

### **General Outlook of identified genomic regions/QTLs associated with salt tolerance traits in wheat (*Triticum aestivum* L.)**

#### **Abstract**

Salinity stress declines plant growth and its efficiency, which is leading to a substantial reduction in crop yield. Presently, the worldwide challenges are to meet the food consumption demand, along with the decreasing crop productivity per unit area at the same time of stress environment. Wheat (*Triticum aestivum* L.) is one of the major cereal grain crops and losses gain yield exceeds the 60% due to salinity stress. Now, it is imperative to develop a comprehensive understanding of salt tolerance contrivances and the assortment of reliable tolerance indices is crucial for breeding salt-tolerant wheat cultivars. The specific chromosomal location of these salt-tolerant genes or genetic loci has also been partially characterized through QTLs mapping that cannot use directly in breeding programs. This information helps the efficient transfer of these genes into other crop cultivars through molecular breeding tools. This review highlights the using association techniques for identifying novel QTLs/genomic regions associated with salinity tolerance in wheat that can help to improve salt tolerance in wheat through marker-assisted breeding programs.

**Keywords:** Association mapping, Next Generation Sequencing, Salinity tolerance, Bread wheat

#### **1. Introduction**

Wheat (*Triticum aestivum* L.) is one of the major cereal crops, grown worldwide supplying nearly 2.5 billion of the total world population. It is the most strategic staple crop, occupying 33% of total food grain production area of the worldwide and providing a source of ~20% protein in the form of the calories and human diet <sup>[1]</sup>. According to the current scenario of the United States Department of Agriculture (USDA), Foreign Agricultural Service, the global wheat production was recorded about 768 million metric tons in the year 2020-21. Among all the crops, wheat is holding the highest position in production by occupying approximately 217 million hectares areas worldwide. India is the second largest producer at 103 million tons and a major exporting country for the wheat after the USA. Among all the Indian states, Uttar Pradesh utilizes largest area with 9.75 million hectare (32%), followed by Madhya Pradesh, Punjab, Rajasthan and Haryana in the wheat production <sup>[1]</sup>. The expanding world population, demands nearly a 70% increase in food production by the end of 2050 <sup>[2]</sup>. On the other hand, climate changes, reduction in cultivated land, increase use of chemicals has subjected the sustainable production of crop plants worldwide. The emerging scenario challenge us to not only develop new methods and strategies that can alleviate the responsible factors but also develop techniques to boost the yield of crop plants is of utmost importance <sup>[1]</sup>.

Salinity is one of the prominent abiotic stress factors affecting crop yields worldwide; approximately 6% of the world's total land area is threatened by salinity, including 20% of arable land and 33% of irrigated land <sup>[3,1]</sup>. The negative impact of salinity on plant growth achieved by dismantling osmotic potential and cellular homeostasis of the plant which directly affects the metabolic functions and hence, resulted into reduced growth of the plants. Primarily, saline conditions disturb sodium and chloride ion exchange within the plants. One of the most effective and feasible ways to minimize the detrimental effects of salinity on crop production is to enhance the salinity-tolerant ability. Crops comprising natural

variants from a spontaneous mutation in their wild relatives and further domestication and breeding impacted their genetic diversity found in modern crops <sup>[4]</sup>. Understanding the crop genetic of phenotypic variations due to domestication and cultivation can be utilized as diverse resources for the improvement of crop productivity.

Salt tolerance in wheat is a complex trait (quantitatively inherited) controlling by multiple genes or QTLs (quantitative trait locus) involving traits such as osmotic adjustment, ions compartmentalization, and morphological and yield associated traits such as plant height, biomass, grain yield, thousand-grain yield, and grain number <sup>[5,6]</sup>. QTLs mapping/Association mapping method has been widely used in the understanding of genetics and molecular basis of salinity tolerance in the crop. Analysis of QTLs has revealed the approximate locations of significant markers associated with salt tolerance traits across the genome and considerable potential for improving salt tolerance of bread wheat by marker-assisted selection. The present study discusses various genetic tools used for the identification of the suitable genes responsible for attributing salt resistance in wheat varieties. This preview can be useful to program future studies and to classify major and stable QTLs that can be considered to be cloned or further examined by researchers for forthcoming applications in plant breeding programs (Figure 1).

## **2. Current status of salinity affected areas**

Salinity is the foremost damaging environmental stress for plant growth and crop production. It can increase hurriedly in the soil by creating challenges for plants in saline conditions <sup>[5]</sup>. Due to high salt levels, about 1.5 million hectares of land are inappropriate for agricultural production. Nowadays, it is growing in such a way that over 50% of global land will be salinized by 2050 <sup>[7,8,9]</sup>.

Including India, other countries such as Bangladesh, Australia, China, Egypt, Mexico, Pakistan and Turkey are also suffering from salt-affected soils. Presently, India covering

about 6.73 million hectares of salt-affected soils including higher area with Gujarat (2.23 mha) followed by Uttar Pradesh (1.37 mha) and Maharashtra (0.61 mha). Due to continuous suffering from marginal quality water and the use of natural resources in the state of Rajasthan, Haryana, and Punjab, the salt-affected area of the country would be increased to 20 million hectares by 2050<sup>[10]</sup>. The ICAR-Central Soil Salinity Research Institute, Karnal entirely working on the development of salt tolerant wheat varieties. Till date, five salt tolerant varieties of wheat (KRL 210, KRL 213, KRL-19, KRL 1-4, KHARCHIA 65) have been developed with yield potential of 43-48 q/ha under irrigated timely sown and salinity stress conditions.

### **3. Sources and Types of salinity**

Salinity is the water-soluble salts dissolved naturally in water and soil and above the soil ECe-4 dS/m increasing soluble salt concentration (salts such as sodium, chloride, calcium, magnesium, and sulfate) is called salinization. Na<sup>+</sup> represents predominant and exchangeable cation which creates toxicity in crops. The first effects of salts, it reduced the water availability that creates difficulties in water uptake since decreased the osmotic potential in root zone comparison in the soil moisture resulting in decreased plant growth. The second effect of salt stress on plants is chlorophyll degradation causing leaf senescence or old leaves death. Third, the Na<sup>+</sup> molecule enters the cytosol of plants causes physiological damage and because of the high level of Na<sup>+</sup> in soil started to compete with potassium (K<sup>+</sup>) ions which essential for plant growth results facing the difficulties to absorbing sufficient K<sup>+</sup>. Hence both toxic effects lead to a reduction in biomass and yield of the crop<sup>[5]</sup>. The sources of salinity have been categorized as primary factors (mineral weathering, capillary rise from shallow brackish groundwater, intrusion or tidal movements of seawater along the coast, estuaries, and salt-laden, sand blown by sea winds, soil erosion or soil degradation and secondary factors (irrigation through poor drainage system, overuse of industrial effluents and

fertilizers, flooding with salt-rich waters, high water tables. Generally, three types of soils are present all over the salt-affected areas: saline soils, sodic soils, and saline-sodic. Saline soil contains high water-soluble salts i.e., NaCl, CaCl<sub>2</sub>, MgCl<sub>2</sub>·6H<sub>2</sub>O, Na<sub>2</sub>SO<sub>4</sub>, MgSO<sub>4</sub> (ECe ≥ 4.0 dS/m ESP < 15, pH < 8.5). Whereas sodic soil contains a high proportion of Na<sup>+</sup> ions ≥ relative to other cations. (ECe ≤ 4.0 dS/m ESP > 15 (or SAR > 13), pH > 8.5). The third, saline-sodic is mostly present in the arid and semiarid zone with neutral soluble salts (ECe ≥ 4.0 dS/m, ESP > 15, pH < 8.5)<sup>[11]</sup>. Plants grow well in high salt concentrations called halophytes however some plants that cannot survive even at 10% of seawater are called glycophytes<sup>[12]</sup>.

#### **4. Mechanisms ascribing tolerance against salinity stress in plants:**

Plants evolve different types of physiological, biochemical and molecular mechanisms to survive in high salinity stress conditions. The mechanisms including such as osmotic balance, ion homeostasis, Na<sup>+</sup> compartmentalization, ion transport/uptake, biosynthesis of compatible solutes, activation of antioxidant enzyme and signaling pathways, hormones regulation, transcriptional/post-translational regulations of genes at different time points. There are three mechanisms of salinity tolerance in plants:

**1. Osmotic tolerance:** Osmotic stress induced by high salt content around the root zone of soil which immediately decreases the osmotic potential inside the soil water as resulting reduces plant growth and stomatal conductance. Many plants are regulating the water loss through stomatal conductance as indicator of plant water status during saline stress condition. Previous studies demonstrated that stomatal closure is assumed to be initiated by ABA synthesis. This osmotic effect is same as in effect of drought stress condition<sup>[5,13,14]</sup>. Plants have an ability to sustain plant growth and stomatal conductance during osmotic stress if they were in non-saline condition, which is referred as osmotic tolerance. Early seedling effects of

osmotic stress such as inhibit cell elongation, thicker leaves, less lateral branches formation then finally reduction in shoot development. Salt affects more on shoot growth in comparison to root but some studies are reported that formation of lateral roots is turn down during salinity<sup>[5,15]</sup>. The mechanism linked with osmotic tolerance is little known but several studies thought that there was a long-distance signal from root controls plant growth reduction mediated by ROS waves, Ca<sup>2+</sup> waves<sup>[5,6]</sup>.

**2. Shoot ion exclusion:** High Na<sup>+</sup> accumulates in leaves rather than roots creating metabolic toxicity of Na<sup>+</sup> that causes dead leaves or cells die due to inhibition of many enzymatic activity and lack of photosynthetic ability in leaf cell. The high Na<sup>+</sup> concentration in leaves causes a metabolic toxicity which compete with K<sup>+</sup>. At cellular level, K<sup>+</sup> as a co-factor playing role in activation of approximately fifty enzymes in cytosol of leave cells are being necessary for protein synthesis<sup>[5,16,17]</sup>. Previous studies were focused on Na<sup>+</sup> exclusion and Na<sup>+</sup> tissue tolerance since Na<sup>+</sup> ion is more toxic than Cl<sup>-</sup><sup>[18,19]</sup>. Whereas, in some crops like soybean, citrus and grapevine, Cl<sup>-</sup> ion is more toxic than Na<sup>+</sup>. To prevent the entry of Na<sup>+</sup> in photo-synthetically active tissues, sodium exclusion mechanisms have been found in crops. The mechanism, Na<sup>+</sup> ion exclusion associated with salt tolerance is already present in some cereal crops such as rice, durum wheat, bread wheat and barley. Na<sup>+</sup> exclusion method generally involves up or down regulations of specific ion channels/transporters allowing control of Na<sup>+</sup> transport in cytoplasm throughout the plant's life. Till now, two transporter genes have been identified which involved in exclusion: SOS (salt overly sensitive) and HKT (high-affinity potassium transporter family). The SOS gene consisting three proteins, SOS1 encoding functional Na<sup>+</sup>/H<sup>+</sup> antiporter which is controlling by generated proton gradient through ATPase activity localized in plasma membrane. Some studies suggested that SOS1 (Na<sup>+</sup>/H<sup>+</sup> antiporter) involved in Na<sup>+</sup> exclusion back to the soil and overexpression of this SOS1 gene in Arabidopsis confers the salt tolerance in transgenic plants. The second protein

SOS2, which encodes a protein serine/threonine kinase consists N-terminal (catalytic) and C-terminal (regulatory) domain involved in  $\text{Ca}^+$  ion signals <sup>[20,21]</sup>. Third protein SOS3,  $\text{Ca}^+$  ion binding protein which contains a myristoylation site at N-terminal, conferring salt tolerance <sup>[22]</sup>. In wheat, two up-regulated SOS gene were also identified, TaSOS1 (a transmembrane  $\text{Na}^+/\text{H}^+$  antiporter) and TaSOS4 (a cytoplasmic pyridoxal (PL) kinase) and their expression analysis were measured in cultivated and wild wheat by using qRT-PCR technique under salinity stress. Second, two classes of HKT (high-affinity potassium transporter family) known for controlling  $\text{Na}^+$  ion distribution in plants, class I:  $\text{Na}^+$  selective transport, class II:  $\text{Na}^+/\text{K}^+$  co-transport. The gene AtHKT1;1 has been found in Arabidopsis which localized in the root stele and overexpression of AtHKT1;1, enhances plants salt tolerance by decreasing  $\text{Na}^+$  transport to the shoot via the transpiration stream <sup>[23]</sup>.  $\text{Na}^+$  exclusion is most effective way to improve salt tolerance in many cereal crops by preventing the entry of  $\text{Na}^+$  ion to the shoot.

**3. Shoot tissue tolerance:** In high salinity stress condition, when plants fail to exclude  $\text{Na}^+$  ion from shoot, plants start to accumulate  $\text{Na}^+$  in vacuoles via cytoplasm in shoot that creates a detrimental effect on various stages or mechanisms of plants growth. Many authors suggested that  $\text{Na}^+$  ion accumulation in the cytosol of plants is very problematic during salinity stress. Therefore, tissue tolerance mechanisms are evolved to tolerate high salt concentration in plants also refers as  $\text{Na}^+$  compartmentalization in the vacuole. To regulate  $\text{Na}^+$  sequestration to vacuole from cytoplasm, several channels/transporters in vacuole have been found such as vacuolar NHX ( $\text{Na}^+/\text{H}^+$ ) transporter involves in the transportation of  $\text{Na}^+$  ion from the cytosol into the vacuole. In vacuolar membrane, two types of  $\text{H}^+$  pumps are present, vacuolar type H -ATPase (V-ATPase) and the vacuolar pyrophosphatase (V-PPase) <sup>[24]</sup>. The V-ATPase is known as dominant due to playing a role in maintaining solute homeostasis using secondary transport and facilitating vesicle fusion under salt stress. A study reported a study in *Vigna unguiculata* that during salinity stress condition, activity of

V-ATPase pump increased in seedlings whereas activity of V-PPase pump was inhibited <sup>[25]</sup>. The functional activity of NHX depends upon tonoplast membrane-localized H<sup>+</sup>-ATPase (V-ATPase) and H<sup>+</sup>-ATPase (V-PPase). Overexpression of vacuolar AtNHX1 or AVP1 is involved in plant salinity tolerance <sup>[22]</sup>. To tolerate the toxicity of Na<sup>+</sup>, plants need some osmotic adjustment in the vacuole and this process can be acquired by continue increasing cytosolic K<sup>+</sup> and accumulation of compatible solutes (low molecular weight soluble compounds) including such as glycine betaine, proline, sugars (sucrose and raffinose), polyols (mannitol and sorbitol) <sup>[5,24]</sup>. The functions of these solutes are protecting the cells by osmotic adjustment and stabilizing the tertiary protein structure by the help of shielded photosynthetic apparatus from stress damages such as reactive oxygen species (ROS). Several genes controlling the biosynthesis rate of these compatible solutes which play a role in enhancing salt tolerance. For example, the rate-limiting enzyme pyrroline-5-carboxylate synthetase (P5CS) gene involved in proline biosynthesis to enhanced the salt tolerance in *Arabidopsis thaliana*. Na<sup>+</sup> sequestration is an important part of tissue tolerance mostly used by both halophytes and glycophytes <sup>[5, 16]</sup>.

Salinity stress can cause disruption of electron transport chains (ETC) in chloroplasts and mitochondria that producing the ROS and oxidative stress. Singlet oxygen (O), hydroxyl radical (OH), superoxide radical (O<sub>2</sub>), and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) are strong oxidizing agents causing harm to cell integrity. Antioxidants such as glutathione peroxidase (GPX), ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD) and glutathione reductase (GR) play a critical role in detoxifying ROS induced by salt stress <sup>[5,16]</sup>. During salt stress, ABA works as cellular signals in plants that increased numbers of salt and water deficit-responsive genes that also correlated with accumulation of K<sup>+</sup>, Ca<sup>2+</sup>, proline and sugars content in vacuoles of roots, which counteract with the uptake of Na<sup>+</sup> and Cl<sup>-</sup>. In wheat, ABA treatment induces the expression of MAPK4, TIP 1 and GLP 1 genes under

salinity stress. Some other compounds having hormonal properties, such as salicylic acid (SA) and brassinosteroids (BR), also participate in salinity responses <sup>[26]</sup>.

## **5. Methods for the Detection of QTLs responsible to salt tolerance**

**1. Phenotyping:** Accurate phenotyping of AM panel is needed for reliable GWAS. An increasing the number of individuals/lines enhance the power of marker trait associations rather than increasing marker numbers for genotyping. The phenotypic data can be improved by more replication/trial's overall locations and years that also increase the power of QTLs detection. Precise phenotyping of large populations in replicated manner will require appropriate field designs with accurate statistics and minimum errors.

**2. Mapping Populations/Association panel:** The success of association mapping largely depends upon varietal population/material used for analysis. Population can be historical germplasm, family-based and breeding populations. For AM, biparental and multiparent populations, can also be used. Biparental crosses included doubled haploid, F3 generations and others generated by random mating or group mating of inbreeds in diallel scheme. In case of multi-parental populations, multiparent advanced generation intercrosses (MAGIC) and nested association mapping (NAM) populations are becoming very popular. Both MAGIC and NAM populations are used for variety development and crop improvement with the help of association mapping and linkage-based mapping. For these lines/genotypes, term used as association mapping population or association mapping panel includes high genetic diversity.

**3. Molecular Markers, High throughput genotyping (NGS):** More knowledge on the genetic variation and determinants of diversity is useful for discovering new genes in crops. Diverse molecular marker systems are now available for genetic mapping in various crops. For characterizing germplasm and genetic diversity analysis different types of molecular markers used previously such as RFLP, RAPD, AFLP, SSR are available. But nowadays,

markers except SSR are not use extensively in crop breeding programs due to inefficient application in marker-assisted-selection. Single-nucleotide polymorphisms (SNPs) are common type of genetic variation among crops and emerged as the most abundant molecular marker that is amenable to high-throughput genotyping. The availability of SNP genotyping platforms would facilitate the genetic dissection of multiples traits for economic importance and the application of marker-assisted selection. Thousand's millions of SNPs reported in various crops such as Arabidopsis, rice, soybean and barley using for evaluating genetic diversity, population structure, and association analysis that gained much interest in the scientific and breeding community. In wheat, several high-density SNP genotyping arrays (35 K, 55 K, 90 K, 660 K and 820 K) have been developed and increasingly being used for molecular dissection of complex traits using GWAS. SNP markers can be developed from sequencing resources such as expressed sequence tags (EST sequence), amplicon sequencing, sequenced genomes and next generation sequencing (NGS) <sup>[27]</sup>.

**4. Association mapping:** Association mapping or "linkage disequilibrium mapping", is a method of mapping quantitative trait loci (QTLs) that takes advantages of historical linkage disequilibrium (LD) to link phenotypes to genotypes for uncovering the genetic associations. Thousands of QTLs associated with significant markers for multiples traits have been identified in various crops by using biparental populations and interval mapping (IM). However, QTLs detected by these biparental and interval mapping methods were relevant for only those breeding programs where researchers were using parents for crossing/breeding program. But actually, in this case, parents may or may not be different from this QTLs as such, detected QTLs through IM could not apply in actual breeding programs, so that these marker–trait associations (MTAs) are partially useful for marker-assisted selection (MAS) in a wider range of crop breeding programs. To overcome these problems, multiple-line cross QTL mapping was started to used <sup>[28]</sup>. Although, due to strong linkage disequilibrium (LD),

the high detection power but poor resolution was found in linkage-based QTL-IM methods which makes difficulty for fine mapping. Moreover, this method is very laborious for used in genetic studies because of development of biparental population which is costly and time taking. LD-based association mapping (AM) is a choice of an alternative method uses set of genotypes (known/unknown ancestral) which carry high genetic variability for the particular trait of interest and product of multiple historic recombination events, thus AM provides higher resolution during QTL mapping<sup>[28]</sup>. However, MTAs detected by association mapping are spurious because of the reason that linkage disequilibrium does not only based on linkage but also includes population stratification and individuals' relatedness. To overcome these problems, family-based AM has been launched and AM used either population-based or family-based<sup>[29]</sup>. For overcome more limitations and exploit the benefits, researches have made efforts to combine both methods (linkage-based QTL-IM with LD-based AM) and conduct joint linkage AM (JLAM) with linkage and LD.

**5. Concept of Linkage Disequilibrium:** Initially LD was concerned for population genetics, later LD recognized in evolutionary biology and human genetics however after passing the time LD rapidly grew fast in 1980 for use in genetic mapping to identified closely linked loci. LD defined as the differences between the observed frequency of a combination of alleles at two loci and the frequency expected for random association of alleles at two loci. It is assumptions that, in the evolutionary time, random recombination events will give equal distribution of alleles at each locus. So that, the frequency of alleles at a given locus will be independent of alleles at other linked loci. In a random mating population, although allelic frequencies remain constant generation to generation but whenever changes occur in gene due to evolutionary factors such as mutation, selection, migration, and random drift then after allelic frequencies will automatically change. For example, a gene has two alleles A, a and their frequencies p and q, then genotype frequencies will be  $p^2$  (AA),  $q^2$  (aa) and  $2pq$  (Aa) at

this locus called Hardy–Weinberg equilibrium. Sometimes this equilibrium may be disturbed by more than one evolutionary factor, then after it can be restored naturally in next generation due to multiple recombination events. Suppose, we have two independent segregating genes (alleles A, a and B, b) then the frequencies of their allelic combinations (AB, Ab, aB, and ab) would be equal to the products of allelic frequencies of two genes. Therefore, the observed allelic frequency AB ( $p_{AB}$ ) will be the product of two allelic frequencies A ( $p_A$ ) and B ( $p_B$ ) and allelic frequency Ab ( $p_{Ab}$ ) will be the product of another allelic frequencies of A and b. So, the four allelic combinations with frequencies will be  $p_{AB}$ ,  $p_{Ab}$ ,  $p_{aB}$ , and  $p_{ab}$ , respectively. At equilibrium level, allelic frequencies ( $p_{AB}$ ,  $p_{ab}$ ) will be equal to the allelic frequencies ( $p_{Ab}$ ,  $p_{aB}$ ) but whenever the equilibrium may be disturbed by any evolutionary factors then after frequencies ( $p_{AB}$ ,  $p_{ab}$ ) does not equal to the frequencies ( $p_{Ab}$ ,  $p_{aB}$ ). The differences between frequencies ( $p_{AB}$ ,  $p_{ab}$  and  $p_{Ab}$ ,  $p_{aB}$ ) is actually referred as disequilibrium (d). The linkage disequilibrium is referring that a distance of one allele at a locus occurs with another allele at the second locus can be more than expected in random assortment of the two loci and these two loci may represent as two markers, two genes/QTLs, or one gene/QTL. In every generation, after random mating, disequilibrium (d) will be decline by the value of  $rd$  ( $r$  = frequency of recombination between the two loci). Actually, this phenomenon known as LD decay since it will be less than the decline in disequilibrium when genes segregating independently. Whenever a mutation occurs, it will reveal complete LD with this mutant allele present with them at flanking regions. Therefore, the more recombination will lead to a decline in the level of LD between the two loci <sup>[30]</sup>.

**6. Integrated approaches for GWAS (Multi-locus Mixed Model):** Generally, GWAS uses single locus model to detect the QTLs/genomic regions associated with particular target traits (Table 2). However, quantitative traits are a complex trait that governed by multiple QTLs. So single locus model test cannot be entirely reliable until test statistic cannot be expanded

with structured populations. This problem could be overcome after adding multiple QTLs as cofactors in the test model. In GWAS, multi-locus mixed model (MLMM) was proposed on the basis of multiple loci as a cofactor in the associations model and it is a simply stepwise mixed regression analysis combined with forward inclusion and backward elimination of loci. The advantage of multi-locus mixed model increases with trait heritability and high-resolution power. MLMM performs better than single locus model combined with structured population and phenotypic traits are governed by multiple QTLs having large significant effects. In MLMM, results are expected with low false discovery rate (FDR) than single locus tests because of performed multiple testing of marker trait association by using Bonferroni correction, which is too conservative so that the result sometimes missed many important genomic regions. To address these problems, multi-locus mixed linear models were developed with high power resolution to detect significant QTNs/genomic regions<sup>[31,32]</sup>. In multi-locus-GWAS models, markers effects are simultaneously tested, these are considered more appropriate genetic model for dissection of complex traits. Six types of models were included in mrMLM packages, ISIS EM-BLASSO<sup>[33]</sup>, FASTmrEMMA<sup>[32]</sup>, pLARmEB<sup>[31]</sup>, pKWmEB<sup>[34]</sup> and FASTmrMLM<sup>[33]</sup>.

## **6. Previous identified QTLs/genomic regions for salt tolerance**

AM has been carried out in many crops and QTLs associated with traits of interest have been identified. QTL Mapping makes a considerable contribution to increase the efficiency of breeding varieties with improved responses for abiotic stresses; e.g. drought and salinity. QTL mapping are applied on various crop species to identify the major QTLs loci/genomic regions associated with salinity tolerance traits. This salt tolerance associated traits are quantitatively inherited, controlled by many genes called as a QTLs, for example, Na<sup>+</sup> exclusion trait was firstly reported in durum wheat which was located on chromosome 4D. Later, in bread wheat, major QTL locus for salinity tolerance has been mapped, Kna1

controlling Na/K discrimination in roots located on the long arm of chromosome 4D and the locus play a role in the transportation of  $K^+$  from roots to leaves. Another major salt tolerance locus Nax 1 (Sodium Exclusion locus 1) has been identified on chromosome 2AL<sup>[36]</sup>. The locus Nax 1 was identified in F2 mapping population which were derived from crosses between Line 169 (salt tolerant durum line) x Tamro (a salt-sensitive Australian durum wheat cultivar 248). Further second locus, Nax2 (Sodium Exclusion locus 1) from line 169 (salt tolerant durum line) was identified on chromosome 5AL. These genes encoded for high-affinity K transporter (HKT) proteins; HKT1;4 (Nax1) and HKT 1;5 (Nax2) and their possible role were in regulating the  $Na^+$  transportation from root to shoot<sup>[36]</sup>. Last few years, QTL mapping has been applied for identification of QTLs/genomic regions in wheat for salt tolerance associated traits such as chlorophyll, seedling biomass, plant dry weights, grain yield, spikelet number, germination, tiller number and leaf injury,  $Na^+$  and  $K^+$  concentration and  $Na^+/K^+$  ratio in shoots<sup>[35-50]</sup>. GWAS applied on a diverse panel of wheat cultivars genotyped using 90K SNP array, 35K SNP array and 660K SNP array for mapping of salt tolerance traits in the seedling hydroponics and adult field condition<sup>[39,40,50]</sup>. List of previously reported QTLs are summarized in Table 1.

## 7. Conclusion

The emerging climatic issues and its effects on saline areas continuously affecting wheat growth and yield in different parts of country. In order to avoid food scarcity in upcoming future, the sustainable and effective production of salt tolerant wheat varieties are needed to develop through marker assisted breeding program. Information gained from our study could be helpful to reveals the details about various appropriate methods and techniques involving association mapping to identify salt tolerance genomic regions in wheat. Availability of sequencing information of QTLs/genomic regions database related to salinity tolerance traits

in wheat allow less time required to identify candidate genes and using in fine mapping, marker assisted selection, QTL cloning and genome editing.

### **Declarations**

### **Availability of data and materials**

The datasets supporting the results of this article are included within the article.

### **Ethics approval and consent to participate**

This study does not contain any research requiring ethical consent or approval.

### **Consent for publication**

Not applicable.

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**Table 1:** Previous identified QTLs associated with salinity tolerance traits in wheat

Traits	Chromosome	Mapping populations	References
Germination	2A, 4A, 7B, 6D	RIL (Pasban 90 X Frontana)	[38]
	2A, 4A, 7B, 6D	RIL (Pasban 90 X Frontana)	[38]

	4A, 4B, 6A, 7A	RIL (Opata 85 X W7984)	[41]
	3AL	150 wheat genotypes	[50]
Seedling vigour	2A, 4A, 7B, 6D	RIL (Pasban 90 X Frontana)	[38]
Membrane stability	3A, 4A, 5B, 7B, 7D, 3D	RIL (Pasban 90 X Frontana)	[46]
	2BL, 3BS, 5AL, 7BL	135 diverse wheat genotypes	[39]
Water Potential	5D, 2A, 5B, 6B, 6A	RIL (Pasban 90 X Frontana)	[46]
Osmotic Potential	2B, 7B, 5D, 7A	RIL (Pasban 90 X Frontana)	[46]
Relative water content	4A, 7A, 2A, 7B	RIL (Pasban 90 X Frontana)	[46]
Chlorophyll	2D, 5A, 5B, 5D	DH (Berkut X Krichauff)	[44]
	7D, 6B, 5A	RIL (Roshan × Sabalan)	[35]
	3D, 5B, 6B, 6D, 7D, 1B, 6A, 3A, 7A, 7B, 1D, 3B, 4A,	RIL (Pasban 90 X Frontana)	[46]
	2AL, 2BS, 3BS, 4AL, 7AS	135 diverse wheat genotypes	[39]
	1A, 2B, 3D, 7A	RIL (Zhongmai X Xiaoyan)	[47]
	6DS	153 diverse wheat genotypes	[40]
Root length	3B, 5B, 6B, 2D, 3D, 4D	RIL (Pasban 90 X Frontana)	[38]
	4D, 6B	RIL (Xiaoyan 54 and Jing 411)	[34]
	1A, 2A, 2B, 2D, 3B, 4A, 7B	RIL (Zhongmai X Xiaoyan)	[47]
	1DS, 2BL, 6BL	135 diverse wheat genotypes	[39]
Shoot length	2D, 3D	RIL (Pasban 90 X Frontana)	[38]
	3A, 5B, 3B, 2B	RIL (Roshan × Sabalan)	[35]
	1A, 2B, 2D, 4B, 5B, 6B	RIL (Zhongmai X Xiaoyan)	[47]
Shoot fresh weight	2A, 1D, 2D	RIL (Pasban 90 X Frontana)	[38]
	1D, 1A, 6B, 2A, 5B	RIL (Roshan × Sabalan)	[35]
	2BL, 2DS, 3AL, 5BL, 7AS, 7AL, 7BL	150 wheat genotypes	[50]
	1B, 1D, 2A, 2B, 4B, 5A, 6D	RIL (Zhongmai X Xiaoyan)	[47]
	1BL, 2AL, 3AL, 4AL, 5AL, 7AL	150 wheat genotypes	[50]
Root fresh weight	2AL, 5BL, 2BL, 2DL, 5BS, 6AS	150 wheat genotypes	[50]
	1AL, 1BS, 5BL, 6AL, 6AS, 7AS	150 wheat genotypes	[50]
Total fresh weight	2DS, 2DL, 6DL, 7AS	135 diverse wheat genotypes	[39]
Root dry weight	4A	RIL (Xiaoyan 54 and Jing 411)	[34]
	2AL, 5BL, 1BL, 5BS, 2BL, 2BS, 2DS, 4AS	150 wheat genotypes	[50]
	2A, 2B, 2D, 5A, 5B, 5D, 6A, 6D, 7D	RIL (Zhongmai X Xiaoyan)	[47]
	2BS, 3AL, 5AL, 1BL, 1DS, 5BL, 4AS, 7AS, 2AL, 4AL	150 wheat genotypes	[50]
	2AL, 3AL, 3BS, 5AL, 5BL, 6AL, 6BL, 7AL, 7AS, 7BS	150 wheat genotypes	[50]
	1AS, 1DS, 5DL, 6AL, 7BL	135 diverse wheat genotypes	[39]
Shoot dry weight	3B, 6A, 6B	RIL (Roshan × Sabalan)	[35]
	7A	RIL (Xiaoyan 54 and Jing 411)	[34]
	1BL, 2AL, 5AL, 1AL, 2DS, 5BL, 7BL, 2BL, 7BL, 1BS	150 wheat genotypes	[50]
	1B, 2A, 2B, 4B, 5A	RIL (Zhongmai X Xiaoyan)	[47]
Total dry weight (Seedling biomass)	1B, 2B, 5D	RIL (Pasban 90 X Frontana)	[38]
	2A, 4B, 5A, 5B, 6A, 6D, 7A	DH (Berkut X Krichauff)	[44]
	3A, 7A	RIL (Xiaoyan 54 and Jing 411)	[34]
	1B, 1D, 2A, 2B, 4B, 5A	RIL (Zhongmai X Xiaoyan)	[47]
	1A, 3A	RIL (Xiaoyan 54 and Jing 411)	[34]
	1AS, 3DS, 7BS	135 diverse wheat genotypes	[39]
Root shoot ratio (DW)	1AL, 6AS	135 diverse wheat genotypes	[39]
Booting	2D	RIL (Opata 85 X W7984)	[41]
Ear emergence time	2D	RIL (Opata 85 X W7984)	[41]

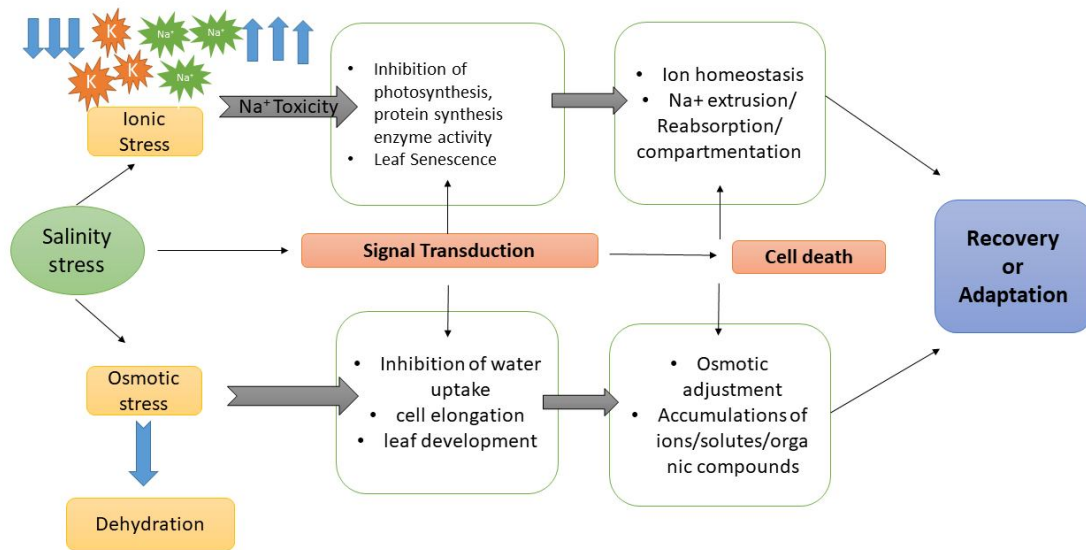
Days of heading	2D, 6D	RIL (Opata 85 X W7984)	[41]
	2BL, 7AL	150 wheat genotypes	[50]
	1A, 2B	RIL (Attila/Kauz X Kharchia)	[48]
	2D, 2A	RIL (Opata 85 X W7984)	[41]
	5A, 5B, 5D	DH (Berkut X Krichauff)	[44]
	1B	RIL (Attila/Kauz X Kharchia)	[48]
	5AL	150 wheat genotypes	[50]
Days of Maturity	5BL	153 diverse wheat genotypes	[40]
Plant height	2A, 2D, 4D, 5A, 5D, 6A, 7A	DH (Berkut X Krichauff)	[45]
	2A, 3B, 5B	RIL (Attila/Kauz X Kharchia)	[48]
	4A, 4D, 5A	191 wheat genotypes	[47]
	2AL, 7AL	150 wheat genotypes	[50]
	1AL, 6BL	150 wheat genotypes	[50]
	1DS,1DL, 2AL, 4AL, 5BL, 6BS	153 diverse wheat genotypes	[40]
Tiller number	2A, 2D, 5A, 6D	RIL (Opata 85 X W7984)	[41]
	1A, 4B, 5A, 5B, 5D	DH (Berkut X Krichauff)	[44]
	2A, 2B, 2D, 5A, 5B, 6D,7A, 7B	RIL (Zhongmai X Xiaoyan)	[47]
	1DS, 6DL, 7BS	153 diverse wheat genotypes	[40]
Ear length	1B,4A,5A,7B,2D	RIL (Opata 85 X W7984)	[41]
	2B, 5B	RIL (Attila/Kauz X Kharchia)	[48]
Ear weight	1B,4A,6B	RIL (Opata 85 X W7984)	[41]
	2A, 6B	RIL (Attila/Kauz X Kharchia)	[48]
Peduncle length	1A,1B, 6B	RIL (Attila/Kauz X Kharchia)	[48]
Spike length	5DL, 7AS, 7BL	153 diverse wheat genotypes	[40]
Spikelet number	4A, 6A, 2D	RIL (Opata 85 X W7984)	[41]
	5A	191 wheat genotypes	[47]
	2AL, 3AS, 5BL, 7DL	153 diverse wheat genotypes	[40]
Grain number	4A, 7B	RIL (Opata 85 X W7984)	[41]
	2B, 2D, 3A, 4B, 4D, 5A, 5B, 5D,6A, 6D, 7A	DH (Berkut X Krichauff)	[45]
	2A, 2B, 3B, 5D, 6B	RIL (Attila/Kauz X Kharchia)	[48]
	5B	191 wheat genotypes	[47]
	3BS, 6BS, 6BS	153 diverse wheat genotypes	[40]
Grain weight	1B	RIL (Opata 85 X W7984)	[41]
	2A, 2B, 6B	RIL (Attila/Kauz X Kharchia)	[48]
1000-grains weight	2D	RIL (Opata 85 X W7984)	[41]
	1A, 2B, 2D, 3A, 4B, 4D, 5A,5B,5D, 6A, 6B, 6D, 7A, 7D	DH (Berkut X Krichauff)	[45]
	3AL, 3AS, 5AS, 5BL 1AS, 1BS, 1BL, 7AL	150 wheat genotypes	[50]
	2BL, 3AL,5AS, 5BL, 5DL, 6BS, 7BS	153 diverse wheat genotypes	[40]
Grain Yield	7B	RIL (Opata 85 X W7984)	[41]
	2A, 2B, 3A, 3B, 4B, 4D, 5A, 5B, 5D, 6A, 6D, 7B	DH (Berkut X Krichauff)	[45]
	2B, 6B, 5D 7A	RIL (Attila/Kauz X Kharchia)	[48]
	6BL	150 wheat genotypes	[50]
	3A, 5A	191 wheat genotypes	[47]
	1BS, 1BL, 2BS, 2DS,5BL,1AL, 1BL, 3AL, 4DL, 5AL, 2AL, 2BL, 3BL, 5AL, 6BL, 6BS	150 wheat genotypes	[50]
	5BS, 6BS, 6DL	153 diverse wheat genotypes	[40]
Biological Yield	7A, 2B	RIL (Attila/Kauz X Kharchia)	[48]
	5B	191 wheat genotypes	[47]
	1BL, 6DL, 7BL	153 diverse wheat genotypes	[40]
Na <sup>+</sup> concentration (Shoot/leaf)	2AL	F2 (Line149XTamaroi)	[36]
	7AS	Two DH (Cranbrook/Halberd & Excalibur/Kukri)	[43]
	2B, 2D, 3D, 4B, 4D, 6D,7A, 7D	Backcross (Aus29639 X	[49]

		Yitpi)	
	2A, 2B, 6A, 7A	DH (Berkut X Krichauff)	[44]
	1D, 3B, 6B, 2A, 2B	RIL (Pasban 90 X Frontana)	[46]
	1A,1D, 2A,2B,2D, 3A,4D,5A,5B,5D,6A,6B,7A,7D	DH (Berkut X Krichauff)	[45]
	2A,7A	DH (Excalibur × Kukri) RIL (Excalibur × Kukri)	[37]
	6BL, 3AL	150 wheat genotypes	[50]
	1BL, 2BL, 3AL, 3BL, 5AL, 5DL, 7BL	135 diverse wheat genotypes	[39]
	1AL, 2AS, 2BS, 6AL, 6DL,	153 diverse wheat genotypes	[40]
Na <sup>+</sup> concentration (Root)	6A	RIL (Zhongmai X Xiaoyan)	[47]
Cl <sup>-</sup> concentration	1D, 2B, 3B,7A	RIL (Pasban 90 X Frontana)	[46]
	2A, 3A	RIL (Excalibur × Kukri)	[37]
K <sup>+</sup> concentration (Shoot/leaf)	1D, 3B, 3D, 4A, 5A, 5B, 5D, 7A, 7D	DH (Berkut X Krichauff)	[44]
	5A, 4A, 6A, 2B	RIL (Pasban 90 X Frontana)	[46]
	1A, 1B, 2A, 3B, 5A, 5D	DH (Berkut X Krichauff)	[45]
	2B, 5A	DH (Excalibur × Kukri)	[37]
	5AL, 1DL	150 wheat genotypes	[50]
	2AL, 5AL, 5DL, 7BL	135 diverse wheat genotypes	[39]
	1D, 2B, 2D, 4B, 5D, 6B	RIL (Zhongmai X Xiaoyan)	[47]
	1AS, 2AL, 2DL, 3BL,3BL,4BL, 5BL, 7BL	153 diverse wheat genotypes	[40]
K <sup>+</sup> concentration (Root)	1D, 3B, 5D	RIL (Zhongmai X Xiaoyan)	[47]
Na <sup>+</sup> /K <sup>+</sup> ratio	4DL	RIL for chromosome 4B/4D	[42]
	1D, 2D, 3A, 4D	RILs (Pasban90 X Frontana)	[46]
	5BL, 6BL, 6DL, 7BS	135 diverse wheat genotypes	[39]
	1BS	153 diverse wheat genotypes	[40]
K <sup>+</sup> /Na <sup>+</sup> ratio	6A	DH (Excalibur × Kukri)	[37]
	2AL	150 wheat genotypes	[50]
	2B, 4B, 6A, 5D	RIL (Zhongmai X Xiaoyan)	[47]
	2DS	RIL (Excalibur × Kukri)	[37]

Table 2: Software packages used in Association mapping

TASSEL	( <a href="http://sourceforge.net/projects/tassel">http://sourceforge.net/projects/tassel</a> ; <a href="http://www.maizegenetics.net">http://www.maizegenetics.net</a> )
EMMAX	( <a href="http://genetics.cs.ucla.edu/emmax/">http://genetics.cs.ucla.edu/emmax/</a> )
GenAMap	( <a href="http://sailing.cs.cmu.edu/genamap/">http://sailing.cs.cmu.edu/genamap/</a> )
GenABEL	( <a href="http://www.genabel.org/packages/GenABEL">http://www.genabel.org/packages/GenABEL</a> )
FaST-LMM	( <a href="http://fastlmm.codeplex.com/">http://fastlmm.codeplex.com/</a> )
GAPIT	( <a href="http://www.maizegenetics.net/gapit">http://www.maizegenetics.net/gapit</a> )
STRUCTURE	( <a href="http://pritch.bsd.uchicago.edu/structure.html">http://pritch.bsd.uchicago.edu/structure.html</a> )
SPAGeDI	( <a href="http://www.ulb.ac.be/sciences/ecoevol/spagedi.html">http://www.ulb.ac.be/sciences/ecoevol/spagedi.html</a> )
EINGENSTRAT	( <a href="http://genepath.med.harvard.edu/~reich/software.html">http://genepath.med.harvard.edu/~reich/software.html</a> )
MTDFREML	( <a href="http://aipl.arsusda.gov/curtvt/mtdfrem.html">http://aipl.arsusda.gov/curtvt/mtdfrem.html</a> )
R	( <a href="http://www.r-project.org/">http://www.r-project.org/</a> )
ASREML	( <a href="http://www.vsni.co.uk/products/asreml">http://www.vsni.co.uk/products/asreml</a> )
GenStat	( <a href="http://www.vsni.co.uk/software/genstat">http://www.vsni.co.uk/software/genstat</a> )
JMP Genomics	( <a href="http://www.jmp.com/software/genomics/">http://www.jmp.com/software/genomics/</a> )
SAS	( <a href="http://www.sas.com">http://www.sas.com</a> )

Figure 1. Overview of salinity stress pathway in wheat



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