Growing wheat on saline lands: Can a dream come true?

Armghan Shahzad[1], Muhammad Iqbal[1], Muhammad Asif[2*], Arvind H. Hirani[3], Aakash Goyal[4]

[1]Plant Biotechnology Program, National Agricultural Research Center, Park Road 45500 Islamabad, Pakistan
[2]Agricultural, Food and Nutritional Science, 4-10 Agriculture/Forestry Centre, Univ. of Alberta, Edmonton, AB T6G 2P5, Canada
[3]Department of Plant Science, University of Manitoba, Winnipeg, Canada
[4]Bayer Crop Science, Saskatoon, Saskatchewan, S7N4N1

*Corresponding author: asifrana@gmail.com

Abstract

Wheat is the third largest cereal produced in the world and it supplies over 20% of calories in human food around the globe. Wheat production and productivity directly influence human survival in developing countries and quality of life in industrial countries. World population is growing at a very rapid pace. It is one of the biggest concerns for current and future food supply due to limitations in the availability of land for agricultural production. The land available for cultivation is also affected by progressive salinization. Food security can only be mitigated by continuous increase in production and productivity of major crops like wheat. Utilization of saline land for wheat production is one of the important strategies to enhance production. Wheat and its wild relatives possess ample diversity for abiotic stresses, especially salt tolerance, however, limited research has been conducted to explore salt tolerance mechanism and its utilization in current and future wheat production. Current review has focused mainly on progressive salinity issues in agricultural land, salt tolerance mechanism in wheat and existence of genetic diversity in wild relatives of wheat and its utilization in breeding for salt tolerance. In addition, this review has highlighted different conventional, molecular and advance genomic strategies to enhance salt tolerance in wheat. Current status of available molecular markers, marker assisted selection/breeding and transgenic approaches for salt tolerance in wheat is discussed along with challenges and future research direction that could be directly employed in wheat breeding programs globally to enhance wheat production.

Keywords: marker assisted selection, molecular marker, QTL, salinity, salinization, wheat.

Introduction

Wheat is next to rice as a main source of food for consumers of developing countries and it is primary source of proteins (Braun et al., 2010). In the developing and under developed world, about 2.5 billion people survive on wheat as an element source of energy (FAO, 2010). Among these, 1.2 billion people are wheat dependent for whom wheat is a staple food and its production involves about 200 million famers and their family members. Wheat production and productivity directly influence human survival in developing countries and quality of life in industrial countries. Since, 1995 till 2006, 1.1% annual increase in yield was recorded in about 85% of 20 wheat producing countries (Dixon et al., 2009). Estimation based on this rate of annual wheat yield increment suggested that wheat yield will increase 17% by 2025, which is significantly lower than the required of 25% to feed the estimated population of that time (Rosegrant and Agcaoili, 2010). Additional wheat yield enhancement can be achieved by using the land resources which are currently unusable or forbidden to produce crop like wheat due to excess salts and other abiotic stresses. Salt stress is one of the major yield limiting factors in irrigated and rain-fed agriculture cropping systems. It is estimated that approximately 45 Mha out of 230 Mha of irrigated lands (20%) in the world is salt affected (FAO, 2005) and one third to half of the irrigated land may be heading towards this fate (Donalds et al., 1998). Irrigated land is comprised of 15% of the total cultivated land, but the productivity of irrigated land is twice as that of rain-fed land and it is known that irrigated land, approximately, produces one third of the total world's food supplies (Munns, 2005). CIMMYT research data suggested that about 10% of the cultivated areas of wheat in India, Pakistan, Iran, Mexico, Libya and Egypt are significantly affected by salinity (Mujeeb-Kazi and Diaz de Leon, 2002). In Australia, about 2 Mha of agriculture land is affected by salinity in which wheat is the major crop of rotation (ABS, 2010). The scenario becomes more persistent when the fact of increasing salinization is coupled with the increasing world population and urbanization especially in developing world. According to FAO estimates, over 200 Mha of crop land will be needed in the next thirty years to feed the booming world's population (Amin, 2002). Despite the positive impact of green revolution, 2 billion people still need access to plentiful nutritious food and out of these, 800 million are chronically malnourished (Fresco and Bauloin, 2002). It is estimated that approximately 60% increase in cereal productions will be to meet the global demand by 2050 (Rosegrant and Coline, 2003). This scenario implicates that food security is still the biggest issue despite innovation and implementation of advanced technologies in current researches. The acquisition of salt tolerance in wheat would increase the ability to tolerate salt, which may increase food supply and income to the small farmers by growing wheat on saline lands. Edward et al. (1998) argued that only 15% of the world's underdeveloped land in the coastal areas and inland salt deserts could introduce up to 130 Mha of new crop land without cutting or diverting more scarce fresh

water for agriculture. Current edge of technology may make Edward and his co-worker's argument true. A continuous spectrum of salt tolerant plants ranging from very sensitive even less than 1/10 of sea water (50 mol m^{-3}) concentration to highly salt tolerant plants that complete their life cycle at 500 mol m^{-3} is observed in the nature. Halophytes are the plants that have been naturally selected to grow in salt environment using a special anatomical design (Edward et al., 1998). The resilience of halophytes to salt accumulation reported by Volkmar et al. (1998), plants inherit the ability to restrict cytoplasmic salt entrance that inhibit the adverse impact on the enzymatic processes. The salt tolerant plants accomplish the favorable water potential gradient from soil solution to that of plant cell. Glycophytes, the plants easily damaged by salinity, on the other hand, are sensitive to salt but have a selective advantage in non-saline environment due to higher growth rate and bigger economic returns. In fact, all major crops are susceptible under saline condition. Crop growth and development in saline soils is affected for two reasons. Firstly, salts reduce the water uptake into the plants and this leads to slower growth and inadequate development, it is generally known as osmotic effect of salinity. Secondly, salts enter into plants via transpiration stream and injure cell processes resulting a reduction in further growth. This is known as ionic toxicity (Munns, 2005). Stress due to salts leads to a two phased response of plant to salinity. In the first phase, plants are exogenously affected by salts and subjected to physiological drought which is much similar response of plants like drought stress. In this case, neither Na$^+$ nor Cl$^-$ accumulates in excess amounts that inhibit growth rather these ions are accommodated in expanding vacuoles in growing tissues when they arrive in xylem. In the case of meristematic tissues which are largely fed in phloem, salt are effectively excluded (Munns, 2002). In the second phase, the plant is largely affected by salt which is accumulated inside the plant. The salt absorbed by plants in excess during water intake is restored in older leaves in the forms of Na$^+$ and Cl$^-$ at very high concentration; resulting leaves die because of gradual decline in enzymatic processes. This injury is probably caused by overloading the vacuolar capacity to compartmentalize toxic salts. Alternatively, they might accumulate in surrounding cell wall and cause relatively higher dehydration (Munns, 2005). The death rate of leaves is very crucial for plant survival. If death rate of leaves is greater than the rate of emerging new leaves, the plants are unable to survive for seed production. Natural and wild species are evolved to adapt to saline soil environment for survival and fitness, which opens an avenue to the breeders to take advantage of the salt tolerance mechanism of wild species to be adapted to some extent in cultivated crop species. Using inter- and intra-specific variations for salt tolerance, especially cultivated species, development of salt tolerant varieties through breeding has been carried out since new scientific knowledge became available. Flowers and Yeo (1995) have discussed the scheme of various breeding strategies such as: (1) incorporation of genes from halophytic relatives of crop species, (2) improving halophytes as crops, (3) selection within phenotypic range of the species or genera, (4) generation through mutation of new phenotypes followed by selection for salt tolerance, and (5) selection based on yield potential disregarding salt tolerance. Numerous breeding programs have been carried out for generating genetically adapted salt tolerant crop species that could maintain productivity. In addition, improvements in salinity tolerance by DNA technologies are very recent, but offer the ability to predict beneficial traits of salt tolerance by combining the skills of plant breeders and plant physiologists with biochemical and molecular genetic analysis.

Mechanism of salt tolerance in wheat

Abiotic stresses including soil salinity affect plant growth and development through ion cytotoxicity that alters cytosolic K$^+$/Na$^+$ ratios and osmotic tension. These phenomena disrupt distribution and homeostasis in the plant cells that lead to denature their functional and structural proteins (Smirnoff, 1998; Serrano et al., 1999; Zhu, 2001). In vitro studies suggested that most enzymes reduce their activity at the Na$^+$ concentration approaching 100mM in moderately salt sensitive plants (Greenway and Osmond, 1972). In contrast to that some plant species evolved to survive in saline soils by one or more mechanisms such as excluding Na$^+$ at the plasma membrane, sequestering Na$^+$ in intracellular vacuoles, and accumulation of osmolites and osmoprotectants (Serrano et al., 1999; Zimmermann and Sentenac, 1999; Blumwald, 2000). By evolving such mechanisms, plants can maintain proper ratios of K$^+$/Na$^+$ and optimum cellular osmolarity to withstand in saline soil environments. Individual salt tolerance mechanisms are regulated by various physiological, biochemical, metabolomic and genomic cross talk during tolerance reactions in plants. Salt tolerance plants exclude Na$^+$ by plasma membrane H$^+$-ATPase, which uses energy of ATP hydrolysis to generate electrochemical H$^+$ gradient to extrusion of Na$^+$. Biochemical studies reported Na$^+$/H$^+$ anti-porters in animals, fungi and several plant species. Similarly, Na$^+$ sequestration also dependent on endogenous H+-ATPase to establish Na$^+$ and Cl$^-$ influx through Na$^+$/H$^+$ anti-porters and chloride channels, respectively.

Enhance tolerance to salt in wheat

Mass and Hoffman (1977) in their classical work on salt tolerance classification had declared wheat (Triticum aestivum L.) as a moderately salt tolerant crop. Several approaches have been proposed to improve salt tolerance of wheat by introducing genes for salt tolerance into adapted cultivars (Munns, 2005), screening of large germplasm collections (Shahzad et al., 2012), detailed field trials of selected cultivars (Munir et al., 2011) conventional breeding methods (Salam et al., 1999) and unconventional crosses with wild relatives (Colmer et al., 2006).

Screening for salt tolerance

In vitro, greenhouse or field trials are most suitable approaches to screen wild, weedy and cultivated wheat germplasm for various levels of salt concentrations to identify salt tolerant genotypes. Based on screening, parental lines can be selected for convention and advanced breeding. Munns and James, (2003) described several salt screening methods based on measuring parameters (i) growth or yield, (ii) damage or tolerance to very high level of salinity, and (iii) physiological mechanisms (Table 1). Colmer et al. (2005) has reviewed that screening of large germplasm collections as reported by Kingsbury and Epstein (1984), Srivastava and Jana (1984), Sayed (1985), and Martin et al. (1994) had identified few lines that could tolerate salt as high as equivalent to 50% seawater. However, these efforts were lacked by subsequent field trials for selecting advanced progenies for cultivar development. A wealth of variation between wheat accessions exist that could be exploited for breeding for salt tolerance (Gorham and Wyn Jones, 2002).

Table 1. Commonly used screening methods for salt tolerance phenotypic selection in wheat.

Screening methods	Measuring parameters		Length of treatments (weeks)	Effects Osmotic/Salt specific	Advantages
Modetate salinity (50-150 mM NaCl)	Growth measurments	Root elongation	1	Osmotic	Appicable at seedling stage
		Leaf elongation	2	Osmotic	Non distrucitve
		Biomass production	4	Both	Related to field
		Yield	16	Both	Related to field
	Injury measurments	Leakage from leaf discs	3-4	Both or either	Non distrucitve
		Chlorophyll content	3-4	Both or either	Non distrucitve
		Chlorophyll fluorescence	3-4	Both or either	Non distrucitve
	Specific trait measurments	Na+ exclusion	1-2	Salt specific	Quick and non distrucitve
		K+/Na+ discrimination	1-2	Salt specific	Quick and non distrucitve
		Cl- exclusion	1-2	Salt specific	Quck and non distrucitve
High level of salinity (200-300 mM NaCl)	Germination	Survival days of seeding	1	Osmotic	Effective and quantitative
	Survival	Variation in salinity tolerance	2-8	Both or either	High tolerance stand out

In a recent effort, Shahzad et al. (2012) had identified few tolerant wheat lines that are being used for introgression of salt tolerance traits in wheat cultivars. They have also conducted extensive field trials using these wheat lines (Ahmad et al., 2011).

Wild germplasm tolerance to salt in wheat

Wild relatives of wheat as potential donors of tolerance to salinity have been investigated in different genetic pools to introgress into adopted wheat genetic background. Aronson, (1989) reported 1554 halophytic species of which, 135 were grasses, among these, 13 shared same tribe as of wheat. Halophytic members of *Triticeae* like tall wheatgrass (*Thinopyrum* spp.) are more salt tolerant than bread and feed wheat (Gorham, 1994; Colmer et al., 2005). Attempts to introgress salt tolerant genes from *T. bessarabicum* were made extensively (King et al., 1997) and a new salt tolerant cereal *Tritipyrum* was developed. Dvorak et al., (1988) studied salt tolerancein solution culture media of the amphidiploid (2n=8x=56) obtained from a cross between Chinese Spring and *Lophopyron elongatum* (2n=2x=14, genomes EE) which is a highly salt tolerant relative of wheat found in Mediterranean salt marshes. It was found that salt tolerance of the amphidiploid was enhanced due to additive genes actions. Genetic study revealed that major effects were associated with *L. elongatum* chromosome 3E and smaller effects with chromosomes 2E, 4E and 7E. Minor enhancement of salt tolerance, however, was also associated with chromosomes 1E and 5E of *L. elongatum* (Dvorak and Gorham, 1992). The study suggested that the greatest effect on Na$^+$ exclusion and K$^+$ accumulation was associated with chromosome 3E. Gorham et al., (1987) observed that salt tolerance traits in bread wheat were mostly associated with the D genome. Nevertheless, Schachtman et al., (1992) found that the salt tolerance of three synthetic hexaploids produced by crossing the tetraploid wheat cultivar, Langdon (2n=4x=28, genome AABB) and three *Triticum tauschii* accessions differed according to the source of their D genome, Langdon x *T. tauschii* CPI 110756, Langdon x *T. tauschii* CPI 110791 were sensitive to saline conditions. Results of these crosses indicate that salt tolerance in wheat is

either quantitatively controlled trait or epistatic interaction may exist between homeologous genes. In order to access genetic benefit of salt tolerance mechanism of wild relatives, dense genetic maps need to be constructed which involved such types of crosses to develop molecular markers linked with major genes or QTLs.

Conventional breeding

Salinity is becoming nowadays one of the major abiotic stress issues worldwide. Limited work, however, has been reported on breeding for salt tolerance in wheat and other cereals. In the public and private organizations, plant breeders mostly emphasize on breeding program related to high yield, biotic stresses and yield potential under drought conditions. Very small scale targeted breeding for salt tolerance has been carried out in some part of India and Pakistan in wheat. In the wheat germplasm screening, a land race, derivatives of Kharchia was identified as salt tolerant by Rana (1986). Similarly Hollington et al., (1994) reported inbred lines; KRL 1-4 and KTDH-19 tolerate salt levels relatively high in India but not in Pakistan. On the other hand, cultivar, LU26S showed improved yield in saline soils in Pakistan but it was susceptible to rust (Qureshi et al., 1980). With an intra-specific cross, two lines, S24 and S36 were developed by crossing LU26s and Kharchia, which displayed increased salt tolerance (Ashraf and O'Leary, 1996). Pedigree selection of SARC-1 derived from cultivar LU26S has demonstrated higher salt tolerance in saline field conditions (Munns et al., 2006).

Advanced molecular breeding

New and innovative technologies are highly recommended and current need for the improvement of wheat yield by overcoming abiotic and biotic stresses (Reynolds and Borlaug, 2006b). Omics studies including genomics, proteomics and metabolomics could dissect the salt tolerance traits into their respective genetic and biochemical components to better understand its utilization. Genetic improvement of wheat using wealth of information derived from the omics approaches could be achieved if comprehensive knowledge of environmental and

developmental factors involved in crop adaptation is used in designing the experiments (Cushman and Bohnert, 2000). In fact, much of this background information has not been empirically used especially for complex traits like salt tolerance. Multidisciplinary approaches are required for the improvement in wheat which can be realised in farmers' field (Snape, 2004; Evans, 2005).

Genetic regulation of salt tolerance in wheat

Conventional breeding has been boosted by immense support of genomic techniques like gene mapping, cloning and functional characterization together with marker assisted selection of important traits. Microarray and subtraction hybridization based information can be used and interpreted to investigate involvement of a gene or a metabolic pathway in salt tolerance mechanisms. Identification of salt tolerance genes and their transformation in cultivated plants including wheat have been accomplished with the advance technology (Munns, 2005). In the salt tolerance mechanism of the plants, compartmentalization of toxic Na^+ into vacuoles provides an efficient mechanism to minimize the toxicity of Na^+ in the cytoplasmic reactions. The vacuole Na^+ accumulation is mediated by a Na^+/H^+ anti-porters that work under electro chemical gradient generated by vacuolar H^+ translocaters, the H^+-ATPase and H^+-Pyrophosphatase (Blumwald, 1987). Overexpression study of $AtNHX1$ gene (Na^+/H^+ anti-porter) in $Arabidopsis$ $thaliana$ were able to tolerate high concentration of salts (Apse et al., 1999). Similarly, introduction of this gene in wheat by transgenic approach resulted in relatively higher salt tolerance in wheat (Xue et al., 2004b). In addition, same gene transformed in tomato (Zhang and Blumwald, 2001), $Brassica$ $napus$ (Zhang et al., 2001), maize (Yin et al., 2004) and tobacco (Soliman et al., 2009) produced salt tolerant plant than control at 200 mM NaCl. Introduction of this gene by transformation did not affect the fruit quality in tomato and seed quality in $Brassica$, however, there was an accumulation of up to 6% Na^+ in leaves on dry weight basis. Similar results were obtained in maize (Yin et al., 2004) and wheat (Xue et al., 2004b) transgenic plants. Overexpression of Na^+/H^+ anti-porter gene from rice in the same species increased yield under salt affected growth conditions (Fukuda et al., 2004). Several research studies on $AtNHX1$ suggested its crucial role in the salt tolerance mechanism in plant that could be employed in the new cultivar development in crop plants including wheat.

Genes involved in ions uptake and transport

Sodium transport from soil to plant and within the plant is regulated by lipid bilayer embedded proteins in the cell. The K^+ channels also act in regulating the Na^+ transport into plant cell as these proteins are involved in Na^+ transport along with K^+. The lipid bilayer embedded proteins also have an indirect role because they have to balance the toxic effect of Na^+ in the cell by maintaining K^+ homeostasis (Munns, 2005). Selective and non-selective channels of ions play their role in passive transport of ions like Na^+, K^+, Ca^{++} and NH_4^+ (Demidchik et al., 2002). The sym-porters and anti-porters are forms of active transporters. Transport occurs under electrochemical potential difference of a coupled solute, usually H^+. Many potential genes are involved in the maintenance of K^+ or Na^+ homeostasis in higher plants and could be considered candidates for genetic manipulations (Munns, 2005). Gene encoding for K^+ channels like $AKT1$, $AKT2$, $KAT1$ (encoding for shaker type single pore inward channels), $SKOR$ (shaker type outward channel), $KCO1$ (two pore channel of KCO family); K^+ anti-porters like KEA, CPA (K^+/H^+ anti-porter); K^+ transporters like $HAK1-10$, $KUP1-4$ and $HKT1$; Na^+ anti-porters like $NHX1-5$ and $SOS1$ and proton pumps like $AHA2$ and $AVP1$ encoding for H^+-ATPase and H^+-PPase (Maser et al., 2002; Reinhold and Guy, 2002; Demidchik et al., 2002; Very and Sentenac, 2003). Some of these candidate genes can be further explored and characterized to use in novel transgenic plants which can enhance salt tolerance. Chen et al. (2003) identified wheat mutant line and genetic characterization revealed that gene $TaGSK1$ induced expression under salt condition compared to wild and suggested that this gene is involved in signal transduction in wheat.

Genes with an osmotic adjustment function

There are four classes of solutes that could have an osmotic or protective effect; N-containing solutes such as proline and glycine betaine, sugars such as sucrose and reffinose, straight chain polyhydric alcohols such as mannitol and sorbitol (polyols) and cyclic polyhydric alcohols such as myo-inositol, ononitol and pinitol. Several genes and their family in higher plants are involved in the osmotic adjustment to maintain cell turgidity. Genes like $P5CS$ known for Δ^{-1} pyroline corboxylate synthetase which is encoding for proline production (Hong et al., 2000), similarly, gene, $codA$ encodes for choline oxidase produces glycine betaine (Sakamoto et al., 1998). Likewise, $otsA$ encodes enzyme trehalose 6-phosphatae synthase and ots8 encodes trehalose 6-phosphatae phosphatase for biosynthesis of trehalose (Gong et al., 2002). The enzyme manitol 1- phosphate dehydrogenase encoding gene, $mt1D$ regulate biosynthesis of mannitol in plants (Abebe et al., 2003). Similarly, gene, $S6PDH$ (sorbital 6- phosphate dehydrogenase) for sorbitol (Gao et al., 2001) and $imt1$ (myo-inositol methyletransferase) for myo-inositol (Sheveleva et al, 1997) have been transformed in plants and their salt tolerance level were elicited. These transformations, however, were mainly carried out in model plants. Further research for these gene functions in eliciting salt tolerance level in crop plant species need to be performed to introduce new high salt tolerance cultivars in non-cultivated salty land (Yamaguchi and Blumwald, 2005). Huang et al., (2012) identified novel gene, $TaSC$ for salt tolerance in wheat and its expression in $Arabidopsis$ revealed elevated level of salt tolerance in transgenic plants. This study suggested that the gene $TaSC$ involved in up-regulating the expression of several genes in the salt tolerance plants via the CDPK pathway.

Genes controlling tissue and cell growth rates

Genes that can increase the growth and development rate of plants under salt stress capable to influence the production of new leaves and roots or they can influence the rate of photosynthesis. Regulation of cell division, cell wall expansion and dimensions for differentiated cells, genes can increase the growth rate. Cross talk signal transduction between the salt tolerance genes and growth rate regulating genes could change the rate of photosynthesis by controlling stomatal aperture or by contracting the dimensions of mesophyll cells. Candidate genes involved in regulation of growth are involved in signalling pathways (Zhang et al., 2004) that initial expression with sensing signals and cross signal with hormone biosynthesis genes, transcriptional factors, protein kinases, protein phosphatases and calmodulin binding proteins. It is highly likely that these genes also confer drought tolerance as well (Chaves et al., 2003). The

work on C-repeat binding factors (CBFs) and ABRE-binding factors (ABFs) that control the set of downstream functional genes in stress acclimation promised increase in salt tolerance along with other abiotic stresses tolerance (Ozturk et al., 2002). Badawi et al., (2007) reported *CBF* genes that have been classified into 10 different sub-families regulating various abiotic stresses including salt stress.

Overview of transcriptomics for salt tolerance

Quantitative traits such as salt tolerance are complex to dissect and interpret at molecular level by using genomic tools. Genomic approaches do not imply impact of gene or pathway and its effect on salt tolerance mechanisms. It, however, is speculated that genomic approaches will pave the way by identifying genes for marker assisted selection for relatively simple traits like disease resistance in the short term and for complex traits like salt tolerance in long term (Reynolds and Borlaug, 2006b). On the other hand, global transcript profiling (cDNA micro-array) experiments in rice and wheat showed that plant responded to salt stress trigger up and down regulation of a significant proportion of coding genome. It is further suggested that apart from transcripts that elevated on salt stress by regulating synthesis of osmolytes and ion transporters, two transcription systems governing synthesis of proteins related to the transcriptional and translational machineries have significant role in salt tolerance (Sahi et al., 2006). Liu et al., (2012) reported that microarray experiment in somatic hybrid of cultivar Jinan 177 and tall wheatgrass (*T. ponticum*) revealed high expression of some genes associated with elevated level of salt. This study suggested that introgression of new genetic materials from *T. ponticum* involved in high salt tolerance mechanism in this study by enhanced unsaturated fatty acid and flavonoid synthesis.

Proteomics study for salt tolerance

In the omics areas of innovation, proteomics is being most recently used for characterization of different types of proteins which are involved in original and/or modified forms. Single gene or gene family could produce several types of proteins to regulate particular trait. Advance technological inventions have been utilized to determine insight mechanisms that regulate complex inhabitant traits. In bread wheat, Peng et al., (2009) evaluated salt tolerance cultivar Shanrong 3 and its parent cultivar Jinan 177 for protein profiles using two-dimensional gel electrophoresis and mass spectrometry. Results revealed that 34 and 6 differentially expressed protein in roots and leaves, respectively, were cultivar specific. Some of these differentially expressed proteins could further be characterized for their functions at metabolomic or signal transduction cross talk for salt tolerance mechanisms in plants. Similarly, Kang et al., (2012) reported that wheat seedlings treated with 0.5 mM salicylic acid, 250 mM NaCl 250 mM NaCl and 0.5 mM salicylic acid for 3 days and showed 39 differentially regulated proteins by 2D-PAGE and 38 proteins determined by MALDI-TOF/TOF MS in both salt and salicylic acid treatments. The study suggested that expressed proteins are involved in different cellular and metabolic processes such as signal transduction, stress defense, metabolism and photosynthesis.

QTL mapping and MAS for salt tolerance in wheat

Since 1960s introduction of isozymes and subsequent development of DNA based molecular markers have revolutionized the genetic analysis of crop plants to improve yield, quality traits and resistance to abiotic and biotic stresses. Flowers and Yeo (1989) reported that salt tolerance is a complex phenomenon controlled by several gene families. Salt tolerance mechanism can be better understood by physiological and biochemical studies, and underpin genetic variations can be determined to be used for salt tolerant cultivar development. Polygenic trait like salt tolerance can be dissected using molecular markers and highly reliable closely linked or functional (gene-specific) markers that can be deployed for combining genes for salt tolerance. Molecular markers can also be used to monitor the introgression of alien genes from chromosome addition lines into the recipient genotypes (Quarrie, 1996). Efforts to improve plant performance under salt stress have not yielded to the expectations due possibly to the fundamental mechanisms of stress tolerance in plants are not fully understood (Yamaguchi and Blumwald, 2005). Biotechnological approaches that are currently being used to improve salt tolerance include: (1) introduction of novel gene(s) or to alter expression level of existing gene(s) to affect the degree of salt tolerance i.e. transgenic approach (Munns, 2005), (2) quantitative trait loci (QTLs) mapping– regions of a genome that are associated with the variation of a quantitative trait (Foolad, 2004; Flowers, 2004) and subsequent marker assisted selection and /or exploitation of natural genetic variation through selection in salt environment. (3) Creation of point mutation and subsequent in vitro selection of large quantity of plants for novel germplasm creation, (4) Wide somatic hybridization and introgress novel genome fragments characterization for salt tolerance functions. Estimation of genetic diversity present in the germplasm of wheat is a prerequisite for planning any successful breeding programs. Establishment of breeding nurseries for any target trait on the basis of morpho-physiological characters was a norm in international collaborative wheat improvement programs (Reynolds and Borlaug, 2006a). The quantitative trait loci (QTLs) mapping in dissecting complex traits within genome involved in the expression of that trait have been a promise for the future wheat improvement (Reynolds and Borlaug, 2006b). A quantitative trait is influenced by many genes across the genome whereas a qualitative trait is controlled by one or a few genes. A QTL is defined as genetic loci where functionally different alleles segregate and cause significant effect on a quantitative trait (Salvi and Tuberosa, 2005). Through the combined use of DNA marker technology and advanced statistical methods chromosomal regions that contain the genes that determine quantitative traits can be identified (Collard et al., 2005; Kearsey and Poni, 1996), these are called quantitative trait loci or QTLs (Flowers and Flowers, 2005). In durum wheat, Lindsay et al. (2004) mapped a locus (*Nax*1) for Na^+ on the long arm of chromosome 2A using a QTL approach. Quarrie et al. (2005) reported QTLs for salt tolerance on chromosome 5B and 5D in an irrigated field experiment in bread wheat. Likewise, Ma et al. (2007) identified a total 47 QTLs in a RIL population derived from a cross between Opapta85 and W7984 in saline hydroponic experiment and suggested that 10 QTLs were effective in salt tolerance in germination and 37 where in seedling stage.

Table 2. Genes/QTLs involved in salt tolerance mechanisms in wheat in different species of wheat.

Genes/QTL	Function	Species	References
TaST	CDPK pathway	*T. aestivum*	Huang et al., (2012)
AeNHX1	Na+/H+ anti-porter	*Th. elongatum*	Qiao et al., (2007)
TmHKT7-A2	Na+ unloading	*T. monococcum*	Huang et. al., (2006)
Kna1	Na+ and K+ accumulation	*T. aestivum*	Dubcovsky et al., (1996)
Nax1 and *Nax2*	Na+ exclusion	*T. monococcum*	James et al., (2006)
Nax1	Na+ exclusion	*T. durum*	Lindsay et al. (2004)
TaSnRK2.8	NaCl and cold stresses	*T. aestivum*	Zhang et al., (2010)
TVP1	H^+ transport, vacuolar acidification	*T. aestivum*	Brini et al., (2005)
TaSOS1	Na+ detoxification	*T. aestivum*	Xue et al., (2004a)
Q.Na2A, Q.Na2B1, Q.Na2B2, Q.Na6A, Q.Na7A	Shoot Na+ concentration	*T. aestivum*	Genc et al., (2010)
Q.K1D, Q.K3B, Q.K3D, Q.K4A, Q.K4D, Q.K5A, Q.K5B, Q.K5D, Q.K7A, Q.K7D	Shoot K+ concentration	*T. aestivum*	Genc et al., (2010)

The genetic locus, *Kna1* controlling Na^+ and K^+ accumulation in shoot was identified on chromosome 4D by Dubcovsky et al., (1996), however, its homologue counterpart has not been identified in the A and B genome of tetra or hexaploid wheat. Recently, James et al., (2006) revealed the genes, *Nax1* and *Nax2* originally inherited from *T. monococcum* with correspondence to the QTL on chromosomes 2A and 5A, respectively. Gene cloning technologies that originally targeted major genes are increasingly including those genes responsible for quantitative, multigenic traits (Salvi and Tuberosa, 2005). Identification of the genes underlying these QTLs is the biggest challenge for plant biologists of this century (Luo et al., 2002) due to their complex interaction as well as variable contribution for the particular trait. It is generally believed that mapping QTLs does not accurately position genes underlying polygenic traits on the genome in primary populations which limits the application of QTL analysis in marker assisted selection and gene discovery (Kearsey and Farquhar, 1998). Advance technology, however, have been cloned and accurately tagged a few QTLs of agronomically important trait in the crop species (Salvi and Tuberosa, 2005). The SSR markers (gpw2181 and gpw2181) closely linked to the gene *Nax1* and *Nax2* for Na^+ exclusion can be deployed in the marker assisted selection to develop novel lines with salt tolerance in different genetic background in hexaploid and tetraploid wheat. List of major genes and/or QTLs identified in different wheat species have been given in Table 2.

Somatic wide hybridization for salt tolerance

Somatic hybridization or protoplast fusion is a way to transfer nuclear as well as cytoplasmic genome fragments from distant plant species with high salt tolerance to wheat. This technique is useful where sexual cross is inconceivable or very difficult to achieve. Asymmetrical protoplast fusion was attempted between bread wheat (*T. aestivum*) and *Agropyron elongatum* (Xia et al., 2003) and between wheat and *Aeleuropus littorulis* (Wei et al., 2001) to transfer salt tolerance genes or genome fragments to common cultivated wheat but there was no success in generating plants from fused calli in both the cases. However, somatic hybridization between wheat and Italian ryegrass proved to be successful in generating hybrid plants (Cheng and Xia, 2004). Suiyun et al. (2004) reported introgression of small segments from *T. ponticum* into cultivated bread wheat through somatic hybrid and two independent lines displayed stable high salt tolerance than parental wheat at two locations.

Challenges and future prospects

Quantitative nature of the salt tolerance mechanism in plants is one of the major challenges to manipulate easily through marker assisted selection or other means. The marker assisted selection pre-requires efficient and reliable markers, which have higher linkage as well as the linked genes or QTLs that should have significant contribution in salt tolerance level in plants. Several studies have been reported for development of molecular markers including SSR, SCAR and SNP involved in salt tolerance mechanism of plant; however, follow up marker assisted selection processes have very limited or no successes. Assessment of salt tolerance in transgenic experiments has been mostly carried out using a limited number of seedlings or mature plants in laboratory experiments. Salt tolerant transgenic plants are yet to be subjected to high salt affected soil conditions such as alkaline soil pH, high diurnal temperature, low humidity and presence of other salt species (Yamaguchi and Blumwald, 2005). The evaluation of field performance under salt stress is difficult because of the variability of salt levels in field conditions (Richards, 1983; Dennialls et al., 2001). Stable transgenic line development with an efficient salt tolerance level is primary challenge and regulatory approval of transgenic cultivars with salt tolerance is another challenge to deal with for commercial cultivation of transgenic salt tolerance. There have been successes in conferring salt tolerance by transformation technologies, but these efforts have been mainly focused on model plants like tobacco, *Arabidopsis* and rice (Grover et al., 2003). There is an urgent need to test these successes in the elite cultivars of other crops such as wheat. There are several other technical and financial challenges in transforming many of crop plants particularly monocots (Yamaguchi and Blumwald, 2005) like transformation and development of series of independent T_2 lines is very labor intensive with very less success. The promoters which are being used for transgenic plant productions are constitutive in nature like CaMV35S (Cauliflower mosaic virus 35S promoter) and ubiquitin and actin promoters (Dennialls et al., 2001), which may reduce the efficiency and overall effect of transgene for the specific function within the plants. Overexpression of salt induced genes under salt induced or tissue specific promoters has shown better results (Zhu et al., 1998; Kasuga et al., 1999), however very limited studies available with respect to promoters role in salt tolerance enhancement. The progressive salinization of irrigated and rain-fed land is one of the major constraint to enhance production and productivity of agricultural land around globe. Ghassemi et al., (1995) estimated that about 20% of irrigated land is being

affected by progressive salinization, which is on the other hand essential to be utilized for food production to meet the current and future demand of food and feed. More resources and additional scientific efforts need to be allocated in the salt tolerance elite germplasm as well as cultivars development through advance technologies in major crops such as wheat, rice and maize. Intra-specific, wide hybridization and subsequent *in-vitro* rescuing of embryos for the introgression of the genetic materials from wild relative of wheat is one of the important strategies to enhance salt tolerance in adopted germplasm or cultivars. Bread wheats have numerous close and distant relative species that can be explored for salinity tolerance to utilize in future breeding programs (Colmer et al., 2006).

Conclusion

Soil salinity drives dramatic modification in plant architect including morphological, biochemical, physiological and metabolomic, which significantly affect plant growth and development resulting in reduced productivity. In the model plant species, *Arabidopsis* and rice, detailed studies have been conducted to explore ion uptake, transport, storage and detoxification mechanism and genetic underpin to these mechanisms. However, in the wheat, limited studies carried out for salt tolerance mechanism, which can be utilized in breeding for salt tolerance. Existence of genetic diversity in wheat and its wild relative could be an advantage to develop somatic wide hybridization based new germplasm as well as introgression of genetic material from wild species to enhance salt tolerance in common bread wheat. Genetic mapping of candidate regions within each genome can be performed in different gene pool to identify genes and QTLs with reliable molecular markers for MAS. Gene sequence information from model plant rice and its comparative analysis with wheat genomes for the development of functional gene markers is achievable for more specificity in MAS. Recent advances in sequencing technology can provide edge to generate large numbers of SNP for association mapping in diverse sets of germplasm to evaluate significant regions for salt tolerance within genome and their interaction with other genomes in wheat. Rapid progress of genome sequence projects in important crop species along with generation of large number of knockout mutants and their characterization in model plant such as rice would support other relative species like wheat. Role of metabolites in salt tolerance mechanisms is to be explored to understand crucial biochemical and physiological processes involved in salt tolerance.

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