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Salinity tolerance in wheat: An overview

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Abstract

Wheat is a grass which is cultivated for its seed, a cereal grain (staple food) and is the most common food crop used worldwide. There is a great impact of abiotic stress onto the growth, metabolism & productivity of plants. One of the most common factors for plant growth and yield is Salinity Stress. There is a high impact of salinity on morphological as well as physiological characteristics. Morphological characteristics may include root length, plant height and number of leaves whereas physiological characteristics include photosynthesis, water relations and chlorophyll contents. Saline land is not appropriate for cropping as plants are less tolerant towards salinity. Plant growth response towards salinity occurs in two phases: - Rapid phase and Slower phase. In rapid (osmotic) phase, there is inhibition of the young leaves whereas in slower (ionic) phase, senescence of mature leaves occur. Salt tolerance of wheat is greatly enhanced by potassium transporter (HKT) genes. Major three types of plant adaptations to salinity are: Osmotic stress tolerance, Na⁺ or Cl⁻ exclusion and tolerance of tissue to accumulated Na⁺ and Cl⁻. However, overall control of Na⁺ accumulation and osmotic stress tolerance is of less molecular understanding. But molecular genetics and functional genomics provide a great opportunity to synthesize molecular and physiological knowledge to improve the salinity tolerance in plants.

Keywords: Salinity, tolerance, wheat

Introduction

Wheat (*Triticum aestivum* L.) being one of the important staple food cereal globally, it provides calories to approximately 30% (4.5 billion) of the world population and 20% of the total protein requirements (Arzani and Ashraf, 2017) ^[1]. It is cultivated to rally the demands of food for population consumption in many countries of the world. However, the wheat productivity per hectare is much lower than its production potential due to many different factors and salt salinity is the most common one.

The most common abiotic stress which tremendously reduces the productivity of agricultural crops in arid and semi-arid regions of the world is Soil Salinity. Approximately 60% of crop production lost is due to the salt stress in the environment. Salinization (Soil Salinity) refers to the addition of soluble salts into the soil. Salts in soil may increase naturally (primary soil salinity) or may be introduced anthropogenically (secondary soil salinity) (Waters *et al.*, 2013) ^[2]. It could arise by soil modifications, irrigation and fertilizers by means of saline water. This may be because very few salt tolerant bread wheat genotypes have been identified till date like, Kharchia-65 (collected from Kharchi, in Pali District of Rajasthan, India) and KRL-210 (Developed at Central Soil Salinity Research Institute, Karnal, India) (Kumar *et al.* 2015) ^[11]. Weathering of parental rocks releases various types of soluble salt such as chlorides of sodium, calcium and magnesium and to lesser extent sulphates and carbonates. The most abundant soluble salt released is Sodium chloride whereas deposition of oceanic salts is carried

out in wind and rain. Salt stress not only leads to the reduction of crop yield but it also affects the metabolic processes in plants through impairment of water potential of cells, ion toxicity, uptake of essential mineral nutrients, membrane integrity and function.

Because NaCl is the most soluble and widespread salt, one of the most detrimental effect of salinity is the accumulation of sodium ion (Na⁺) in plant tissues. The uptake of essential micronutrients such as Potassium (K⁺) and Calcium (Ca⁺) from soil is inhibited by higher concentration of Na⁺ (Very and Sentenac, 2003) [20]. K⁺ is required for growth or development of plants and for maintaining high K⁺/Na⁺ ratio in shoot which is the major strategy adopted by plants to cope up with salt stress. K⁺ and Na⁺ though having similar chemical properties, both have different physiological impact on plant growth. Different mechanisms for salt tolerance helps in reducing Na⁺ accumulation in the cytoplasm by limiting Na⁺ entry into the cell, actively transporting Na⁺ out of the cell, and compartmentalizing Na⁺ into the vacuole. High-affinity potassium transporters (HKTs) are most active at level of plasma membrane and acts as Na⁺/K⁺ symporters as well as Na⁺ selective uniporter. Major two subfamilies of HKTs: HKT1 and HKT2 are being analysed phylogenetically (Platten *et al.*, 2006) [16]. HKT1 are only permeable to Na⁺ but HKT2 are permeable to both Na⁺ and K⁺. The families of HKTs belonging HKT/Trk/Ktr-type K⁺ transporter superfamily are found mainly in microorganisms and plants. In most plants, Na⁺ and Cl⁻ are excluded by roots and water is taken up from the soil.

This exclusion at higher salinities is maintained by Halophytes (natural flora of highly saline soils). For example, sea barleygrass, *Hordeum marinum*, excludes both Na⁺ and Cl⁻ until at least 450 mM NaCl.

Reactive oxygen species (ROS), produced during the stress cause chlorophyll degradation and membrane-lipid peroxidation, Malondialdehyde (MDA) is one of the final products of peroxidation of polyunsaturated fatty acids in the cell membranes. The increase in free-radicals causes the overproduction of MDA which is the most common marker of the oxidative stress.

Plants accumulate an array of metabolites on exposure to stressful conditions. The significant changes under abiotic stress are shown by soluble sugar, proline, phenolic compounds, chlorophyll contents, K⁺/Na⁺, shoot-root biomass ratio etc. Total soluble sugar is a main component of carbohydrate metabolism. Thus it shows a close relationship between photosynthesis and plant productivity and reflects the ability of grains to use assimilates. Proline, the only amino acid act as excellent osmolyte and also serve as metal chelator, anti-oxidative defence molecule and signalling molecule (Flowers *et al.*, 1992) [6]. Thereby it maintains concentration of ROS in normal range and prevent oxidative burst in plants. Phenolic compounds also show important role in neutralising the free radicals, quenching singlet oxygen and decomposing peroxides. So hereby in this chapter we will provide an outline of the mechanisms of wheat salinity tolerance and present an outlook on prospective key research on this topic.

HKT-type transporters

Tetraploid wheat is less soil tolerant than bread wheat because bread wheat is able to maintain a higher ratio of potassium concentration to sodium concentration in the leaves. Class 1HKT genes are involved in regulating transport of Na⁺ in higher plants. Several HKT1 genes including HKT1; 1/2-like,

HKT1; 3-like, HKT1; 4-like, and HKT1; 5-like, have been identified and mapped to wheat homologous chromosome groups 2, 6, 2 and 4 respectively. Among these, Nax1 in chromosome 2AL co-segregated with sodium transporter gene HKT1; 4-A2, which was shown to control Na⁺ unloading from xylem in roots and sheaths and therefore was proposed as the functional candidate. Nax2 was mapped to the distal region of chromosome 5AL that is homologous to a region on chromosome 4DL containing Kna1 (Lauchli A. 1984) [12]. Based on synteny and phylogeny analysis with Nax2, TmHKT1; 5-A significantly reduced leaf sodium content and increased durum wheat grain yield by 25% compared to lines without the Nax2 locus. Furthermore, decreased expression of TaHKT1; 5-D, which is homoeologous to TmHKT1; 5-A and underlies Kna1 locus in bread wheat, caused by target-specific RNA interference-induced silencing (RNAi) led to an accumulation of Na⁺ in leaves, strongly suggesting that TaHKT1; 5-D should be the candidate gene of Kna1.

A major mechanism in salinity tolerance of wheat is Na⁺ exclusion mediated by HKT genes. AtHKT1 is regulated by small RNA and DNA methylation. Moreover, DNA methylation also participates in the response of TaHKT1; 5s. Transcription factors such as AtAB14 and OsMYBc were shown to regulate HKT genes in plants, offering more candidate targets for enhancing salinity tolerance.

ROS homeostasis involved insanity tolerance of a somatic hybrid

Transcriptomic, proteomic and metabolic comparisons of SR3 with its wild type cv.jinan177 (JN177) wheat parent suggested that reactive oxygen species homeostasis was the major biochemical basis for the salt tolerance of cv. SR3. A mapping analysis localized a tolerance QTL on chromosome arm 5AL, at a position containing TaSRO1, a gene encoding a poly (ADP ribose) polymerase (PARP) domain protein. PARP proteins have been implicated in modulation of redox homeostasis (TD Colmer *et al.*, 2005) [4]. Sequence variation between the TaSRO1 alleles present in both was predicted to affect the catalytic activity that is significant for DNA repair under oxidative stress. The transgenic constitutive expression of the allele from cv. JN17, a sensitive cultivar, enhanced the level of salinity and ROS tolerance, while RNAi-induced knock-down of the gene in cv. SR3 compromised the level of tolerance. Thus TaSRO1 was considered to be a strong candidate for the salt tolerance QTL in cv. SR3 (GM Xia, 2004) [22].

Somatic hybridisation introduces a minimum of exogenous chromatin into a recipient genome, but causes genomic shock that induces high frequencies of both point mutation and indels (insertion and deletions) encoding sequences and is thus capable of generating eite alleles. Genetic analysis indicated the remarkable salinity tolerance of SR3 by modulation of ROS homeostasis that was accomplished by a polygene effect. A Zinc finger transcription factor, TaCHP, was activated in SR3 with much higher transcript abundance than in JN177. TaCHP facilitated salinity tolerance in wheat through improved leaf peroxidase (POD) activity to enhance ROS scavenging ability. In animals, the status of DNA-methylation is affected by the level of ROS content. It is essential to determine whether the divergence of ROS accumulation and ROS homeostasis maintenance between SR3 and JN177 is associated with DNA-methylation, and its effect on expression patterns of salt stress responsive genes (Qiu QS *et al.*, 2002) [17]

Genes involved between salinity response and other environmental and developmental signals in wheat

When there is high concentration of salt, complex physiological responses such as phytohormone signaling pathways and developmental signals are triggered to adapt the stress. Therefore it is essential to identify the environmental and developmental signals. First of all an attempt was performed by looking at phytohormones, as most phytohormones are regulatory factors of both developmental process and stress response. For example, the wheat gene TaAOC1, encoding cyclase involved in jasmonic acid synthesis, was induced by high salinity. Constitutive expression of TaAOC1 in both wheat and *Arabidopsis* restricted root growth, but enhanced salt tolerance and JA content. These findings firstly indicate the different branches of metabolic pathway participate in a single process but controlled by different mechanisms.

Light is a basic factor that positively affects the growth and development of plants. TaGBF1, a blue light specific responsive G-box binding factor, was induced after exposure to salt. TaGBF1 caused salt sensitivity and promoted light blue mediated photomorphogenesis, showing that it was a common component of the blue light and salt stress-responsive signalling pathways. Interestingly genetic analysis suggested that the role of TaGBF1 in response to salt relied on AB15, a key component of ABA signalling pathway (Flowers *et al.*, 1992)^[6]

Salinity effects on wheat

Salinity stress shows elevated extent of variations with respect to seed germination, morpho-physiological, anatomical, molecular, biochemical and genetical impacts.

• Germination and seedlings growth

Salinity stress is one of the major problem which affects plant's seedling, development and finally productivity. Elevated level of salt progressively increased the time to sprouting and decreases the sprouting percentage and affected the seedlings growth (Naz *et al.*, 2010)^[14]. Some scientists showed that salinity stress suppressed and delayed the germination of seeds in different wheat cultivars (Khan *et al.*, 2009)^[9].

• Morphological characteristics

The morphological characters of wheat crop are affected at all stages of growth in saline conditions which include leaf (shape, size, area, senescence, cuticle tolerance and waxiness), root (length, root hairs, root area, fresh and dry weight, density) and vegetative (plant height, diameter and fresh and dry biomass). Thus it is essential to understand wheat's response at every phase of growth which can be helpful in improving or developing salt tolerant varieties. According to the study of salinity (Zheng *et al.*, 2008)^[23] stress decrease the plant leaf area which as a result reduces the rate of photosynthesis that resulted in low biomass production (Kingsbury *et al.* 1984)^[10].

Root is actually the first important organ since it has the potential to shift in order to salt avoidance. Under stress conditions, the production of ramified root system is vital to above ground biomass. A developed root system can contribute advantages to maintain plant growth for the period of early growth phases and extracts water and micro-nutrients through the soil. In conclusion, increased level of salt concentrations adversely affects root length and its viability to absorb water (Bacilio *et al.*, 2004)^[2].

• Physiological characteristics

Wheat's physiology is interrupted by salinity at both cellular and whole plant levels through ionic and osmotic stress. These physiological processes include alteration in plant development, mineral distribution and membrane variability resulting from calcium dislocation by sodium and membrane permeability (Mehta *et al.*, 2010)^[13]. High salt concentration in soil decreases wheat leaf water potential, turgor pressure loss and stomata closure, decline in CO₂ conductivity through stomata, alteration in cell wall integrity, increase of oxidative stress and enhances development of toxic metabolites which ultimately lead to plant's death. It also affects the plants photosynthetic activity that results in low growth yield production.

Through salinity stress, the stomatal pattern of gases conductance reduces and lead to inadequate availability of CO₂ to the wheat plants which lead to more reactive oxygen species (ROS) production.

Chlorophyll as well as carotenoides pigments content are reduced due to salt stress. Chlorophyll and carotenoides amount co-relate with the crop yield under salinity stress.

• Biochemical characteristics

Salinity stress increases the formation of reactive oxygen species (ROS). ROS are generally produced in thylakoid, mitochondria and peroxisome during photosynthetic process, Electron transport chain and glyoxylate cycle respectively.

There are many enzymatic mechanisms used by plants for scavenging of salinity induced ROS. The enzymes which are overproduced comprises of glutathione reductase (GR), glutathione synthetase, superoxide dismutase (SOD) and ascorbate peroxidase (APX). This continuous overproduction of ROS retards the anti-oxidant defense system of plants. This may lead to damaging effects on the macromolecules such as DNA, RNA, proteins and lipid and may also lead to the impairment of plant function.

Many solutes including proline, glycine betaine, abscisic acid and polyol in the plant cell are required to retain the osmotic potential in the vacuoles against the ion toxicity accumulated in the cell compartments. These are also known as compatible solutes or metabolites as they help in protection of plant against salt stress (Sharma *et al.*, 1983)^[18].

Earlier it has been accounted that there is reduction in chlorophyll content in plants cultivated in saline environment ND chlorophyll content has been recommended one of the attributes for salinity tolerant in plants. Salinity stress enlarged the accumulation of Na⁺ and Cl⁻ contents in wheat different parts especially in roots and leaves whereas reduced the K⁺ and Ca⁺ contents. Na⁺ and Cl⁻ ions provoke toxicity which has negative effects on vegetative and reproductive phases of growth.

• Molecular and genetical characteristics

Various genes and proteins that have been identified in different salinity-stress induced plants are Sal1, Bnd22, 25 KDa protein P 150, 27KDa protein, RAB 21 KDa, vitronectin and fibronectin. Dehydrins, Vacuolar acid invertase, late embryo abundant proteins, ABA genes expression and proline induction have been two identified in salinity influenced plants.

Wheat acclimation to salinity is resulted by abscisic acid (ABA) which enhances tolerance against salt shocks (Bray, 1997)^[3] sion of the ESI (early salt induced) genes in the roots, Many changes in 3A and 3D (homologous chromosomes)

which provoke stress tolerance were also observed (Noaman *et al.*, 2002) ^[15]

Proline which is an essential protein performs very important function in salt stress tolerance. During salt stress concentration of 26KDa protein and proline content was observed significantly high in wheat which show it is induced by salt-responsive gene to protect the plant during salinity (Elshintinawy and Elshourbagy, 2001) ^[5]

In summarizing, elevated salt concentration brings changes at molecular and genetic level which retards the plants functioning properly.

• Anatomical characteristics

Relationship of wheat's anatomical traits which shows tolerance against salinity stress is essential to identify the salinity influences at all levels (cell tissue and organ). These may include decrease of overall cell dimensions of root, stem and leaf parts. Other structural variation under salt-stressed condition include change in size and number of stomata, cuticle thickness, early lignification and changes in number and diameter of xylem vessels and decrease in the leaf dermal cells expansion in wheat.

Similarly, there were many variations observed among ground and vascular tissue which showed a significant reduction in cortex cell area and interveinal, vascular bundle area and Meta xylem vessels area.

Smaller xylem vessels thickness or area has been accounted earlier in rice roots with increase osmotic stress. Therefore, smaller and narrow xylem cells are physiologically more confined against cavitation. The risk of cavitation is reduced which results in better water uptake.

Variation in genotypes for salinity tolerance

As reflected the different growth responses, plants differ greatly in their tolerance in salinity. Rice is the most sensitive and barley is the most tolerant if you see the cereals. Bread wheat is moderately tolerant and Durum wheat is less so. The variation in salinity tolerance in dicots is even greater than monocot species. Some legumes are very sensitive than rice, alfalfa is very tolerant. It continues to grow well at salinities greater than that of sea water.

Plant response can occur in different phases through time i.e. osmotic phase, Ionic phase and Both Ionic and Osmotic phase.

Types of plant response or tolerance

Tolerance to osmotic stress

The osmotic stress leads to reduction in cell expansion in root tips and young leaves and also causes stomatal closure. This reduction would lead to greater leaf growth and stomatal conductance but it also increases leaf area which is only beneficial for the plants that have sufficient soil water.

Growth

The decreased rate of leaf growth after an increase in soil salinity is primarily due to the osmotic effect of the salt around the roots. This sudden increase in soil salinity causes leaf cells to lose water, but this loss of cell volume and turgor is transient. Within hours, cell regains its original volume and turgor, but despite this, there is reduction in the rates of cell elongation. After few days, there is slower leaf appearance and smaller final size. Cell dimension changes, with more reduction in area than depth, so leaves are smaller and thicker. The reduction in leaf development is due to the salt outside the roots, this reduction is largely due to the osmotic effect of

the salt which is supported by experiments using mix salts such as KCl, mannitol, polyethylene glycol and Hoagland's solution.

The reduction in leaf growth can be regulated by signals which are long distant in the form of hormones, because the reduced leaf growth rate is independent of water status and carbohydrate supply. The reduction occur in the absence of nutrient deficiency and ion toxicity, as evidenced by very low concentration of Na⁺ and Cl⁻ in expanding cells that do not correlate with growth rate (Khan *et al.*, 2009) ^[9]

Root growth is usually less affected than leaf growth, and root elongation rate recovers remarkably well after exposure to NaCl. Recovery from a moderate stress of up to 0.4MPa of mannitol, KCl and NaCl is complete within an hour. With time reduced initiation of lateral roots probably occurs, but little is known about this.

Photosynthesis and stomatal conductance

The most dramatic plant response to salinity is a decrease in stomatal aperture. Stomatal responses are induced by the osmotic effect of the salt outside the roots. Salinity affects stomatal conductance immediately, firstly and transiently owing to perturbed water relations and shortly afterward owing to the local synthesis of ABA. This stomatal response is regulated by many root signals.

Rates of photosynthesis per unit leaf area in salt treated plants are often unchanged. When photosynthesis is expressed on chlorophyll basis rather than leaf area basis, reduction is seen due to salinity. A feedback signal to down regulate photosynthesis is generated due to leaf expansion reduction which results in build-up of unused photosynthate in growing tissues (Sharma *et al.*, 1983) ^[18].

Oxidative stress

There is increase in the formation of reactive oxygen species (ROS) and increase in the enzyme activity due to reduced rate of photosynthesis. When plants acclimate to a changed environment, they undergo changes in the leaf morphology, chloroplast pigment composition and the activity of biochemical processes that prevent oxidative damage to photosystems. The enzyme activity of the multiple forms in different cell compartments leads to the balance between the rate of formation and removal of ROS and maintain hydrogen peroxide, at the levels required for cell signalling.

More than 150 genes make up the complex ROS network in *Arabidopsis*. Recently *Arabidopsis* mutants lacking either or both a cytosolic and chloroplastic ascorbate peroxidase were found to be actually more tolerant of salinity stress, illustrating the plasticity of ROS regulatory pathways and the redundancy of pathways for ROS regulation and protection (Mehta *et al.*, 2010) ^[13].

Cellular signalling

Plants respond directly and specifically to the addition of Na⁺ within seconds. The extracellular Na⁺ is either sensed at the plasma membrane, or if it is sensed intracellularly then it must first cross the plasma membrane. Thus a plasma membrane protein must either be the sensor or be immediately upstream of the sensor (Qiu QS *et al.*, 2002) ^[17]

The first recorded response to an increase in Na⁺ around roots is an increase in cytosolic free Ca²⁺. The changes in the cytosolic free Ca²⁺ are quite complex, and are modulated by differences in extracellular composition, including Na⁺ concentration. The best characterized signalling pathway involves the Na⁺ increase in cytosolic free Ca²⁺, which is

sensed by a calcineurin B-like protein (CBL4), originally identified as SOS3. There is physiological increase in cytosolic Ca^{2+} which facilitates the dimerization of CBL4/SOS3 and the interaction with CBL-interacting protein kinase (CIPK24, originally identified as SOS2). The CBL4/CIPK24 (SOS3/SOS2) complex is targeted to the plasma membrane via a myristoyl fatty acid chain covalently bound to CBL4 enabling the phosphorylation and thus the activation of the membrane bound Na^{+}/H^{+} antiporter (SOS1) (Flowers *et al.*, 1992) [6].

1. Accumulation of sodium ions in shoot

The main site of Na^{+} toxicity for most plant is the leaf blade where Na^{+} accumulates after being deposited in the transpiration stream rather than in the roots. Most Na^{+} that is delivered to the shoots remains in the shoot, because for most plants, the movement of Na^{+} from the shoot to the roots in the phloem can re-circulate only the small proportion of the Na^{+} that is delivered to the shoot. The net delivery of Na^{+} to the xylem can be divided into four distinct components:-

- Influx into cell in the outer half of the roots.
- Efflux back out from the cell to the soil solution.
- Efflux from cells in the inner half of the root to the xylem.
- Influx back into these cells from the xylem before the transpiration stream delivers the Na^{+} to the leaf blade.

Thermodynamics of Na^{+} transport

The thermodynamic analysis assumes cytosolic Na^{+} concentrations of 30mM and an electrical potential of -20mV, but even if values differ by factor of two, the principle remain unchanged (Waters *et al.*, 2013). For example, at the xylem parenchyma the efflux of Na^{+} from the cells would be active even if the xylem Na^{+} concentrations were nearly ten times lower than cytosolic Na^{+} concentration. With a xylem Na^{+} free concentration of 10mM and a potential difference between the xylem parenchyma cells cytoplasm and xylem apoplast of -60mV, active influx of Na^{+} into the xylem parenchyma cells would only be necessary with cytoplasmic free Na^{+} concentration greater than 100mM.

The thermodynamics of Na^{+}/H^{+} antiporter is simpler, because the electroneutral exchange this antiporter catalyses is unaffected by membrane potential. Thus, the direction of Na^{+} movement is determined by the differences in free concentration of Na^{+} and H^{+} (Arzani and Ashraf, 2017) [1].

The various processes of Na^{+} transport are discussed below.

• Net Na^{+} influx into the outer half of roots

Na^{+} enters root via voltage independent non-selective cation channels and via members of the high-affinity K^{+} transporter (HKT) family. High affinity Na^{+} influx is also mediated by some member of the HKT transporter family in low salt roots, but it repressed by moderate concentration of Na^{+} . From X-ray micro analysis it has been identified that the vacuolar Na^{+} and Cl^{-} concentration decrease across the cortex, are highest in the epidermis and sub epidermis and lowest in the endodermis.

Most of Na^{+} which enters root cells are pumped back again via plasma membrane Na^{+}/H^{+} antiporter. Plasma membrane Na^{+}/H^{+} antiporter activity has been demonstrated for the Arabidopsis protein SOS1. Na^{+} which are left in the roots can be transported to the shoots or sequestered in vacuoles. There is passive leakage of Na^{+} back to the cytosol from vacuole which requires constant re-sequestration of Na^{+} into the vacuoles.

• Na^{+} loading into and retrieval from the xylem

Na^{+} moves in the symplast across the endodermis, is released from stellar cells into the stellar apoplast and moves to the xylem in the transpiration stream. SOS1 is expressed in stellar cell and is involved in the efflux of the stellar cells into the xylem.

There is an evidence for role of members of the HKT gene family in the retrieval of Na^{+} from the xylem. In the Arabidopsis root, AtHKT1; 1 is involved in the retrieval of Na^{+} from the xylem before it reaches the shoot.

Furthermore good evidence exists that a closely related gene, TmHKT1; 4-A2, is the candidate gene for the Na^{+} exclusion (Nax1) locus in the durum wheat which is associated with Na^{+} exclusion and a high leaf K^{+}/Na^{+} ratio. These subfamilies reflects differences in a selectivity-determining amino acid residue in the first so called pore loop region of the protein and the differences in the cation selectivity.

2. Tissue tolerance of sodium ions

High amounts of Na^{+} and Cl^{-} arriving in the leaves can be tolerated by anatomical adaptations and intracellular partitioning. The two types of Anatomical adaptations of dicot halophiles are salt-induced increase in cell size due to increase in the vacuole volume (succulence), and the excretion of Na^{+} and Cl^{-} by salt glands or bladders.

• Intracellular Compartmentation of Na^{+}

Na^{+} are partitioned within the cells so that the cytoplasm concentration are kept at low (10 to 30mM). There is no direct measurement of cytosolic concentration in the leaves but in roots, direct measurement of cytosolic Na^{+} in salt treated plants via the use of ion sensitive micro electrode indicates cytosolic Na^{+} concentration range of 10 to 30mM. *In vitro* studies explain that Na^{+} starts to inhibit most enzyme at concentration of 100mM.

Ideally, Na^{+} and Cl^{-} are largely sequestered in the vacuole of the cell. The differences in the expression level of AtNHX1 may affect the potential to sequester Na^{+} in vacuoles of the leaves. Increased vacuolar Na^{+} concentrations require an increase in the osmotic pressure of the subcellular compartments to maintain their volume. This can be achieved by an increase in the concentration of K^{+} to sub-toxic levels, as well as the concentration of compatible solutes.

• Increased accumulation of compatible solutes

Compatible solutes are the organic solutes that are compatible with metabolic activity even at higher concentration. These compatible solutes must accumulate in the cytosol and organelles to balance the osmotic pressure of the ions in the vacuole. Most common compounds that accumulate are Sucrose, proline and glycine betaine (Lauchli A *et al.*, 1984) [12].

Accumulation of these compatible solutes occur under drought stress and also sometimes under other stresses that reduces growth, such as low temperatures. There are many genes which play an essential role in tolerance to abiotic stress. For example, the lower expression of a gene encoding proline dehydrogenase (PDH) may contribute to the higher salt tolerance of *Tbellungiella halophila*.

Tolerance of leaf tissue to high Na^{+} concentrations is an adaptive mechanism which can tolerate at least 400mM Na^{+} in leaf blades. The high Na^{+} and Cl^{-} allows to maintain turgor in the face of high salt salinities. This is the 'cheapest' form of osmotic adaptation.

New trends in functional genomics studies of salinity tolerance in wheat Omics networks

The rapidly improving technical capacity of next generation sequencing (NGS) and genomic information of wheat will also enable identification of the role of epigenomics in salinity tolerance. Analysis between SR3 and JN177 reveal that multiple salt stress responsive genes were regulated by DNA methylation. Furthermore, bulks of small RNAs in response to salt stress were discovered through miRNome analysis. Such studies will facilitate the functional study of wheat small RNAs in response to salt stress (James RA *et al.*, 2002)^[7].

Omics network will greatly promote the exploitation of functional genes and give us more understanding of salinity tolerance. Moreover, multi-regulatory genetic bases of salinity tolerance together with other environmental or developmental stimuli will be easily identified from the network intersections.

1. Salt tolerant germplasm

Specific germplasms, including T. monococcum and the somatic hybrid introgression, elaborated two major mechanisms of wheat salinity tolerance. Few elegant systems need to be applied to generate novel salt-resistant germplasm for gene discovery and breeding. Further analysis of the genes in transgenic wheat is needed (Kapulnik Y *et al.*, 1989)^[8].

Another recent trend in functional genomics is the establishment of comprehensive EMS mutant libraries of tetraploid wheat and hexaploid wheat. Based upon phenotype screening, novel genes involving salinity tolerance will be easily identified. Moreover, selected mutants can be used to validate the functions of salt stress responsive genes, hopefully avoiding the tedious process of wheat transformation.

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