-6-deoxy-D-glucose 3,5-epimerase UDP-4-keto-L-rhamnose-reductase RHM1	AX-94432552	Nucleotide-sugar metabolism	3.69
quinolinate chloroplastic isoform X3	AX-95629102	Fe S cluster binding; aerobic respiration	3.38
omega-3 fatty acid chloroplastic-like	AX-94817182	Unsaturated fatty acid biosynthesis	3.38
Glutathione S-transferase GSTU6	AX-94408415	Glutathione metabolism; Antioxidant	3.18
Sugar-phosphatase	AX-95154997	Sugar phosphorylation	2.77
O-methyltransferase ZRP4	AX-94868846	Aromatic compound biosynthesis	2.77
alcohol dehydrogenase class-3	AX-95016894	Alcohol dehydrogenase	2.77
serine decarboxylase 1	AX-95630547	Carboxylic acid metabolism; antioxidant	2.62
4-hydroxyphenylacetaldehyde oxime monooxygenase-like	AX-95184143	Electron transport chain	2.46
E3 ubiquitin- ligase PRT6	AX-94381520	Ubiquitin-dependent protein catabolism	2.46
Acyl-coenzyme A oxidase peroxisomal	AX-94749649	Long-chain fatty acid metabolism	2.36
Cysteine ase 1	AX-94659431	Proteolysis for protein catabolism	2.18
WEB family chloroplastic	AX-94950749	Synaptonemal complex	2.15
WEB family chloroplastic	AX-95199286	Synaptonemal complex	2.15
bifunctional 2	AX-94395869	Oxidation-reduction process	2.15
Peroxidase 12	AX-94777280	Oxidation-reduction process	2.15
phosphoglycerate mutase family	AX-94717107	Oxidation-reduction process	2.15
Secologanin synthase	AX-95198133	Oxidation-reduction process	2.15
NADPH HC toxin reductase	AX-94995929	Oxidation-reduction process	2.15
WD repeat-containing 6	AX-94927055	Oxidation-reduction process	2.15
26S proteasome non-ATPase regulatory sub 7A	AX-94545384	Proteosome; Protein catabolism	2.15
Protease 2	AX-94963121	Proteolysis	2.15
Beta-galactosidase 15	AX-95166572	Carbohydrate metabolism	2.05
Glycerol kinase	AX-94696230	Fatty acid beta-oxidation	2.05
Glycerol kinase	AX-95018915	Fatty acid beta-oxidation	2.05
vacuolar-processing enzyme	AX-94543147	Proteolysis for protein catabolism	2.02
fatty acid amide hydrolase	AX-94634369	Long-chain-acyl ethanolamine deacylase	2.00
Flavonoid 3 -monooxygenase	AX-95684819	Flavonoid metabolism; antioxidant	2.00
Adenosinetriphosphatase	AX-94413298	ATPase activity	0.49
2-oxoglutarate mitochondrial-like	AX-94844146	Kreb cycle	0.48
Cysteine ase inhibitor 3	AX-95140632	Protein stability	0.46
Beta-amylase	AX-95249422	Polysaccharide catabolism	0.46
benzoxazinone:UDP-Glc glucosyltransferase	AX-94460120	Flavonoid biosynthesis; antioxidant	0.42
Glutathione S-transferase F11-like	AX-95190148	Glutathione metabolism; Antioxidant	0.41
amine expressed	AX-94725002	Oxidation-reduction process	0.38
deoxyribodipyrimidine photolyase	AX-95152679	Oxidation-reduction process	0.38
deoxyribodipyrimidine photolyase	AX-95206273	Oxidation-reduction process	0.38
proteasome subunit alpha type-7-B	AX-94893418	Ubiquitin-dependent protein catabolism	0.38
12-oxophytodienoate reductase 1	AX-94957092	Oxidation-reduction process	0.34

tRNA-guanine transglycosylase	AX-94490431	Redox homeostasis	0.31
TIC 20- chloroplastic	AX-95243312	Protein import into chloroplast stroma	0.31
1-phosphatidylinositol-4-phosphate 5-kinase	AX-94888472	Carbohydrate metabolism	0.31
Glucan 1,3-beta-glucosidase	AX-94528205	Carbohydrate metabolism	0.31
Glucan 1,3-beta-glucosidase	AX-95630543	Carbohydrate metabolism	0.31
2-oxoglutarate mitochondrial-like	AX-94738192	Kreb cycle	0.31
probable O-methyltransferase 2	AX-94840887	Aromatic compound biosynthesis	0.31
ACT domain-containing ACR10	AX-95099408	Amino acid binding and metabolism	0.31
asparagine synthetase 2	AX-95025076	Amino Acid biosynthesis	0.31
26S proteasome non-ATPase regulatory sub13	AX-95069434	Proteosome; Protein catabolism	0.31
ubiquitin-conjugating enzyme E2 2	AX-95216912	Ubiquitin-dependent protein catabolism	0.31
zinc C3HC4 type family	AX-94940509	Ubiquitin-dependent protein catabolism	0.31
O-acyltransferase WSD1	AX-94548774	Triglyceride biosynthesis	0.31
Anthocyanidin 5,3-O-glucosyltransferase	AX-95226726	Flavonoid biosynthesis; antioxidant	0.31
Anthocyanidin 5,3-O-glucosyltransferase	AX-94787659	Flavonoid biosynthesis; antioxidant	0.31
lil3	AX-95628663	Endo-alpha-N-acetylgalactosaminidase	0.31
S-norcoclaurine synthase 1	AX-94614751	Oxidation-reduction process	0.21
TPR repeat-contain thioredoxin TTL1	AX-94909913	Chloroplast fission	0.15
WEB family At2g38370-like	AX-94632321	Chloroplast avoidance movement	0.15
WEB family At5g55860	AX-94987158	Chloroplast avoidance movement	0.15
TPR repeat-contain thioredoxin TTL1	AX-94596653	Cell redox homeostasis	0.15
TPR repeat-contain thioredoxin TTL1	AX-94811352	Cell redox homeostasis	0.15
allene oxide partial	AX-94639509	Oxidation-reduction process	0.15
Bifunctional riboflavin kinase FMN phosphatase	AX-94383420	Vitamin B biosynthesis	0.10

Table 2.20: Could be vital genes for plant growth both under normal and saline conditions

Annotated Gene	Linked SNP	Annotated Function	NACT	NAST
sucrose synthase	AX-95247320	Sucrose synthesis	61	347
IQ-DOMAIN 32	AX-94832924	Response to ABA	6	34
Dihydrolipoyl dehydrogenase	AX-94631903	e- transport chain; cell redox homeostasis	6	33
Histone-lysine N-methyltransferase ASHH2	AX-94561348	Histone-lysine methylation	4	21
aspartic ase oryzasin-1	AX-95629375	Proteolysis	23	118
Actin-7	AX-95073456	Cytoskeleton	8	40
NADPH--cytochrome P450 reductase	AX-94442784	Monooxigenase; Oxidation-reduction	6	30
Auxin response factor 21	AX-95128343	ARF21 transcription factor	8	39
chaperone chloroplastic	AX-94624047	Chloroplastic proteolysis	8	39
Nuclease S1	AX-94391628	Nucleic cid binding; DNA catabolism	11	51
ABC transporter B family member 21	AX-94433809	Basipetal auxin transport	14	58
ABC transporter B family member 21	AX-94667626	Basipetal auxin transport	14	58
Pyrophosphate-energized proton pump	AX-94936984	Proton Pump	17	68
tubulin alpha-1 chain	AX-94755340	Microtubuler cytoskeleton	88	349
eukaryotic translation initiation factor 5B	AX-94555977	Translation initiation factor	11	43
eukaryotic translation initiation factor 5B	AX-94654870	Translation initiation factor	11	43
eukaryotic translation initiation factor 5B	AX-94745465	Translation initiation factor	11	43
ABC transporter B family member 21	AX-94645208	Basipetal auxin transport	15	58

ABC transporter B family member 21	AX-94845848	Basipetal auxin transport	15	58
Aldehyde dehydrogenase (NAD(P)(+))	AX-94842084	Oxidation-reduction process	11	42
spindle pole body component 110-like	AX-94460113	Vesicle	8	30
O-methyltransferase ZRP4	AX-94889337	Aromatic compound biosynthesis	14	52
importin-5 isoform X1	AX-94506088	Transports Protein to nucleus	10	37
transaldolase 2	AX-94785822	Glycolytic process; Pentose-phosphate shunt	13	48
asparagine synthetase	AX-94459250	Amino acid catabolism	27	99
sucrose: fructan 6-fructosyltransferase	AX-95018710	Carbohydrate metabolism	34	123
BEL1-Like homeodomain 7; BLH7	AX-94935882	Transcription factor activity; DNA binding	12	42
zinc- peroxisomal	AX-94568269	Zn ion binding; protein processing	7	24
ATP synthase subunit mitochondrial	AX-94384299	Transports proton	22	75
TRANSPARENT TESTA 12	AX-94613383	Transports Drug	11	37
TRANSPARENT TESTA 12	AX-95085345	Transports Drug	11	37
polyamine oxidase 2	AX-95229444	Oxidation-reduction process	10	33
Peroxidase 1	AX-95236907	Oxidation-reduction process	8	26
Adenosine kinase 2	AX-94898852	Nucleic acid metabolism	16	51
BEL1-like homeodomain 7; BLH7	AX-95012249	Transcription factor activity; DNA binding	12	37
E3 ubiquitin- ligase UPL3-like	AX-94990291	Ubiquitin-dependent protein catabolism	12	37
E3 ubiquitin- ligase UPL3-like	AX-95094605	Ubiquitin-dependent protein catabolism	12	37
carotenoid 9,10-cleavage dioxygenase	AX-95072390	Oxidation-reduction process	19	58
glycerol-3-phosphate transporter 1	AX-94775993	Transports anions	8	24
nuclear-pore anchor isoform X2	AX-94937975	Transports Protein to nucleus	18	54
casein kinase I	AX-94384227	Wnt Signalling pathway	8	24
Cyclin-dependent kinase F-4	AX-94948602	Protein Ser/Threo kinase; Phosphorylation	15	45
phosphoinositide phosphatase SAC2	AX-94670884	Vacuole organization	10	30
26S proteasome non-ATPase subunit 1	AX-95109622	Proteosome; Protein catabolism	10	30
endoplasmin homolog	AX-95010896	salt and drought tolerance	32	95
microtubule-associated futsch-like	AX-94902381	Microtubule based Chitin binding	13	38
Calmodulin-binding transcription activator3	AX-94526913	Transcription activation; Ca signalling	11	31
ATP binding	AX-95629497	Microtubule motor	14	39
Eukaryotic translation initiation factor 5	AX-94779961	Translation initiation factor	12	33
succinyl-ligase [ADP-forming] subunit	AX-94603887	Kreb cycle	12	33
ADP, ATP carrier mitochondrial	AX-94686993	Transembrane transport	69	185
sucrose: fructan 6-fructosyltransferase	AX-94437052	Carbohydrate metabolism	45	113
Eukaryotic translation initiation factor 5	AX-94563631	Translation initiation factor	12	30
catalase 3	AX-94765678	Oxidation-reduction process	15	37
Sucrose-phosphate synthase	AX-94732905	Sucrose metabolism	10	24
ATP-dependant-Zn metalloprotease FTSH	AX-94413158	chloroplast thylakoid membrane	8	19
60S ribosomal L27	AX-95629960	Translation; structural part of ribosome	6	14
3-dehydroquinate synthase	AX-95630550	Aromatic Amino acid, steroid synthesis	6	14
3-dehydroquinate chloroplastic	AX-95208240	Aromatic Amino acid synthesis	6	14
calcium permeable stress-gated channel 1	AX-95069958	Transports Ca	7	16
ATP synthase subunit mitochondrial	AX-94486290	Transports proton	19	43
NETWORKED 1A-like	AX-95133096	Kinase activity; Phosphorylation	9	20
sucrose phosphate synthase partial	AX-94820825	Sucrose synthesis	7	15
Tubulin alpha chain	AX-95113198	Microtubuler cytoskeleton	19	40
sucrose-phosphate synthase 4	AX-94679411	Sucrose synthesis	13	27

Table 2.21: The genes showing high expression under salt stress conditions

Annotated Gene	Linked SNP	Annotated Function	NACT	NAST
nuclear pore complex NUP98A	AX-94428682	RNA export from nucleus	0	28
cullin-3A-like isoform X1	AX-94646265	Ubiquitin-depend protein catabolism	0	23
NRT1 PTR FAMILY -like	AX-94766675	Transports Oligonucleotide	0	16
F-box FBD LRR-repeat At5g56810	AX-94910271	Not annotated	0	15
methyl- -binding domain-13	AX-95002995	DNA binding	0	14
multiple inositol phosphatase 1	AX-94504126	Dephosphorylation	0	13
zinc finger CCCH domain-containing 13	AX-94515637	Metal ion binding	0	13
LRR receptor Ser/Threo-kinase MRH1	AX-94742692	Protein Kinase; Phosphorylation	0	12
heat shock STI1-like	AX-94970814	Heat shock protein	0	12
serine threonine- kinase WNK9	AX-94774467	Intracellular signal	0	11
E3 ubiquitin- ligase RNF4-like	AX-95118008	Ubiquitin-depend protein catabolism	0	11
LONGIFOLIA 2-like	AX-95104040	Monopolar cell growth	0	11
hypothetical protein F775_09884	AX-94780062	hydrolase activity on ester bonds	0	11
UDP-N-acetylglucosamine--peptide N-acetylglucosaminyltransferase SPINDLY	AX-95660165	GA mediated signaling pathway	0	10
inactive poly polymerase SRO1-X1	AX-94621492	Post-translational protein modification	0	10
FYVE zinc finger actin-binding-domain	AX-95180535	Metal ion binding; Ruffle assembly	0	10
Reticuline oxidase	AX-95202607	Oxidation-reduction process	0	10
Hippocampus abundant transcript 1	AX-95197645	Transmembrane transport	0	9
F-box FBD LRR-repeat At4g00160-X1	AX-94395821	DNA methylation	0	9
F-box FBD LRR-repeat At4g00160-X1	AX-94532057	DNA methylation	0	9
Geranylgeranyl transferase type-2 alpha	AX-94532669	Protein prenylation	0	9
Reticuline oxidase	AX-94720629	Oxidation-reduction process	0	9
Flowering time control FPA	AX-94866715	Flowering Time	0	9
U2 small nuclear ribonucleo B	AX-94436961	Splicosomal complex; mRNA splicing	0	8
Methionine adenosyltransferase 2 beta	AX-95132187	Transferase; isomerase activity	0	8
NAD kinase 1	AX-94700897	NADP biosynthesis; NAD+ kinase activity	0	8
Cinnamyl-alcohol dehydrogenase	AX-94457592	Systemic Acquired Resistance	0	8
Not annotated	AX-95197905	Not annotated	0	8
Not annotated	AX-95086353	Not annotated	0	8
bHLH13 TF	AX-94465976	Response to ABA; DNA binding	0	7
factor of DNA methylation 1	AX-94464278	mRNA based gene silencing	0	7
glycine-rich RNA-binding RZ1C-X1	AX-94699353	Nucleic cid binding	0	7
transposon Mutator sub- expressed	AX-94639463	Zn ion binding	0	7
Tyrosine- kinase BAZ1B	AX-94509108	Metal ion binding	0	7
zinc finger C-x8-C-x5-C-x3-H type	AX-94813805	Metal ion binding	0	7
WPP domain-interacting tail-anchored1	AX-95152949	Membrane component	0	7
E3 ubiquitin- ligase PRT1	AX-94453517	Ubiquitin-depend protein catabolism	0	7
F-box kelch-repeat OR23	AX-95012377	Protein ubiquitination, catabolism	0	7
E3 ubiquitin- ligase XBOS34	AX-94874865	Protein ubiquitination, catabolism	0	7
Mitochondrial	AX-95200381	Auxin polar transport; ovule development	0	7
Histone-lysine N-methyltransferase ATX2	AX-95223925	Veg/Rep transition; SiRNA in RNAi	0	7

2.4. Discussion

F_2 populations show maximum segregation among mapping population types, which is measured in terms of phenotypic variation for morphophysiological traits, and polymorphism at the DNA marker level [12,29], which makes them valuable material for dissecting the genetic architecture of quantitative traits such as salt tolerance. The Wheat 35K Array used in this study was constructed using exome-captured SNPs; therefore, we used the flanking sequences of polymorphic SNPs to locate the genes containing these markers. The functional annotation of genes revealed that genes associated with various molecular functions and biological processes could be involved in salt tolerance mechanisms in wheat. The wheat sequences of genes containing polymorphic SNPs were highly similar to those from *A. tauschii* (480/1306 genes) and *T. Urartu* (290/1306 genes) when compared to other species, which could be linked to close ancestral relationships of *A. tauschii, T. Urartu* with bread wheat [47]. Similarly, high sequence similarity of annotated genes was found with *H. vulgare*, which is considered the most salt tolerant cereal, indicating that shared salt tolerance mechanisms may be found in both species. Among the annotated genes, there were 44 ion transporters, which are likely to be involved in ion homeostasis under salt stress conditions. Among them, seven K^+ transporters/channels were annotated; of these, high-affinity potassium transporters (HKTs), i.e. HKT1;4 *Nax1* and TmHKT1;5-A *Nax2,* are reported to exclude sodium in order to confer salt tolerance [2,26], Jacalin-related lectin 3 (TaJRL3) is known for its role in the salt tolerance response [52], and TaNHX2 or K^+/H^+ exchanger acts as K^+/H^+ antiporter in wheat [53]. Conversely, K^+ outward-rectifying channel (KORC) is responsible for negative regulation of salt tolerance through K^+ efflux in plant roots under salt stress [54]; however, the role of the remaining K^+ channels in conferring salt tolerance is unknown.

Two chloride channels (CLC-e and CLC-g) were also among the annotated ion channels which are likely to be related to the reported Cl^- QTLs, including a major QTL on chromosome 5A in wheat under salt stress [28]. Similarly, four annotated proton transporters and a Pyrophosphate-energized proton pump (H^+-PPase or TVP1) were among the identified ion channels. The proton pump and transporters are reported to confer salt tolerance by inducing the sequestration of Na^+ into the vacuole and thus acting as Na+/H+ antiporters in wheat [55]. However, the role of 30 anion, Ca, cations, Cu, Fe, Mg, metal, nitrate, Sulfate and Zn transporters under salt stress is not known and should be characterized in the future. Similarly, the roles of 50 SNP-associated genes annotated for ATP, drug, fatty acids, glucose, hexose, malate, mRNA, nucleoside, polyamine, proline, proteins and transmembrane transport for maintaining cell homeostasis under salt stress should be investigated.

The abiotic stress signaling pathways are complex in nature as they involve cross-talk between several genes, transcription factors, microRNAs (miRNAs) and metabolites [15,56]. Therefore, understanding of signaling pathways is crucial for planning a successful breeding program. We located 92 SNPs on genes for signaling processes and pathways such as signal transduction and recognition; Wnt signaling and cell surface receptor signaling, which are responsible for the identification of stress stimuli. The annotated genes for apoptotic signaling are likely to be involved in the cell death to mitigate stress-induced injuries [57]. Another 14 annotated genes were found to be SA and JA-mediated; and ethylene and ABA-activated signaling pathways. These pathways have been reported to be involved in salt tolerance mechanisms [49]. Additionally, some individual ethylene, JA and ABA signaling genes also confer salinity tolerance in bread wheat [58,59]. Among the annotated signaling genes, a maximum of 22 genes were for auxin-activated signaling pathways, auxin biosynthesis and bi-directional transport. However, the functions of auxin signaling pathways and genes under salt stress has not been studied and need to be investigated in future.

Transcription factors are functional proteins responsible for regulating the expression of growth, development and stress tolerance genes in crop plants [15]. Herein, 63 annotated SNPs were linked to 52 TFs belonging to 35 TF classes e.g. ABI3, ARF3, ARF5, ARF21, bZIP17, bHLH140, EIN3, GATA26, MYB44, NAC17, NAC78, WRKY16, WRKY70, ZNFX1-NFXL1 etc. The ABI3, ARF3, ARF5, ARF31, bHLH140, EIN3, GATA26, and ZNFX1-NFXL1 TFs are predicted to be the major salt stress-responsive TFs on the bases of their GO annotations. The ABI3 and ARF3 TFs were previously found to confer salt tolerance and signaling in wheat [58,60]. The remaining TFs need further investigation to ascertain their role in salt tolerance mechanisms. Similarly, 51 SNPs found on genes for Ca, Fe, metal, Mg and Zn ion binding proteins also need to be investigated, as metal ions are essential cofactors for enzymes that could have direct or indirect roles in salt tolerance mechanisms.

Interestingly the second largest group, comprising 198 SNPs, was found within genes for several nucleic acid processes such as DNA repair mechanisms, helicases, DNA replication, transcription, translation and chromatin modifications. The comparison of these genes with published transcriptome data showed over-expression for some of these genes under salt stress [49]. Among these 198 genes, some genes were for epigenetic (rRNA, tRNA, histone-lysine and DNA methylation) or epi-transcriptomic (mRNA splicing, mRNA-based gene silencing, splicing site recognition and splicosomal complexes) processes. As these genes code for core/essential cellular functions; therefore, some of these may be involved in regulating the expression of salt tolerance genes [61]. Similarly, 166 SNPs were found within genes with catalytic (isomerase, hydrolases, kinase, ligases and transferases) or protein modification activities. The biggest group among these genes consisted of 77 protein serine/threonine kinases, protein kinases and kinases which are responsible for post-translational protein modifications and protein phosphorylation processes. Some of these protein kinases and protein serine/threonine kinases have been reported to be involved in ABA signaling and conferring salt tolerance in wheat [60,62] and the rest are candidate genes to be investigated in future. Similarly, other annotated genes for post-translational processes (protein dimerization, de-phosphorylation, and glycosylation) also need to be investigated.

The largest group of annotated SNPs (275) were found within genes for metabolic processes. Among them, the biggest subgroup of 65 SNPs was associated with genes coding for chloroplast fission/organization, Photosystem (PS) I and II complex, photosynthesis regulation, PS II assembly, chlorophyll biosynthesis/catabolism, Carbon/energy pathway, light reaction, photosynthetic sugars (fructose, galactose, glucose, mannose) biosynthesis and chloroplast DNA synthesis/translation. The functions of these genes in photosynthesis under salt stress has not been investigated in wheat. However, 22 photosynthetic proteins were found to be differentially expressed in wheat under salt stress [63] implying that there is a need for studying photosynthesis and photosynthetic genes under salt stress in wheat. Another 45 genes were involved in oxidation-reduction (Redox) processes, a core function thought to be the backbone of cell functions and cellular mechanisms. Among them, redox gene 12-oxophytodienoate reductase 1 (OPR1) has been reported to confer salinity tolerance in wheat through enhanced reactive oxygen species (ROS) scavenging and ABA signaling [64]. The other annotated redox genes such as peroxidase 1, 2 and 12, and 19; and genes for biosynthesis of antioxidants (carboxylic acid, cytokinin, flavonoid, glutathione, lactate and vitamin B and E) are candidate genes for ROS scavenging in salt-induced osmotic stress. Similarly, genes for proteasome, ubiquitin and proteolysis-dependent protein catabolism may be responsible for destroying the unwanted proteins. Similarly, functions of 21 respiratory genes (glycolysis, Kreb cycle, respiratory chain complex I and II and photorespiration) and 64 genes for lipids, carbohydrates, and proteins biosynthesis should be investigated. Finally, among 92 SNPs linked with genes of root/shoot and reproductive development (pollen germination, flowering time, ovule development etc.) could be involved in growth under saline conditions.

The potential functional roles of the annotated genes in salt tolerance mechanism, the sequences of IWGSC genes associated with segregating SNPs were aligned to the only published transcriptomic data in wheat [49]. The *in-silico* transcriptomics data showed that as many as 122 annotated genes were differentially up-regulated. The upregulated genes included drug, cations, nitrate, auxin, protein and mRNA transporters; signal transduction, auxin and ABA-activated signaling; REDOX process, proteolysis, flavonoid metabolism, defense response, kinases, xylem and cell wall development etc. The genes that showed the highest differential expression under salinity included eukaryotic translation initiation factor 6-2, Midasin, transparent Testa 12, phospholipase D delta/pathogenesis-related 1, quinolinate chloroplastic isoform X3, trehalose-phosphatase, transport SEC1 B-like, casein kinase family, Kinesin KIF22, serine threonine kinase, and Ran-specific GTPase-activating 2 genes which coded for embryo development, protein desumoylation, drug transport, ABA activated signaling pathway, iron Sulphur cluster binding/aerobic respiration, trehalose biosynthesis/dephosphorylation, protein/mRNA transport, Wnt Signaling pathway, microtubule motor, pphosphorylation, and protein transports from nucleus. On the other hand, genes with lowest expression under salt stress among the 136 down-regulated genes included genes for vitamin B biosynthesis, endocytosis, sulfate transport, transcription factor SPT20 homolog, programmed cell death, Chloroplast avoidance movement, nucleoside transporter and vesicle transport. The only published transcriptome under salinity is for wheat root; therefore, several annotated genes like photosynthesis related genes did not show differential expression.

2.5. Conclusions and prospects

Around 1,300 segregating SNPs were found within genes of active slat tolerance genes such as ion channels, macromolecule transporters, transcriptional factor and diverse signaling pathways; and passive mechanisms such as ion binders, cellular biosynthesis processes, growth related processes, tolerance mechanisms, genetic factors, functional genes/enzymes and metabolic pathways. Among these, 258 genes belonging to all above groups were differentially expressed under salinity, hinting their involvement in salt tolerance mechanisms in wheat. However, the published transcriptome used for *in-silico* transcriptomic analysis in based on Roche 454-GS FLX sequencing reads and transcriptomic analysis of salt stressed wheat using higher throughput technology such as Illumina will help in understanding the gene expression under salt stress and salt tolerance mechanisms. The characterization of the annotated genes in general and expressed genes particularly will help to understand the salt tolerance mechanisms in wheat; and will guide future breeding for salt tolerant wheat.

Chapter 3: QTL Validation & TFs Expressed under Salt Stress

3.1. Introduction

In previous chapters, the QTL mapping for all micro and macronutrients in wheat i.e. NAX, K^+ Mg^{2+}, Ca^{2+}, Boron, Cu, Fe, Mn, S, P and Zn concentrations in wheat root and shoot under 300 mM NaCl salinity were reported in WSTD91 × Wn64 F_2 population. For the purpose, genotyping of 154 F_2 lines by using Axiome Wheat Breeders Genotyping Array was performed and genotyping data was used to construct a high-density genetic linkage map (consisting of 988 SNPs) for 21 wheat chromosomes. The QTLs were mapped on 12 chromosomes including five each belonging to the A and B genome and two on the D genome. However, mapping more populations is required to map novel and major QTLs for salt tolerance; therefore, QTLs for NAX, K, DRW and DSW were mapped QTLs in WSTD91 × Wn64 and Millet-2011 × Wn64 F_2 populations for the purpose.

Transcription factors (TFs) are important regulators of gene expression under abiotic stress in plants as well as for plant growth and development. Wide range of TFs such as AP2/EREBP (ERF), MADS-box TFs, MYB like TFs, AP2 containing *DREB1*, ARF, *WRKY1*, *MYB101, MYB33, TaNAC69, TaWRKY16, TaWRKY17, TaWRKY10, TaWRKY19-C, TaWRKY24, TaWRKY59, TaWRKY61* etc. regulate the expression of genes coding for abscisic acid or ABA-dependent and independent; salicylic acid, jasmonic acid, IP3 and ethylene signaling pathways under drought stress, and thus confer drought tolerance in wheat [15]. Although, the roles of TFs under drought stress have been frequently studied, little information about their expression under salt stress has not been studied for most of the members of these TF gene families e.g. TdWRKY1, TdWRKY3, TdWRKY2, TdWRKY5 and TdWRKY4 TFs showed differential expression under salt stress in durum wheat [65].

The functional roles of bread wheat TFs such as TaWRKY93 [66], TaWRKY10 [67], TaWRKY44 [68], TaNAC2 [69], TaNAC29 [70], TaNAC69-1 [71], and MYB73 [72] in conferring salt tolerance were characterized by their over-expression in model plants *Arabidopsis thaliana* and tobacco. For MADS box TF, whole genome analysis and expression under several pathogen attack, drought and heat were studied in bread wheat [73]. Therefore, the comparative and expression analysis of NAC, WRKY, MYB, MYB-related, MADS-box and AP2-containing TF gene families in bread wheat will provide novel insights into TFs potentially involved in salt tolerance in wheat. This study was designed to profile the phylogenetic relationship between members of above mentioned five TF gene families, *in-silico* expression analysis under salt stress, and to analyze the conserved genome regions of these TFs which could be used for conserved domain analysis.

3.2. Materials and Methods

3.2.1. Plant material

Three contrasting wheat accessions i.e. two salt tolerant (WTSD91 and Millet-20111) and one susceptible (WN-64) genotype selected from a hydroponics screening of 150 genotypes at 300 mM NaCl [7] were crossed in the field during the growing season of 2011-12. The crossed seeds were harvested and grown during 2012-13 to raise F_1 hybrids. The wheat spikes were covered with butter paper bags at anthesis stage to ensure purity and F_2 seeds were obtained.

3.2.2. Growth conditions

The experiment was conducted at 40° 53′ 25″ N, 29° 22′ 47″ E in Sabanci University, Istanbul in a Venlo-type greenhouse capable of computerized control for evaporative cooling, supplemental lighting and heating. The temperatures were regulated to be 25 ± 4 °C and 20 ± 4 °C during day and night throughout the experimental period. A total of 160 F_2 lines each for WTSD91 × WN64 (pop 1) and Millet-2011 × Wn64 (pop 1) F_2 populations were grown in inert perlite for five days. After removal of residual endosperm from the seedling roots, 152 uniformly growing seedlings were transplanted to 2.7-L hydroponic pots containing aerated nutrient solution as explained previously [40]. On the following day, NaCl amounting to 140 mM salinity was added to hydroponic pots. The nutrient solution was changed every five days and the salinity level was enhanced to 280 mM NaCl on the next solution replacement. The plants were kept under salt stress for 30 days including 25 days at 280 mM salinity.

3.2.3. Phenotyping

Based on phenotypic variation, both F_2 lines' plants were categorized into tolerant (T), moderately tolerant (MT), moderately susceptible (MS) and highly susceptible (HS) groups as explained in chapter 1. A total of 152 plants for each population were pooled into 16 groups (4 × 8 T plants, 4 × 8 MT plants, 4 × 10 MS plants, 4 × 12 HS plants) for measuring mineral nutrients. For the purpose, plant roots and shoots were washed thrice in running dH_2O and were oven dried at 65 °C for 96 hours. Subsequently, dry root and shoot weights (DRW and DSW) were recorded and dried root and shoot were cut into small pieces using ceramic scissors.

For mineral analysis, ~0.15-0.2 g shoot for each pool was added with 2 ml of 30% H_2O_2 and 5 ml of 65% HNO_3 and digested in a closed-vessel microwave system (Mars Express; CEM Corp; NC, USA). Milli-Q water was added to digested tissues to make a final volume of 20 ml and K, and Na concentrations in tissues were measured using inductively coupled plasma optical emission spectrometry (ICP-OES; Vista-Pro Axial; Varian Pty Ltd; Mulgrave, Australia) [41].

The 20 ml digested solution was further diluted 50 times (1:50) to measure Na^+ concentration. To exclude any unexpected variation, ICP-OES data for minerals was also measured for standard durum wheat flour (SRM 8436, NIST, Gaithersburg, MD). The concentration values for all traits was obtained by multiplication of ICP-OES values by the dilution factor and dividing the result by the dry shoot weight used for digestion. As sodium exclusion means less uptake of Na^+ by shoot, Na^+ concentration values were multiplied by -1 to obtain values for shoot Na exclusion (SNAX).

3.2.4. Genotyping, genetic linkage map and QTL mapping

The Axiom Wheat Breeder's Genotyping Array (Affymetrix, Santa Clara, CA, USA) or “wheat 35K array” was utilized for genotyping the WTSD91 × WN64 population. The genotyping data was used for construction of the genetic linkage map using MapDisto 2.0 b93 [23] program. The genetic linkage map consisted of 988 SNP markers assigned to all the 21 wheat chromosomes. Total 342, 562 and 84 markers were assigned to A, B and D sub-genomes, respectively; while total length of linkage map was 2317.88 cM including 975.56, 1133.16 and 209.16 cM lengths for A, B and D sub-genomes, respectively. Further information about the map is available in first chapter and our published paper [46]. Single salinity treatment phenotypic data was utilized for mapping additive QTLs for all traits, by the composite interval mapping (CIM) method. For this purpose, LOD threshold= 2 and walking speed= 1-cM was used in the QTL IciMapping V4.1.0 program [43].

3.2.5. Transcription factor sequences, phylogenetic and expression analysis

The coding sequences (CDS) of 86 NAC, 71 WRKY, 87 MYB, 103 AP2-containing/ERF and 87 MADS box-containing transcription factors were down-loaded from publicly available NCBI nucleotide database. The sequences of 40 MYB-related transcription factors were got from wheat transcription factor database (http://planttfdb_v1.cbi.pku.edu.cn:9010/web/index.php?sp=ta). The multiple sequence alignments, conserved genome regions and phylogenetic relationship among the members of a TF gene family were performed by CLC Genomics Workbench 11. Testing for differential expression of the annotated genes under salinity was performed by alignment of the TF CDS with transcriptome reads expressed under saline and normal conditions in wheat [49] using the BLASTN 2.6.1+ program. The alignments with alignment scores >200 were recorded, and differential expression values were obtained by dividing salt/normal alignment counts ratio by 3.25 (347,200/106,600 spots expressed under salinity/control). The genes which showed 2-fold increase or decrease in expression were taken as differentially expressed under salt stress.

3.3. Results and discussion

3.3.1. Phenotypic variation in two mapping populations

The significant phenotypic variation among the four tolerance groups in both mapping populations. The DRW for both populations showed around 2-fold decrease when T group was compared with HS plants. Similarly, DSW in pop1 varied from 126.667 mg in T group to 48.125 mg in HS group while it ranged between 92.125 to 45.21 mg in pop2. The SKC in pop1 was recorded 2.333, 2.212, 1.848 and 1.095% in T, MT, S and HS plant groups and it ranged from 2.333 to 0.985% in pop2. Similarly, SNAX ranged between -3.196 to -6.957% and -3.456 to -7.109% in pop1 and pop2, respectively. Thus, showing higher SKC and SNAX in tolerant plants as compared to the susceptible plants (Table 3.1). Such phenotypic variation in mapping populations for DRW, DSW, SKC and SNAX have previously been reported [26–28,74]; and is the prerequisite for QTL mapping.

Table 3.1: Phenotypic variation in four diverse tolerance groups of two wheat F_2 mapping populations for salt tolerance

Plant Traits	Tolerant	Moderately Tolerant	Susceptible	Highly Susceptible
DRW Pop1 (mg)	26.667	23.333	16.667	12.229
DRW Pop2 (mg)	30.125	26.666	20.495	15.3125
DSW Pop1 (mg)	126.667	103.333	78.333	48.125
DSW Pop2 (mg)	92.125	80.333	63.812	45.521
SKC Pop1 (%)	2.536	2.212	1.848	1.095
SKC Pop2 (%)	2.333	2.139	1.615	0.985
SNAX Pop1 (%)	-3.196	-4.014	-5.927	-6.957
SNAX Pop2 (%)	-3.456	-4.231	-5.119	-7.109

3.3.2. QTL mapping for salt tolerance in two mapping populations

Total 27 QTLs for salt tolerance were mapped in WTSD91 × WN64 F_2 population for salt tolerance related traits. These included six, four, six and eleven QTLs for DRW, DSW, SKC and SNAX, respectively. In D genome, only one QTL each in pop1 and pop2 were mapped for DSW and SKC, respectively. In B genome, a major QTL for DSW was mapped on chromosome 6B with LOD of 11.65 while a DRW QTL was mapped on chromosome 2B. Two major QTLs for SNAX were mapped on chromosome 1B with LOD of 12.85 and 9.55 while one, two and three QTLs were mapped on chromosome 4B, 2B and 7B (Table 3.2). For Millet-11 × WN64 F_2 population or pop2, total 41 QTLs were mapped including 14, 13, six and eight QTLs for DRW, DSW, SKC and SNAX QTLs on 1B, 2B, 3B and 5B (Table 3.3).

For both the populations, QTLs for DRW, DSW, SKC and SNAX were co-localized on chromosome 2A, 5A and 7A including QTLs clusters on chromosome 2A and 7A. Such co-localization of SNAX, SKC, DRW and DSW QTLs was also reported previously [24,74]. Reduced Na^+ uptake or sodium exclusion (NAX) is one of the most important salt tolerance mechanisms in wheat, and QTL mapping for salt tolerance in wheat has largely been focused on mapping NAX QTLs [24,26,27]. Our mapped QTLs on chromosome 2A coincided with previously reported NAX QTLs on chromosome 2A in bread wheat [26] and a major NAX QTL *Nax1* (*HKT1;4*) in durum wheat [29]. Similarly, QTLs mapped on chromosome 5A also coincided with a reported *Nax2* (*HKT1;5*) QTL in durum wheat [75]. Another NAX QTL on chromosome 4B coincided with a reported SKC QTL [26]. The QTLs pairs were identified on homologous chromosome 1A, 1B, 2A, 2B, 6A, 6B, 7A and 7B indicating synteny. The novel/major QTLs mapped on chromosome 1B and 6B, along with above mentioned QTLs will be a valuable source for future wheat breeding for salt tolerance.

Table 3.2. QTL mapping for salt tolerance in WTSD91 × WN64 F_2 lines at 280 mM NaCl

Trait	Chromosome	Marker Interval	Position (cM)	LOD
DRW	2A	AX-94496850--AX-94696230	32.49-36.16	3.8
	2A	AX-95114316--AX-94878691	118.1-121.02	2.52
	2B	AX-95071189--AX-95129847	109.89-111.18	2.22
	5A	AX-94730618--AX-94390591	44.87-51.15	2.35
	7A	AX-95248570--AX-95002995	64.79-66.44	2.75
	7A	AX-94785878--AX-94738383	166.00-175.59	2.62
DSW	2A	AX-94496850--AX-94696230	32.49-36.16	3.7
	6A	AX-94547709--AX-94774725	32.16-59.57	4.65
	6B	AX-94668676--AX-94883829	51.44-57.07	11.65
	7A	AX-95248570--AX-95002995	64.79-66.44	3.03
SKC	2A	AX-95096362--AX-94433820	3.74-4.42	2.25
	2A	AX-94496850--AX-94696230	32.49-36.16	2.15
	2A	AX-95018915--AX-94842940	173.29-174.96	2.35
	2D	AX-94485593--AX-95151743	23.20-40.78	2.05
	7A	AX-95248570--AX-95002995	64.79-66.44	2.25
	7A	AX-94785878--AX-94738383	166.00-175.59	2.03
SNAX	1A	AX-94416982--AX-94733833	37.28-52.01	3.25
	1B	AX-94394039--AX-95629900	94.84-95.50	12.85
	1B	AX-95629900--AX-95126764	95.50-98.15	9.55
	2A	AX-94496850--AX-94696230	32.49-36.16	2.35
	2B	AX-94725996--AX-95188301	4.99-7.95	2.45
	2B	AX-94435221--AX-94592204	177.42-181.17	2.65
	4B	AX-94632604--AX-95148627	51.12-51.45	2.1
	7A	AX-94976788--AX-95130204	33.70-45.41	3.2
	7B	AX-95074259--AX-94735540	96.31-101.58	2.02
	7B	AX-95654169--AX-94442619	113.33-117.26	2.55
	7B	AX-94442619--AX-94664270	117.26-123.33	2.25

Table 3.3. QTL mapping for salt tolerance in Millet-11 × WN64 F_2 lines at 280 mM NaCl

Trait	Chromosome	Marker Interval	Position (cM)	LOD
DRW	1B	AX-95198133--AX-95629053	11.38-14.46	2.2
	1B	AX-94681412--AX-94476532	164.52-168.19	2.05
	1B	AX-94385896--AX-94801647	169.51-173.3	2.07
	2A	AX-95096362--AX-94433820	3.74-4.42	2.05
	2A	AX-95114316--AX-94878691	118.1-121.02	2.15
	2B	AX-95071189--AX-95129847	109.89-111.18	2.55
	3B	AX-94405214--AX-94545746	69.57-77.64	2.75
	4A	AX-94840887--AX-94656195	18.50-21.47	2.87
	5A	AX-94440814--AX-94382552	3.64-12.30	2.15
	5A	AX-94730618--AX-94390591	44.87-51.15	2.45
	5B	AX-95100010--AX-94582897	52.94-60.76	2.02
	7A	AX-95130204--AX-94633304	45.42-64.46	2.15
	7A	AX-95248570--AX-95002995	64.79-66.44	2.85
	7A	AX-94785878--AX-94738383	166.00-175.59	2.22
DSW	1B	AX-95198133--AX-95629053	11.38-14.46	2.05
	2A	AX-94496850--AX-94696230	32.49-36.16	2
	2A	AX-95114316--AX-94878691	118.1-121.02	2.35
	2A	AX-95018915--AX-94842940	173.29-174.96	2.05
	2B	AX-95071189--AX-95129847	109.89-111.18	2.5
	2D	AX-94485593--AX-95151743	23.20-40.78	2.1
	3B	AX-94405214--AX-94545746	69.57-77.64	2.03
	4A	AX-94840887--AX-94656195	18.50-21.47	2.21
	5A	AX-94730618--AX-94390591	44.87-51.15	2.95
	5B	AX-94713620--AX-94390404	87.65-96.86	2.4
	7A	AX-95130204--AX-94633304	45.42-64.46	2.35
	7A	AX-95248570--AX-95002995	64.79-66.44	2.55
	7A	AX-94785878--AX-94738383	166.00-175.59	2.4
SKC	2A	AX-94496850--AX-94696230	32.49-36.16	2.6
	2A	AX-94696230--AX-94577588	36.16-38.86	2.65
	5A	AX-94730618--AX-94390591	44.87-51.15	2.05
	7A	AX-95130204--AX-94633304	45.42-64.46	2.75
	7A	AX-95248570--AX-95002995	64.79-66.44	2.6
	7A	AX-94785878--AX-94738383	166.00-175.59	2.7
SNAX	2A	AX-95096362--AX-94433820	3.74-4.42	2.05
	2A	AX-94496850--AX-94696230	32.49-36.16	2.1
	2B	AX-95071189--AX-95129847	109.89-111.18	3.55
	5A	AX-94730618--AX-94390591	44.87-51.15	2.15
	5A	AX-94406590--AX-94909932	79.53-85.66	2.2
	7A	AX-95130204--AX-94633304	45.42-64.46	2.31
	7A	AX-95248570--AX-95002995	64.79-66.44	2.12
	7A	AX-94785878--AX-94738383	166.00-175.59	2.23

3.3.3. Phylogenetic relationship among the members of TF gene families

The NAC TFs were classified into group I, II, and III, which were further divided to IIa, IIb, IIIa, IIIb, and IIIc sub-groups while TaNAC55, TaNAC35, TaNAC11, TaNAC19, TaNAC62, TaNAC73bL and TaNAC75 had the most similar sequence (Figure 3.1). The WRKY TFs were classified into group I and II, which were further divided to Ia, Ib, Ic, IIa, and IIb sub-groups while TaNAC78, TaNAC23, TaNAC24, TaNAC44, and TaNAC26 had the most similar sequence (Figure 3.2). The MYB related TFs were classified into group I and II, which were further divided to Ia, Ib, IIa, IIb and IIc sub-groups while six of these TFs had very similar sequence (Figure 3.3).

The AP2 containing TFs were classified into group I, II and III, which were further divided to Ia, Ib, IIa, IIb, IIIa and IIIb sub-groups. Interestingly, TaCBF5 and TaCBF7 had more similarity with TaDREBs and TaERFs sequence (Figure 3.4). The MADS box TFs were classified into group I and II, while II was further divided to Ia, Ib, Ic, and Id sub-groups (Figure 3.5). Such sequence-based grouping in TF gene families has also been reported previously [69,72,73] and are useful for domain analysis in TF gene families.

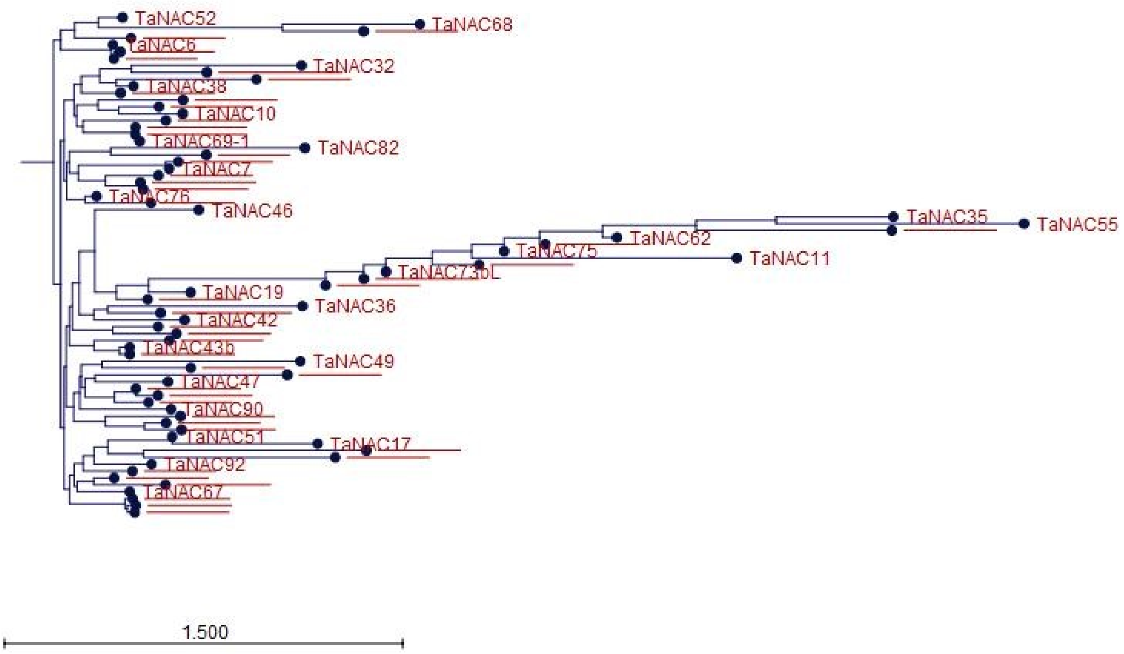

Figure 3.1. Phylogenetic relationship among the members of the NAC TF gene family

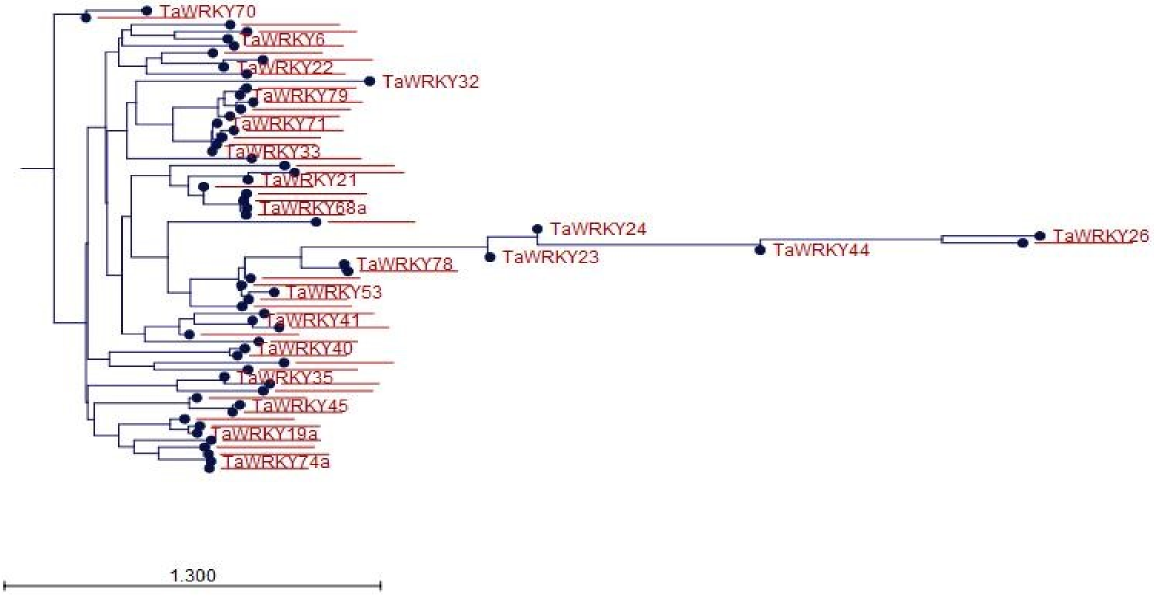

Figure 3.2. Phylogenetic relationship among the members of the WRKY TF gene family

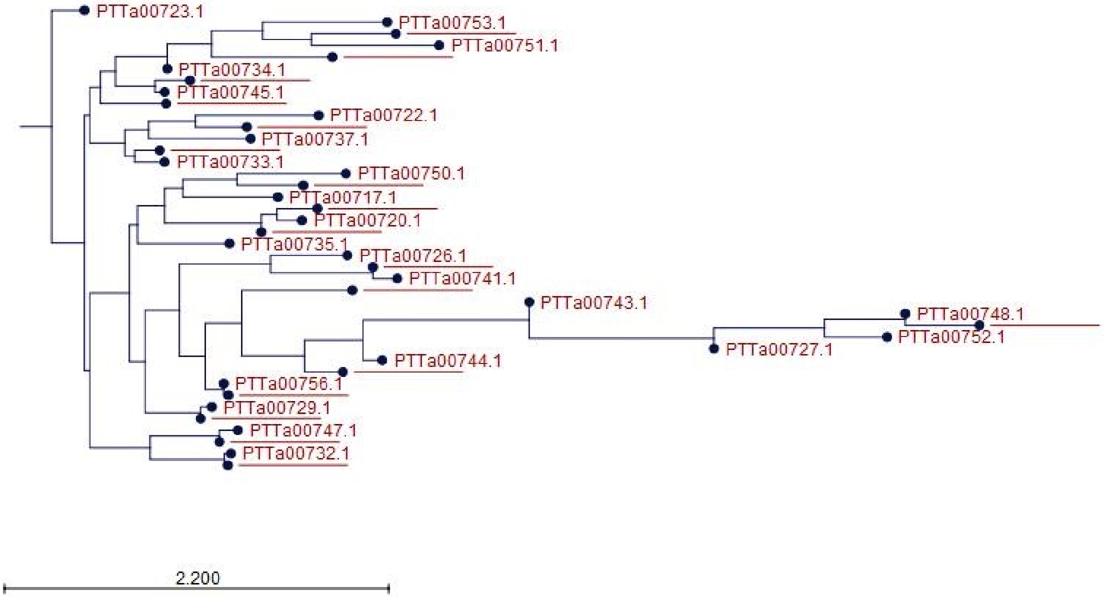

Figure 3.3. Phylogenetic relationship among the MYB-related TF gene family members

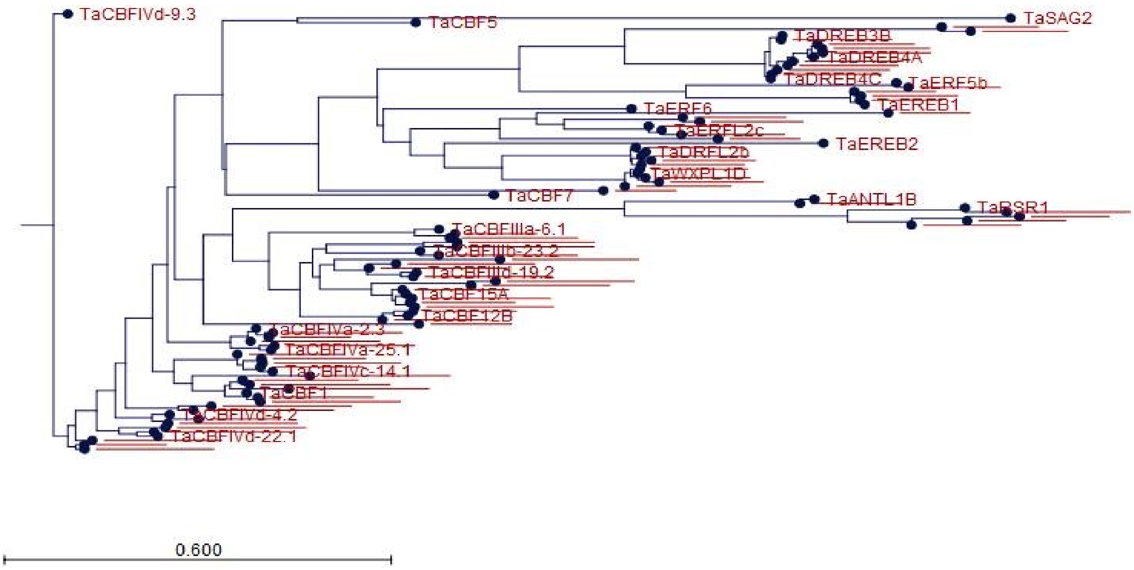

Figure 3.4. Phylogenetic relationship among the AP2-containing TF gene family members

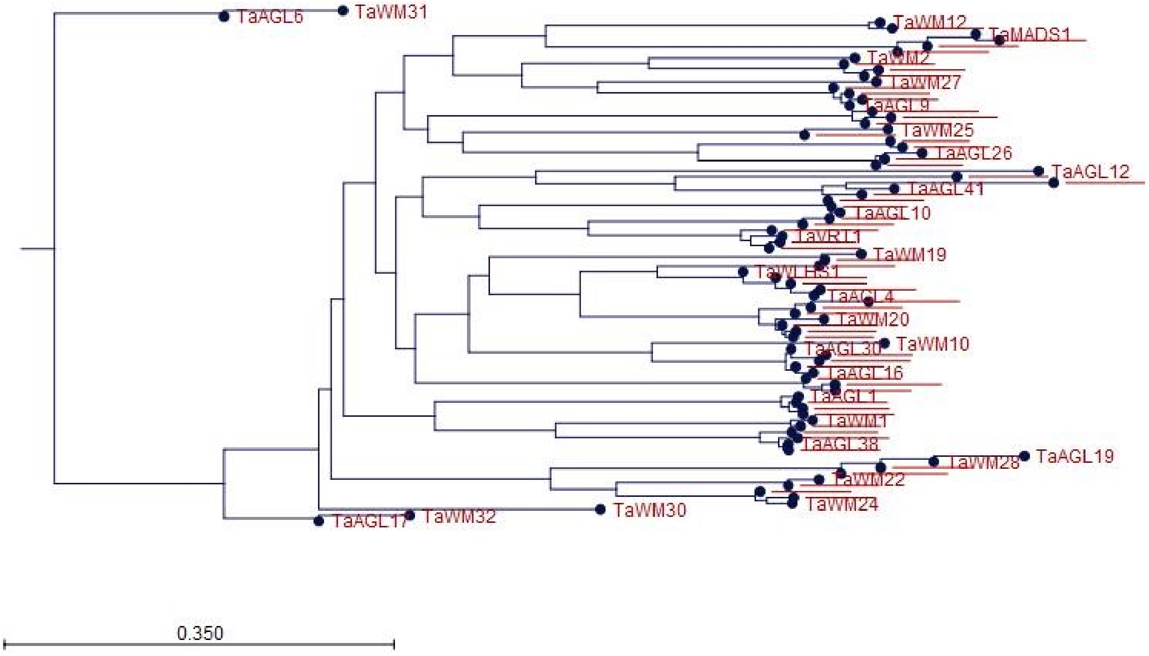

Figure 3.5. Phylogenetic relationship among the members of MADS box TF gene family

3.3.4. Differential expression of TFs under salt stress

Total 40 NAC TFs were differentially expressed under salt stress in wheat. TaNAC67, TaNAC47, TaNAC1, TaNAC2, TaNAC3, TaNAC4, TaNAC6, TaNAC29, TaNAC20, TaNAC71, TaNAC69-1 etc. being the most up-regulated and TaNAC41, TaNAC15, TaNAC8, TaNAC78a, TaNAC51, TaNAC39, TaNAC35 etc. being the most downregulating TFs (Table 3.4). Only the roles of TaNAC2 [69], TaNAC29 [70], and TaNAC69-1 [71] for conferring salt tolerance was known previously.

Among the 32 TaWRKY TFs differentially expressed under salinity, TaWRKY14, TaWRKY78, TaWRKY17, TaWRKY2, TaWRKY53a, TaWRKY53b, TaWRKY20, TaWRKY21, TaWRKY29 etc. were the most up-regulated and TaWRKY45, TaWRKY19a, TaWRKY19b, TaWRKY15, TaWRKY27 etc. were the most downregulating TFs (Table 3.5). Only the roles of TaWRKY93 [66], TaWRKY10 [67], and TaWRKY44 [68] for conferring salt tolerance was known previously.

Among the 40 differentially expressed TaMYB TFs, TaMYB73, TaMYB44, TaMYB71, TaMYB16, TaMYB51, TaMYB32, TaMYB17, TaMYB19, TaMYB33 etc. were the most up-regulated and TaMYB39, TaMYB23, TaMYB62, TaMYB65, TaMYB15 etc. were the most downregulating TFs (Table 3.6). Only the role of MYB73 [72] for conferring salt tolerance was known previously. Among the 45 differentially expressed AP2 containing TFs, TaERF-L2b, TaERF-L2c, TaDREB5, TaERF3, TaERF2 etc. were the most up-regulated, and TaDBP, TaWCBF2-2, TaCBF2, TaEREB2, TaERF5 etc. were the most downregulating TFs (Table 3.7). The role of TaDREB1 and TaERF2 [15] for conferring salt tolerance was studied.

Among the 24 differentially expressed novel MADS box TFs, TaWM14, TaAGL1, TaAGL18, TaAGL20, TaAGL21, TaAGL23 etc. were the most up-regulated, and TaWM32, TaMADS11, TaWAG2, TaVRT1, TaMADS2 etc. were the most downregulating TFs (Table 3.8). As the differential expression of most of TF is reported first time, it provides a novel resource for wheat improvement for salt tolerance. The characterization of differentially expressed in wheat and their over-expression in other crops could help to identify their roles in complex signaling cascades, and thus may help to develop salt tolerant wheat.

Table 3.4. Differentially expressed NAC TFs under salt stress in bread wheat

S. No.	TF Name	TF ID	Seq. Length	NACET	NASET	FCE
1	TaNAC67	KF646593.1	1022	1	24	7.385
2	TaNAC47	KT345698.1	1277	1	19	5.846
3	TaNAC2	GQ231950.1	1295	2	26	4.000
4	TaNAC2	JN621240.1	1222	2	26	4.000
5	TaNAC3	HQ630373.1	1520	1	13	4.000
6	TaNAC4	HQ872051.1	1453	1	12	3.692
7	TaNAC2D	GQ231952.1	1213	2	23	3.538
8	TaNAC3	HM037183.1	1406	2	23	3.538
9	TaNAC6	HM027571.1	1233	2	23	3.538
10	TaNAC6	AK332540.1	1633	2	23	3.538
11	TaNAC1	HM037182.1	1481	1	11	3.385
12	TaNAC2A	HM027577.1	1335	2	20	3.077
13	TaNAC4	HQ630374.1	1515	1	10	3.077
14	TaNAC29	KT783450.1	1198	1	10	3.077
15	TaNAC6	HM037185.1	770	3	27	2.769
16	TaNAC20	KY461026.1	1696	2	18	2.769
17	TaNAC29	KP657687.1	897	2	18	2.769
18	TaNAC6L	KY461013.1	1646	3	26	2.667
19	TaNAC4	GQ985329.1	1076	2	17	2.615
20	TaNAC71	KY461044.1	2038	2	17	2.615
21	TaNAC2	HQ872050.1	1449	1	8	2.462
22	TaNAC2	HQ630372.1	1566	1	8	2.462
23	TaNAC29	KY461030.1	1819	1	8	2.462
24	TaNAC48	KY461059.1	1991	3	23	2.359
25	TaNAC69-1	AY625682.1	1423	2	15	2.308
26	TaNAC69-3	DQ022843.1	1335	2	15	2.308
27	TaNAC47	KY461040.1	919	4	29	2.231
28	TaNAC2B	GQ231951.1	1214	3	21	2.154
29	TaNAC36	KY461064.1	263	1	7	2.154
30	TaNAC69-1	FB787703.1	1065	2	14	2.154
31	TaNAC69-2	DQ022842.1	1319	2	14	2.154
32	TaNAC34	KY461058.1	1097	2	13	2.000
33	TaNAC75	KY461076.1	790	2	3	0.462
34	TaNAC35	KY461032.1	1401	1	1	0.308
35	TaNAC39	KY461033.1	1533	1	1	0.308
36	TaNAC51	KY461070.1	385	1	1	0.308
37	TaNAC78a	KY461049.1	1846	1	1	0.308
38	TaNAC8	HM027573.1	1275	3	2	0.205
39	TaNAC15	KY461023.1	1489	2	1	0.154
40	TaNAC41	KY461034.1	1115	4	2	0.154

NACET: no. of alignments with control expressed transcriptome; **NASET:** no. of alignments with salt expressed transcriptome; **FCE:** fold change expression

Table 3.5. Differentially expressed WRKY TFs under salt stress in bread wheat

S. No.	TF Name	TF ID	Seq. Length	NACET	NASET	FCE
1	TaWRKY14	EU665427.1	1605	1	14	4.308
2	TaWRKY78	HM013818.1	1716	1	14	4.308
3	TaWRKY17	EU665429.1	1839	1	13	4.000
4	TaWRKY53a	EF368357.1	1509	1	12	3.692
5	TaWRKY2	EU665425.1	1407	1	11	3.385
6	TaWRKY53b	EF368364.1	1436	1	11	3.385
7	TaWRKY20	EU665445.1	375	1	10	3.077
8	TaWRKY21	EU669657.1	354	1	10	3.077
9	TaWRKY29	EU669660.1	813	1	10	3.077
10	TaWRKY53	KC174859.1	3138	1	10	3.077
11	TaWRKY13	EF397614.1	1054	1	9	2.769
12	TaWRKY79	JX047374.1	1297	1	9	2.769
13	TaWRKY41	EU669665.1	456	1	8	2.462
14	TaWRKY51	JX277054.1	1295	2	15	2.308
15	TaWRKY68b	EF397617.1	1168	4	30	2.308
16	TaWRKY16	EU665428.1	1050	1	7	2.154
17	TaWRKY46	KR827397.1	791	1	7	2.154
18	TaWRKY48	KR827399.1	978	1	7	2.154
19	TaWRKY68a	EF368360.1	1041	4	28	2.154
20	TaWRKY70	KY784578.1	912	1	7	2.154
21	TaWRKY53	KR827404.1	942	4	27	2.077
22	TaWRKY4	EU665433.1	1164	2	13	2.000
23	TaWRKY8	DQ323885.1	1360	2	13	2.000
24	TaWRKY9	EU665438.1	1011	4	26	2.000
25	TaWRKY33-3	KX756970.1	1047	2	13	2.000
26	TaWRKY80	JX679079.1	1619	2	13	2.000
27	TaWRKY11	EU665440.1	675	2	3	0.462
28	TaWRKY27	EU665431.1	1686	3	4	0.410
29	TaWRKY15	EU665442.1	688	1	1	0.308
30	TaWRKY19a	EF368362.1	972	1	1	0.308
31	TaWRKY19b	EF397616.1	996	1	1	0.308
32	TaWRKY45	KR827396.1	1569	3	2	0.205

NACET: no. of alignments with control expressed transcriptome; **NASET:** no. of alignments with salt expressed transcriptome; **FCE:** fold change expression

Table 3.6. Differentially expressed MYB & MYB-related TFs under salinity in bread wheat

S. No.	TF Name	TF ID	Seq. Length	NACET	NASET	FCE
1	TaMYB73	JN969051.1	1200	1	14	4.308
2	TaMYB44	JF951927.1	1893	1	12	3.692
3	TaMYB71	JF951954.1	726	1	12	3.692
4	TaMYB16	KU674899.1	978	1	11	3.385
5	TaMYB51	JF951934.1	798	2	21	3.231
6	TaMYB32	JF951915.1	735	1	9	2.769
7	TaMYB16	JF951918.1	978	2	17	2.615
8	TaMYB17	JF951901.1	867	1	8	2.462
9	TaMYB19	JF951903.1	744	2	16	2.462
10	TaMYB33	JN584645.1	1040	1	8	2.462
11	TaMYB72	JF951955.1	1068	1	8	2.462
12	TaMYB86	KM066946.1	961	1	8	2.462
13	TaMYB4	JF746995.1	1070	3	23	2.359
14	TaMYB1	AY615198.1	849	2	15	2.308
15	TaMYB61	JF951944.1	1005	2	15	2.308
16	TaMYB56	JF951939.2	731	11	79	2.210
17	TaMYB46	JF951929.1	2256	1	7	2.154
18	TaMYB59	JF951942.1	1452	1	7	2.154
19	TaMYB83	KY475611.1	795	1	7	2.154
20	TaMYB20	JF951904.1	774	12	83	2.128
21	TaMYB13-3	JN191347.1	971	11	76	2.126
22	TaMYB29	JF951912.1	786	3	20	2.051
23	TaMYB13-2	JF288935.1	1352	11	73	2.042
24	TaMYB28	JF951911.1	816	2	13	2.000
25	TaMYB74	KU674898.1	1077	3	4	0.410
26	TaMYB41	JF951924.1	879	2	3	0.462
27	TaMYB49	JF951932.1	1077	6	8	0.410
28	TaMYB15	JF951900.1	990	1	1	0.308
29	TaMYB65	JF951948.1	816	2	2	0.308
30	TaMYB62	JF951945.1	838	6	5	0.256
31	TaMYB23	JF951906.1	618	5	4	0.246
32	TaMYB39	JF951922.1	1959	5	4	0.246
1	MYB-related	PTTa00717.1	990	2	21	3.231
2	MYB-related	PTTa00723.1	1086	1	8	2.462
3	MYB-related	PTTa00743.1	1342	1	8	2.462
4	MYB-related	PTTa00726.1	626	1	7	2.154
5	MYB-related	PTTa00747.1	539	2	3	0.462
6	MYB-related	PTTa00754.1	1316	10	12	0.369
7	MYB-related	PTTa00738.1	962	3	3	0.308
8	MYB-related	PTTa00739.1	1655	1	1	0.308

NACET: no. of alignments with control expressed transcriptome; **NASET:** no. of alignments with salt expressed transcriptome; **FCE:** fold change expression

Table 3.7. Differentially expressed AP2 containing TFs under salt stress in bread wheat

S. No.	TF Name	TF ID	Seq. Length	NACET	NASET	FCE
1	TaERF-L2b	DQ334410.1	782	1	11	3.385
2	TaERF-L2C	DQ334411.1	797	1	11	3.385
3	TaDREB5B	AY781359.1	2210	2	21	3.231
4	TaDREB5A	AY781358.1	2354	2	20	3.077
5	TaDREB5C	AY781357.1	2263	2	20	3.077
6	TaERF-L2a	DQ334409.1	1064	1	10	3.077
7	TaERF3	EF570122.1	1478	1	10	3.077
8	TaERF-L1d	DQ334415.1	920	1	8	2.462
9	TaWAP2D	AB697002.1	1347	1	8	2.462
10	TaANTL1B	DQ286570.1	1577	2	15	2.308
11	TaANTL1A	DQ286569.1	1474	2	14	2.154
12	TaAPETALA2	AJ577368.1	412	1	7	2.154
13	TaDREB3B	AY781350.1	1181	2	14	2.154
14	TaDREB4A	AY781354.1	1638	2	14	2.154
15	TaWEREB2	AB193608.1	1456	2	14	2.154
16	TaERF-L1b	DQ334413.1	1005	1	7	2.154
17	TaERF6	JN681188.1	698	1	7	2.154
18	TaWAP2AQ	AB697000.1	1344	1	7	2.154
19	TaWXPL1B	KX611870.1	855	9	63	2.154
20	TaDRF-L1a	DQ334407.1	1662	10	69	2.123
21	TaWXPL1AD	KX611871.1	855	9	60	2.051
22	TaDRF-L1b	DQ334408.1	1081	9	59	2.017
23	TaWXPL1A	KX611869.1	855	9	59	2.017
24	TaDREB2	HQ171443.1	1282	2	13	2.000
25	TaDREB3A	AY781349.1	1325	2	13	2.000
26	TaDREB4B	AY781355.1	1494	2	13	2.000
27	TaSAG2	AB539586.1	2469	2	13	2.000
28	TaCBFIVd-4.1	EF028780.1	872	2	3	0.462
29	TaCBFIVd-9.1	EF028782.1	1080	2	3	0.462
30	TaCBFIVd-9.2	EF028783.1	999	2	3	0.462
31	TaCBFIVd-9.3	EF028784.1	1063	2	3	0.462
32	TaCBFIVd-22.1	EF028785.1	1219	2	3	0.462
33	TaCBFIVd-22.2	EF028786.1	1252	2	3	0.462
34	TaCBFIVd-22.3	EF028787.1	1211	2	3	0.462
35	TaCBF4	DQ286550.1	936	2	3	0.462
36	TaCBF9	AY785905.1	1073	2	3	0.462
37	TaERF5a	JN681191.1	1416	18	27	0.462
38	TaERF5b	JN681192.1	1432	18	26	0.444
39	TaEREB2	AY781353.1	1794	7	10	0.440
40	TaCBF2	AY785900.1	945	1	1	0.308
41	TaCBFIVa-2.1	EF028769.1	950	1	1	0.308
42	TaCBFIVa-2.2	EF028770.1	822	1	1	0.308
43	TaCBFIVb-20.3	EF028774.1	980	2	2	0.308
44	TaWCBF2-2	AB178167.1	941	2	2	0.308

Table 3.8. Differentially expressed MADS box TFs under salt stress in bread wheat

S. No.	TF Name	TF ID	Seq. Length	NACET	NASET	FCE
1	TaWM14	AM502880.1	1035	1	8	2.462
2	TaAGL1	DQ512330.1	908	1	7	2.154
3	TaAGL18	DQ512335.1	970	1	7	2.154
4	TaAGL20	DQ512338.1	911	1	7	2.154
5	TaAGL21	DQ512339.1	963	1	7	2.154
6	TaAGL23	DQ512340.1	1003	1	7	2.154
7	TaAGL38	DQ512354.1	921	1	7	2.154
8	TaWM18	AM502883.1	967	1	7	2.154
9	TaWM30	AM502900.1	1118	1	7	2.154
10	TaAGL10	DQ512331.1	1146	2	3	0.462
11	TaWM7	AM502870.1	1111	2	3	0.462
12	TaAGL17	DQ512368.1	402	1	1	0.308
13	TaAGL25	DQ512342.1	1076	3	3	0.308
14	TaAGL29	DQ512346.2	1091	1	1	0.308
15	TaAGL39	DQ512355.1	959	1	1	0.308
16	TaWM2	AM502863.1	1101	1	1	0.308
17	TaWM6	AM502869.1	1163	3	3	0.308
18	TaWM8	AM502871.1	1255	1	1	0.308
19	TaWM29	AM502898.1	1141	1	1	0.308
20	TaMADS2	DQ534490.1	1102	3	3	0.308
21	TaVRT1	AY280870.1	1210	3	3	0.308
22	TaWAG2	AB465688.1	1278	1	1	0.308
23	TaMADS11	AB007504.1	1161	3	2	0.205
24	TaWM32	AM502904.1	1012	2	1	0.154

NACET: no. of alignments with control expressed transcriptome; **NASET:** no. of alignments with salt expressed transcriptome; **FCE:** fold change expression

3.3.5. Conserved genome regions among the differentially expressed TFs

The most conserved regions for differentially expressed NAC TFs were at 600-650 bp, 720-760 bp, 960-1020 bp and 1080-1140 bp (Figure 3.6). The most conserved regions for differentially expressed WRKY TFs were at 1920-1980 bp, 2280-2340 bp and 2469-2580 bp (Figure 3.7). The most conserved regions for differentially expressed MADS box TFs were at 240-360 bp, and 600-720 bp (Figure 3.8). The most conserved regions for differentially expressed AP2 containing TFs were at 1140-1320 bp (Figure 3.9). Such conserved sequence/genome regions in members of TF gene families contain conserved domain/motif of TF proteins [69,73].

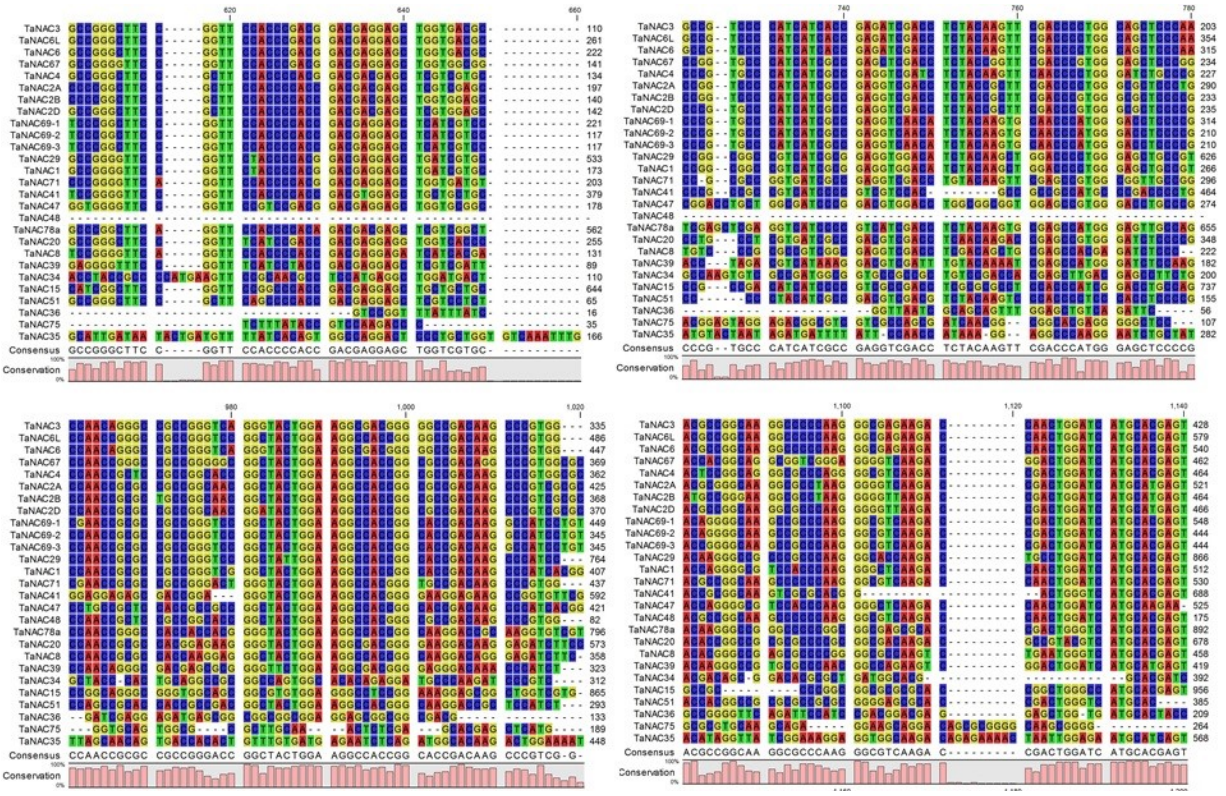

Figure 3.6. Graphical illustration of conserved genome regions in NAC TF gene family

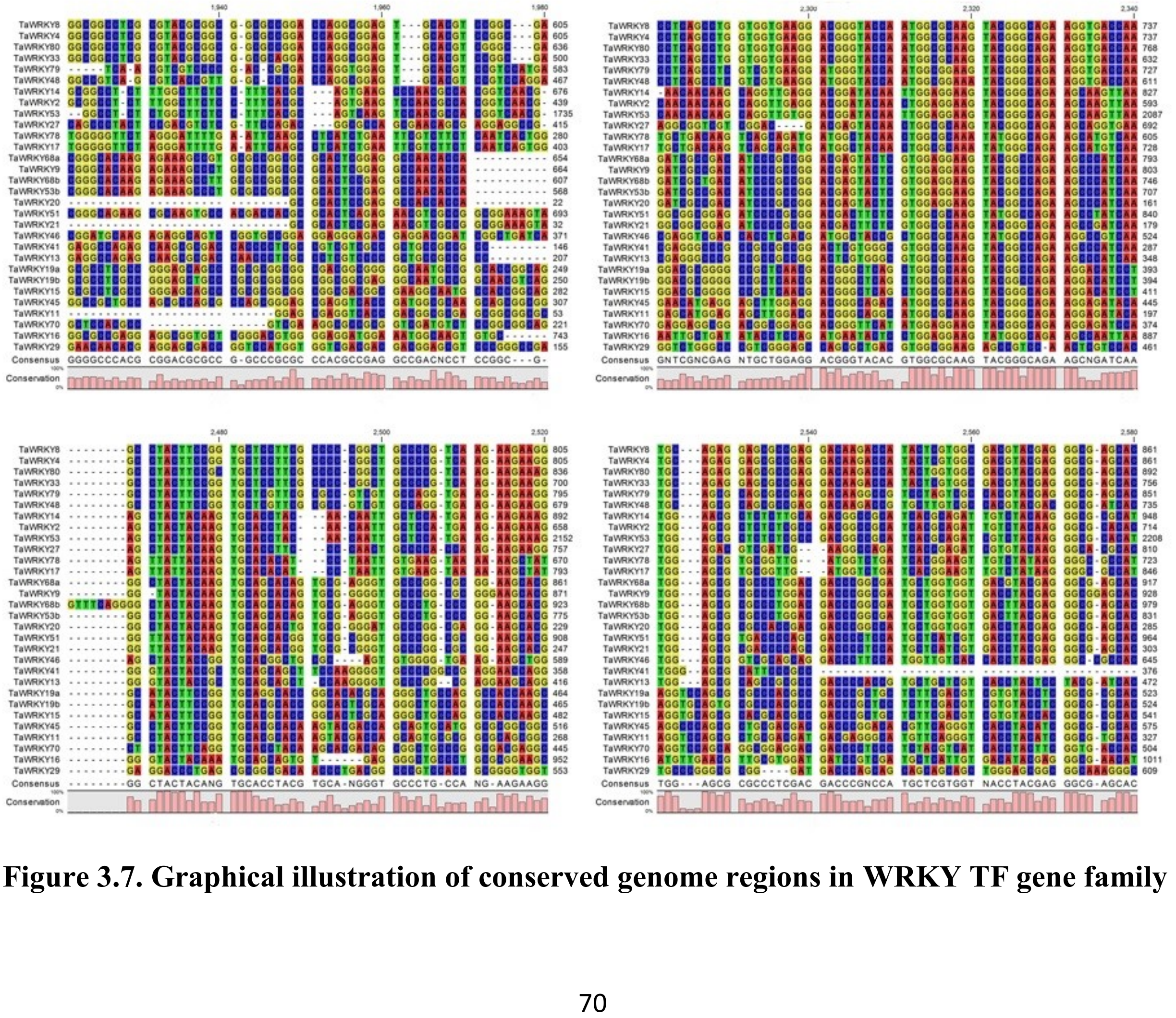

Figure 3.7. Graphical illustration of conserved genome regions in WRKY TF gene family

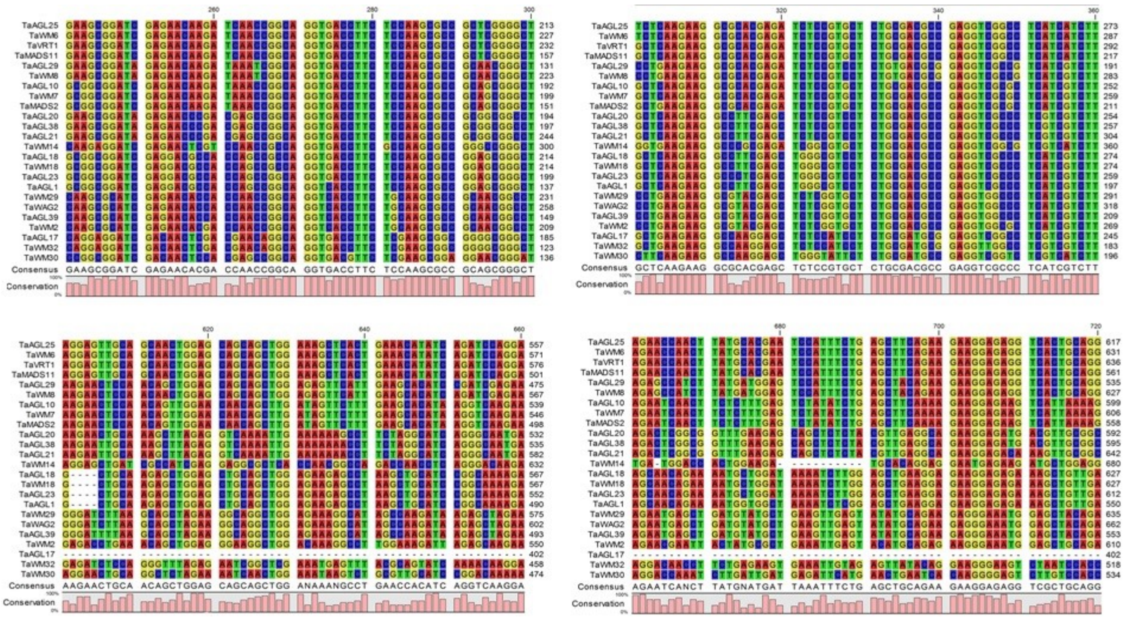

Figure 3.8. Graphical illustration of conserved regions in MADS box TF gene family

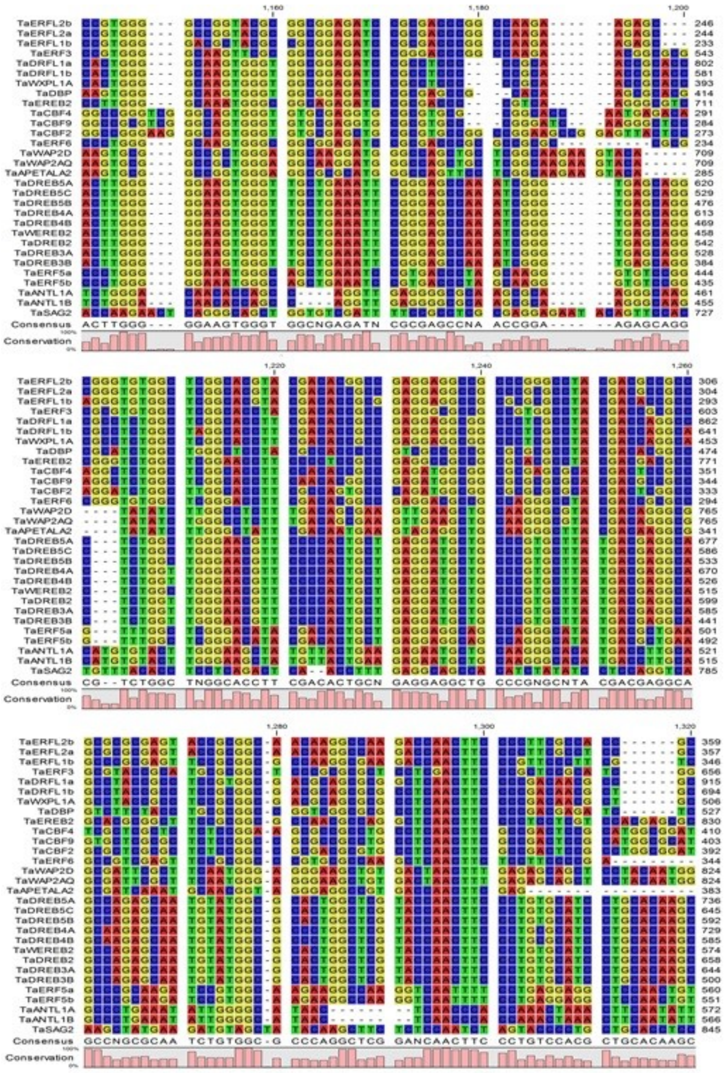

Figure 3.9. Graphical illustration of conserved regions in AP2 containing TF gene family

References

1. Wicke B, Smeets E, Dornburg V, et al. The global technical and economic potential of bioenergy from salt-affected soils. Energy Environ. Sci. 2011; 4:2669–2681

2. Munns R, James RA, Xu B, et al. Wheat grain yield on saline soils is improved by an ancestral Na+ transporter gene. Nat. Biotechnol. 2012; 30:360–364

3. Tilman D, Balzer C, Hill J, et al. Global food demand and the sustainable intensification of agriculture. Proc. Natl. Acad. Sci. 2011; 108:20260–20264

4. Munns R, James RA, Läuchli A. Approaches to increasing the salt tolerance of wheat and other cereals. J. Exp. Bot. 2006; 57:1025–1043

5. Horie T, Karahara I, Katsuhara M. Salinity tolerance mechanisms in glycophytes: An overview with the central focus on rice plants. Rice 2012; 5:1–18

6. Ali Z, Khan AS, Azhar FM, et al. The response of genetically distinct bread wheat genotypes to salinity stress. Plant Breed. 2012; 131:707–715

7. Hussain B, Khan AS, Ali Z. Genetic variation in wheat germplasm for salinity tolerance at seedling stage: improved statistical inference. Turkish J. Agric. For. 2015; 39:182–192

8. Oyiga B, Sharma C, Shen J, et al. Identification and characterization of salt tolerance of wheat germplasm using a multivariable screening approach. J. Agron. Crop Sci. 2016; 2016:1–14

9. Budak; H, Shearman RC, Gulsen O, et al. Understanding ploidy complex and geographic origin of the Buchloe dactyloides genome using cytoplasmic and nuclear marker systems. Theor. Appl. Genet. 2005; 111:1545–1552

10. Castillo A, Budak H, Varshney RK, et al. Transferability and polymorphism of barley EST-SSR markers used for phylogenetic analysis in Hordeum chilense. BMC Plant Biol. 2008; 9:1–9

11. Vogel JP, Tuna M, Budak H, et al. Development of SSR markers and analysis of diversity in Turkish populations of Brachypodium distachyon. BMC Plant Biol. 2009; 11:1–11

12. Hussain B. Modernization in plant breeding approaches for improving biotic stress resistance in crop plants. Turkish J. Agric. For. 2015; 39:515–530

13. Ali Q, Elahi M, Hussain B, et al. Genetic improvement of maize (Zea mays L .) against drought stress : An overview. Agric. Sci. Res. Journals 2011; 1:228–237

14. Hussain B, Lucas SJ, Budak H. CRISPR / Cas9 in plants : at play in the genome and at work for crop improvement. Brief. Funct. Genomics 2018; 2018:1–10

15. Budak H, Hussain B, Khan Z, et al. From genetics to functional genomics : improvement in

drought signaling and tolerance in wheat. Front. Plant Sci. 2015; 6:1–13

16. Filiz E, BS O, F B, et al. Molecular, morphological, and cytological analysis of diverse Brachypodium distachyon inbred lines. Genome 2009; 52:876–890

17. Lucas SJ, Hana Š, Jan Š, et al. Functional features of a single chromosome arm in wheat (1AL) determined from its structure. Funct. Integr. Genomics 2012; 12:173–182

18. Akpinar BA, Lucas S, Budak H. A large-scale chromosome-specific SNP discovery guideline. Funct. Integr. Genomics 2017; 17:97–105

19. Winfield MO, Allen AM, Burridge AJ, et al. High-density SNP genotyping array for hexaploid wheat and its secondary and tertiary gene pool. Plant Biotechnol. J. 2015; 14:1–12

20. Allen AM, Winfield MO, Burridge AJ, et al. Characterization of a Wheat Breeders ' Array suitable for high-throughput SNP genotyping of global accessions of hexaploid bread wheat (Triticum aestivum). Plant Biotechnol. J. 2017; 15:390–401

21. Lucas SJ, Salantur A, Yazar S, et al. High-throughput SNP genotyping of modern and wild emmer wheat for yield and root morphology using a combined association and linkage analysis. Funct. Integr. Genomics 2017; 1–19

22. Leonforte A, Sudheesh S, Cogan NOI, et al. SNP marker discovery , linkage map construction and identification of QTLs for enhanced salinity tolerance in field pea (Pisum sativum L.). BMC Plant Biol. 2013; 13:161

23. Lorieux M. MapDisto: fast and efficient computation of genetic linkage maps. Mol. Breed. 2012; 30:1231–1235

24. Masoudi B, Mardi M, Hervan EM. QTL mapping of salt tolerance traits with different effects at the seedling stage of bread wheat. Plant Mol. Biol. Report. 2015; 33:1790–1803

25. Peleg Z, Cakmak I, Ozturk L, et al. Quantitative trait loci conferring grain mineral nutrient concentrations in durum wheat 3 wild emmer wheat RIL population. Theor. Appl. Genet. 2009; 119:353–369

26. Genc Y, K O, AP V, et al. Sodium exclusion QTL associated with improved seedling growth in bread wheat under salinity stress. Theor. Appl. Genet. 2010; 121:877–894

27. Xu Y, Li S, Li L, et al. Mapping QTLs for salt tolerance with additive, epistatic and QTL x treatment interaction effects at seedling stage in wheat. Plant Breed. 2013; 132:276–283

28. Genc Y, Taylor J, Rongala J, et al. A major locus for chloride accumulation on chromosome 5A in bread wheat. PLoS One 2014; 9:e98845

29. Lindsay MP, Lagudah ES, Hare RA, et al. A locus for sodium exclusion (Nax1), a trait for salt tolerance, mapped in durum wheat. Funct. Plant Biol. 2004; 31:1105–1114

30. Lucas SJ, Šimková H, Šafář J, et al. Functional features of a single chromosome arm in wheat (1AL) determined from its structure. Funct. Integr. Genomics 2012; 12:173–182

31. Wang S, Wong D, Forrest K, et al. Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. Plant Biotechnol. J. 2014; 12:787–796

32. Jin H, Wen W, Liu J, et al. Genome-Wide QTL Mapping for Wheat Processing Quality Parameters in a Gaocheng 8901/Zhoumai 16 Recombinant Inbred Line Population. Front. Plant Sci. 2016; 7:

33. Hussain B, Khan MA, Ali Q, et al. Double Haploid Production is the Best Method for Genetic Improvement and Genetic Studies of Wheat. IJAVMS 2012; 6:216–228

34. Hussain B, Khan MA, Ali Q, et al. Double Haploid Production in Wheat through Microspore Culture and Wheat X Maize Crossing System : An Overview. IJAVMS 2012; 6:332–344

35. Hussain B, Khan AS, Ali Z. Genetic variation in wheat germplasm for salinity tolerance atseedling stage: improved statistical inference. TURKISH J. Agric. For. 2015; 39:182–192

36. Azadi A, Mardi M, Hervan EM, et al. QTL Mapping of Yield and Yield Components under Normal and Salt-stress Conditions in Bread Wheat (Triticum aestivum L.). Plant Mol. Biol. Report. 2014; 102–120

37. Genc Y, Oldach K, Gogel B, et al. Quantitative trait loci for agronomic and physiological traits for a bread wheat population grown in environments with a range of salinity levels. Mol. Breed. 2013; 32:39–59

38. Oyiga BC, Sharma RC, Baum M, et al. Allelic variations and differential expressions detected at quantitative trait loci for salt stress tolerance in wheat. Plant. Cell Environ. 2018; 41:919–935

39. Krishnappa G, Singh AM, Chaudhary S, et al. Molecular mapping of the grain iron and zinc concentration , protein content and thousand kernel weight in wheat (Triticum aestivum L .). PLoS One 2017; 12:e0174972

40. Ova EA, Kutman UB, Ozturk L. High phosphorus supply reduced zinc concentration of wheat in native soil but not in autoclaved soil or nutrient solution. Plant Soil 2015; 393:147–162

41. Zarcinas BA, Cartwright B, Spouncer LR. Nitric acid digestion and multi-element analysis of plant material by inductively coupled plasma spectrometry. Commun. Soil Sci. Plant Anal. 1987; 18:131–146

42. Rice WR. Analysing tables of statistical tests. Evolution (N. Y). 1989; 43:223–225

43. Meng L, Li H, Zhang L, et al. QTL IciMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. Crop J. 2015; 3:269–283

44. Voorrips RE. MapChart: Software for the graphical presentation of linkage maps and QTLs. J. Hered. 2002; 93:77–78

45. Zhang Z, Liu Z, Hu Y, et al. QTL analysis of kernel-related traits in maize using an immortalized F 2 population. PLoS One 2014; 9:

46. Hussain B, Lucas SJ, Ozturk L, et al. Mapping QTLs conferring salt tolerance and micronutrient concentrations at seedling stagein wheat. Sci. Rep. 2017; 7:15662

47. Akpinar BA, Yuce M, Lucas S, et al. Molecular organization and comparative analysis of chromosome 5B of the wild wheat ancestor Triticum dicoccoides. Sci. Rep. 2015; 5:1–13

48. Akpinar BA, Lucas SJ, Vrana J, et al. Sequencing chromosome 5D of Aegilops tauschii and comparison with its allopolyploid descendant bread wheat (Triticum aestivum). Plant Biotechnol. J. 2015; 13:740–752

49. Goyal E, Amit SK, Singh RS, et al. Transcriptome profiling of the salt-stress response in Triticum aestivum cv. Kharchia Local. Sci. Rep. 2016; 6:1–14

50. IWGSC. A chromosome-based draft sequence of the hexaploid bread wheat (Triticum aestivum) genome. Science (80-.). 2014; 345:286–287

51. Götz S, Terol JMG-GJ, Williams TD, et al. High-throughput functional annotation and data mining with the Blast2GO suite. Nucleic Acids Res. 2008; 36:3420–3435

52. Song M, Xu W, Xiang Y. Association of jacalin-related lectins with wheat responses to stresses revealed by transcriptional profiling. Plant Mol. Biol. 2014; 84:95–110

53. Xu Y, Zhou Y, Hong S, et al. Functional characterization of a wheat NHX antiporter gene TaNHX2 that encodes a K+/H + exchanger. PLoS One 2013; 8:1–12

54. Cuin TA, Betts SA, Chalmandrier R, et al. A root’s ability to retain K+ correlates with salt tolerance in wheat. J. Exp. Bot. 2008; 59:2697–2706

55. Brini F, Gaxiola RA, Berkowitz GA, et al. Cloning and characterization of a wheat vacuolar cation/proton antiporter and pyrophosphatase proton pump. Plant Physiol. Biochem. 2005; 43:347–354

56. Akpinar BA, Avsar B, Lucas SJ, et al. Plant abiotic stress signaling. Plant Signal. Behav. 2012; 7:1450–1455

57. Kuzuoglu-Ozturk D, O CY, BA A, et al. Autophagy-related gene, TdAtg8, in wild emmer wheat plays a role in drought and osmotic stress response. Planta 2012; 236:1081–1092

58. Li S, Xu C, Yang Y, et al. Functional analysis of TaDi19A, a salt-responsive gene in wheat. Plant Cell Environ. 2010; 33:117–129

59. Zhao Y, Dong W, Zhang N, et al. A wheat allene oxide cyclase gene enhances salinity tolerance via jasmonate signaling. Plant Physiol. 2014; 164:1068–1076

60. Zhang H, Mao X, Wang C, et al. Overexpression of a common wheat gene TaSnRK2.8 enhances tolerance to drought, salt and low temperature in Arabidopsis. PLoS One 2010; 5:2–13

61. Karijolich J, Yu Y. The new era of RNA modification. RNA 2015; 21:659–660

62. Chen Ping G, Shi W, Jing Z, et al. Isolation and characterization of TaGSK1 involved in wheat salt tolerance. Plant Sci. 2003; 165:1369–1375

63. Xu W, Lv H, Zhao M, et al. Proteomic comparison reveals the contribution of chloroplast to salt tolerance of a wheat introgression line. Sci. Rep. 2016; 6:1–13

64. Dong W, Wang M, Xu F, et al. Wheat oxophytodienoate reductase gene TaOPR1 confers salinity tolerance via enhancement of Abscisic Acid signaling and reactive oxygen species scavenging. Plant Physiol. 2013; 161:1217–1228

65. Yousfi F, Makhloufi E, Marande W, et al. Comparative Analysis of WRKY Genes Potentially Involved in Salt Stress Responses in Triticum turgidum. Front. Plant Sci. 2017; 7:

66. Qin Y, Tian Y, Liu X. Biochemical and Biophysical Research Communications A wheat salinity-induced WRKY transcription factor TaWRKY93 confers multiple abiotic stress tolerance in Arabidopsis thaliana. Biochem. Biophys. Res. Commun. 2015; 464:428–433

67. Wang C, Deng P, Chen L, et al. A Wheat WRKY Transcription Factor TaWRKY10 Confers Tolerance to Multiple Abiotic Stresses in Transgenic Tobacco. PLoS One 2013; 8:

68. Wang X, Zeng J, Li Y, et al. Expression of TaWRKY44 , a wheat WRKY gene , in transgenic tobacco confers multiple abiotic stress tolerances. Front. Plant Sci. 2015; 6:1–14

69. Mao X, Zhang H, Qian X, et al. TaNAC2, a NAC-type wheat transcription factor conferring enhanced multiple abiotic stress tolerances in Arabidopsis. J. Exp. Bot. 2012; 63:2933–2946

70. Guo W, Zhang J, Zhang N, et al. The Wheat NAC Transcription Factor TaNAC2L Is Regulated at the Transcriptional and Post-Translational Levels and Promotes Heat Stress Tolerance in Transgenic Arabidopsis. PLoS One 2015; 10:1–11

71. Baloglu MC, Oz MT. Expression Analysis of TaNAC69-1 and TtNAMB-2 , Wheat NAC Family Transcription Factor Genes Under Abiotic Stress Conditions in Durum Wheat (Triticum turgidum). Plant Mol. Biol. Report. 2012; 2:1246–1252

72. He Y, Li W, Lv J, et al. Ectopic expression of a wheat MYB transcription factor gene ,

TaMYB73 , improves salinity stress tolerance in Arabidopsis thaliana. J. Exp. Bot. 2018; 63:1511–1522

73. Ma J, Yang Y, Luo W, et al. Genome-wide identification and analysis of the MADS-box gene family in bread wheat (Triticum aestivum L .). PLoS One 2017; 12:e0181443.

74. Ren Y, Teng W, Li B. QTLs for seedling traits under salinity stress in hexaploid wheat. Cienc. Rural 2018; 48:1–9

75. James R, Davenport R, Munns R. Physiological characterization of two genes for Na+ exclusion in durum wheat, Nax1 and Nax 2. Plant Physiol. 2006; 142:1537–1547

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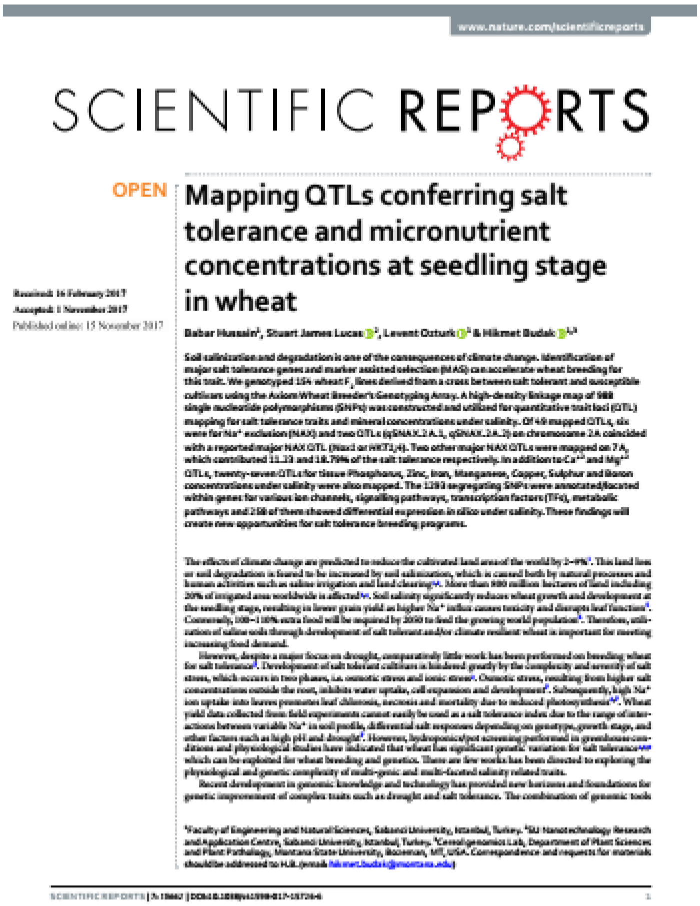

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