*Review Article*

# Salinity induced stress in wheat

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## Abstract

**Wheat is the major cereal crop confronting serious abiotic factors that pose an alarming situation regarding its global biomass production and distribution. Salinization is one of those ecological stresses that impair its growth and developmental processes thereby decreasing the crop production. Nearly twenty percent agricultural land encompasses high salt concentration, moreover due to global warming even more arable land is under salinization. Different management solutions have recently been proposed to reduce the negative impacts of salt stress and optimize wheat productivity and nutritional content. This review emphasis on the physio-chemical alterations in wheat under salinity. The reported data revealed that salinity negatively affect multiple processes in wheat during its germination, growth, and maturity. Various adaptive mechanisms to salt stress at the cellular, metabolic, and molecular phases, while the processes governing salinity tolerance remain unknown. Therefore, studying the salt-induced injury and approaches for boosting salt tolerance in wheat is crucial.**

**Keywords:** Salinity, Wheat, Abiotic Stress, tolerance, Biomass production

## 1. Introduction

Wheat is one of the high ranked staple crops cultivated all over the world which feeds around 36% of the world's population because of its nutritional value. Pakistan, United States (US), United Kingdom (UK), Argentina, China, Turkey, India, Australia, France, Canada, Kazakhstan, Ukraine, Germany, and Argentina are the major producer of wheat (Bennett and Smith 1976; Amuruganathan 1991). Bread wheat genome is extensively complicated (Liu *et al.,* 2012), and researchers are progressively focusing on it for genomic studies as well as yield and quality enhancement. Common wheat (*Triticum aestivum* L. BBAADD) has an allopolyploid genome (Baum *et al.,* 2009). Polyploidy have the potential to modulate physio-morphological characteristics such as photosynthetic ability, flower colors, and improved tolerance to a variety of ecological and pathogenic stresses (Yang *et al.,* 2018). Various kinds of wheat including diploid (2x) like *T. monococcum*, tetraploid (4x=28) like *T. turgidum*, and hexaploid (6x=42) like *T. aestivum*. Wheat varieties such as *T. turgidum* as well as *T. aestivum* are grown as a significant staple crop all over the world (Baum et al., 2009). *T. turgidum* is grown in around 25 million tonnes per year (Li et al., 2018). The haploid DNA content of hexaploid wheat is about 100 times more than that of Arabidopsis, approximately 6x bigger than that of *Zea mays*, and nearly 40x greater than that of *Oryza sativa* (Bennett and Smith 1976; Amuruganathan 1991). Due to polyploidy and extensive doublings, the wheat genome is very massive, with about 80% of the genome consisting of repetitive DNA sequences (Smithand, 1974). Temperature, drought, water logging, nutrient depletion, and saline conditions are the fundamental environmental constraints that pose a barrier in plant

development. Moisture buildup and salts are inextricably related in various soils. Irrigation water in many areas, such as Pakistan, has high levels of salt (high sodium absorption ratio), which might be the cause of textured soil degradation. Less water loss increases salinity issues, whereas more water encourages crop fall (Qureshi and Barrett-Lennard 1998).

Due to reduction in cultivated area, constantly changing environment, and extensive utilization of fertilizers, the sustainable cultivation of wheat is declining in several areas of the planet earth. Salinity being a major strain in many states of the globe has a detrimental impact on plant development and crop production. Around 20% of agricultural areas contain high salt concentration, and due to global warming, even more cultivable area is turning out to be salty. Because of diverse pressures, such as salinity, cause yield decreases of up to 50% and because of the growing global population, a 70% expansion in food production by 2050 is required (Miransari and Smith 2019). Many agricultural plants, including wheat, are sensitive to salt stress as wheat production begins to decline at saline concentration of 6–8 dS m$^{-1}$ (Royo and Abió 2003). As a result, it's critical to identify strategies and procedures that can help alleviate stress and produce stress-tolerant wheat varieties. Such examinations, as well as their outcomes, are critical for both substantially and development objectives. While creating novel strategies for considerable wheat production in salt stressed conditions, clean and affordable production of wheat must also be addressed (Miransari and Smith 2019). Researchers have continued to use breeding to generate stress-tolerant genotypes as a significant strategy for growing wheat (Kumar *et al.,* 2019). The use of seed strengthening strategies, such as seed priming, is a parallel relatively brief methodology that has gained a lot of consideration. Seed priming has been proposed as a viable method of reducing salt stress. To combat salt, temperature fluctuations, hypoxia, and reduced soil moisture, wheat seed priming methods comprising hydro-, redox-, hormone-, and osmo-priming are used. (Hussain *et al.,* 2018). In this review we have discussed the effect of salinity on physiological and chemical processes of wheat, impact of hormones and transcription factors on plants growing in high saline conditions. Furthermore, possible strategies to develop salt tolerance are discussed.

## 2. Effect of salinization on wheat plants

Salinization is the brutal ecological factor affecting plant production and yield. Mortality and decreased output are the detrimental effects of excessive saline soil observed in several plants. These plants are developing mechanisms for either removing salt or bearing its excessive concentration in the interior of cell. Salinity disrupts overall chief operations in plants, involving protein production, photosynthesis, and lipid biosynthesis (Shrivastava and Kumar 2015). Wheat is a major agricultural crop that is consumed as a staple food all over the world. It is farmed on a large scale; however, its production is hampered mostly by salt stress (Zhang et al., 2011). When the plant encounters high saline environment its growth and metabolic activities are diminished that results in the decline of seed germination. The process of germination being a complex process resumes, and plant development occurs normally and without stress when the salinity issues in controlled. The variations in salt resistance in wheat cultivars were used to test the growing reaction to salts had 2 phases. The 1st phase is a significant decline of development rate due to salts existing in the soil surrounding the root, which is known as the osmotic reaction. In the later phase, it will cause excessive decline in growth owing to the salinity that has formed a toxic threshold in the plants, which is known as a particular saline response. If this concept is right that various wheat genotypes can eliminate and tolerate higher salt concentrations, then they should not be impacted by salinity stress for some time (Hasan *et al.,* 2020).

## 3. Physio-chemical mechanisms for salt tolerance in wheat

Salt stress diminishes the yield of crop and influences physicochemical characteristics of the soil. The impacts of salinity are caused by intricate associations among biochemical, morphological, and physiological processes such as sprouting percentage, plantlet health, nutrient and water intake (Shrivastava and Kumar 2015). Salt tolerance is a complex polygenic feature whose expression is affected by various physiological, genetic, and ecological factors. Wheat is also known as a salt excluder, indicating that it mitigates level of salts by removing as much Sodium ions (Na+) from the shoot as feasible. Hexaploid wheat genotypes have a substantially greater ability to exclude Na+ than tetraploid genotypes (Saddiq *et al.,* 2021). Salinity induces morphological, physiological, and biochemical alteration in plants as discussed follows (Fig 1).
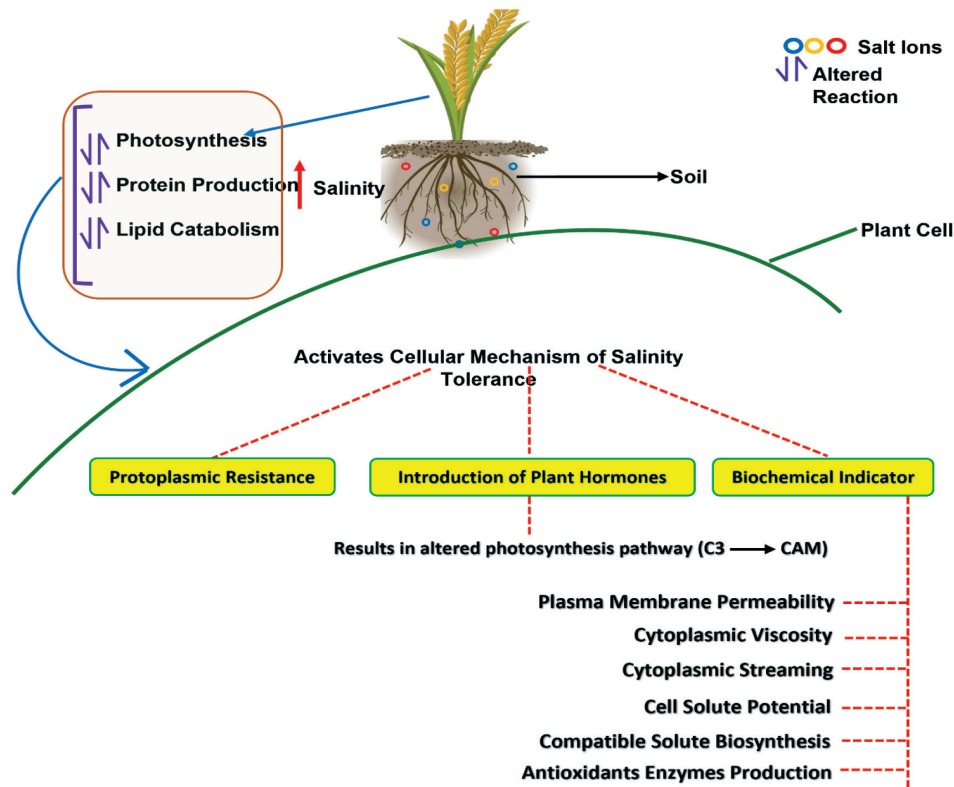
Figure 1. Activation of cellular mechanism in plants when salt ions concentration increased in soil and leads to accumulate in plant shoot.

## 3.1 Salinity and Biochemical indicators

Higher plants have compatible biochemical indicators such as soluble sugars, polyglycols, N-containing compounds (Proline, leucine, glutamine, ornithine, asparagine), proteins, and ammonium compounds that confer substantial resistance under salinity. The proteins that are accumulated by plants under salt situations have the ability to contribute stored Nitrogen that can be utilized after stress conditions is over and might accounts in osmotic regulations (Ashraf and Harris 2004).

3.1.1 Salinity and Protoplasmic characteristics

The salinity endurance of certain halophytes and glycophytes is related with cell susceptibility to extreme saline conditions. Experiments are carried out in saline environments employing cell protoplasm to examine salinity consequences at cell level (Mansour 2000). The protoplasmic properties considered in certain experiment are as follows:

### a. Permeability of Plasma membrane (PM)

PM facilitate ionic and molecular transport across the cell. The changes in its permeability differed substantially amongst salt tolerant cultivars. It was noted that after salt treatment, the permeability of PM to nonelectrolytes (Urea, Chloroform, and sugars) increased whereas that of water dropped significantly in salt sensitive grain crops. Salt stress stimulates the generation of reactive oxygen species, inflicting damage to nucleotides, proteins, and cellular membranes. ROS damages proteins and membrane lipids, causing peroxidation of lipids and peptide degradation by their oxidation, that affects cell wall integrity and so therefore permeability. As a result, salt-tolerant species are likely to boost antioxidant mechanisms, reducing lipid peroxidation and oxidative protein modifications and thereby maintaining membrane physiology and structure (Mansour 2000).

### b. Cytoplasmic viscosity

Cytoplasmic viscosity under salinity stress was investigated by Russian researchers. When the salt resistant crops were examined under high elevated cytoplasmic viscosity, they exhibited increased concentration of cytoplasmic proteins and macromolecules. They supermolecules might already be present there or developed under salt stress. Glycophytes (species that grow under low salts) have

reduced cytoplasmic viscosity as compared to tolerant species, and it also reduces under salty environments. The proteins generated in the cytoplasm under salt stress are consistent with variations in cytoplasmic characteristics in both halophytic and glycophytic cultivars (Hasan *et al.,* 2020, Mansour 2000).

## c. Cytoplasmic streaming

This phenomenon is not well examined in salinity conditions. In plant cell it is related to actin-mayosin pathways that demands ATP (Magdy *et al.,* 1994). Under no salinity stress, streaming is considerable in salt-sensitive vegetation. Cytoplasmic streaming is minimal in salt-tolerant species, which may be linked to a low ATP reserve and other variables in the cytoplasm which influence streaming. Plants acclimatize towards salinity by limiting their metabolism, and thus the development of salt-resilient flora is retarded resulting in a weak streaming of cytoplasm (Hasan *et al.,* 2020, Kuiper *et al.,* 1989).

## 3.1.2 Salinity and hormones regulation

Plants exposed to significant salinization result in Abscisic acid (ABA) as well as cytokinin production (Thomas *et al.,* 1992, Vaidyanathan *et al.,* 1999). ABA is supposed to undergo gene modification under salinity stress. Salinity tolerance is upregulated in salt susceptible *T. aestivum* when it is exposed to salt stress gradually rather than abruptly (Hasan *et al.,* 2020). Auxins have important function in controlling apical dominance, tissue development, plant growth, and cell elongation. It is observed that in plants it react towards salt stress (Kaya *et al.,* 2009).

Crop production is negatively impacted by abiotic elements. Drought and salinity have an impact on plant physiology, and crop productivity (Rontein *et al.,* 2002). One of the fundamental goals of plant biosynthetic pathways was to improve crop resistance to osmotic stressors, and it is still the primary objective currently (Le Rudulier *et al.,* 1984, Maucieri *et al.,* 2018, Sakamoto and Murata 2001). Many processes aid in the reduction of salinity. The creation and storage of osmolytes, which minimize the consequences of salinity and osmotic pressure, is one of them (Yancey 2001).

The generation of osmoprotectants is due to the marginalization of organic osmolytes from the hydrated sphere of proteins (Timasheff 1992). Salt quickly penetrates the hydration sphere and destroys the protein 3D structure by directly interacting with their surfaces if osmoprotectants are not created (Kosar *et al.,* 2019). When NaCl is encountered, plants produce and accrue different solutes e.g., proline (Pro). With the addition of various osmoprotectants, he noticed variations of solute content in NaCl treated *O. sativa* L. During three days of stress, he saw a modest deposit of trehalose in the rice seedlings (Garcia *et al.,* 1997).

## 3.2 Osmoprotectants

Osmoprotectants is a collection of small organic molecules synthesized in plants. They don't have any charge and are easily dissolved (Fang *et al.,* 2015). They act as a shield for cells and organelles from desiccation and keep osmotic balance in the cell. They don't disrupt the biochemical processes taking place within the cell (Hasegawa *et al.,* 2000). These solutes also work as a defensive mechanism against stress in plants by a different mechanism such as detoxification of ROS, membrane stabilization, and maintenance of inherent protein confirmation (Blum 2017). High moisture stress is the result of high salt concentration in soil, inhibition of cell enlargement, plant development, and influx of water. Consequently, High uptake of $Na^+$ ions leads to leaf chlorosis, necrosis, and mortality (Hussain *et al.,* 2011).

## 3.2.1 Types of Osmoprotectants

Osmoprotectants guard several osmotically delicate structures in the cell, moreover each osmoprotectant varies in size, shape, and charge from one another (Garcia *et al.,* 1997). Osmoprotectants contain different compounds, i.e., proline (Ahmad *et al.,* 2021, Hoque *et al.,* 2007) trehalose and quaternary ammonium compounds like glycine betaine, choline-o-sulfate, proline betaine and sugar as sucrose and polyols (Ashraf *et al.,* 2007). There are three different types of Osmoprotectants including betaine along with associated compounds i.e., dimethyl sulfoniopropionate and choline; some amino acids e.g., proline and exotoxin; and polyols and some non-reducing i.e., trehalose (Rontein *et al.,* 2002).

In different plant species, osmoprotectants agents are produced and stored in different concentrations. Some osmolytes are managed by the production of the solute by an irreversible mechanism inside the cell or by a grouping of other pathways, some of which involve synthesis and others breakdown. The quantity of osmolytes rises with

the increase of peripheral osmolarity. Hence, increasing the osmolyte concentration helps to maintain osmotic balance thus essential for growth in the stress of salt conditions. The amount of anti-osmotic chemicals in a plant is determined by the osmolarity of the surrounding environment because production and accumulation are frequently stress-induced to some degree, plants that natively retain osmoprotectants in cells have a fresh weight of 5–50 µmol $g^{-1}$ (∼6–60 mM based on plant moisture) and it is increased with response to salt stress (Bohnert *et al.,* 1995).

### 3.2.2 Trehalose

Trehalose functions as an osmoprotectant. It's a non-reducing disaccharide that helps maintain metabolic homeostasis, osmotic balance, and improves saline resilience in a variety of organisms. Trehalose-6-phosphate synthase that catalyzes the glucose component of UDP-glucose is transferred to G6P, giving uridine diphosphate and T-6-P in the trehalose biosynthesis (Cabib and Leloir 1958, Goddijn and Smeekens 1998). Trehalose is formed when trehalose-6-phosphate phosphatase dephosphorylates T-6-P. Trehalose production is a stress-relieving approach since it promotes plant development facing water shortage and excessive salt levels (Orozco-Mosqueda *et al.,* 2019).

In reaction to salt and drought stress, elevated concentrations of trehalose were detected in *Phaseolus vulgaris* and *Medicago truncatula* root nodules. This suggests that trehalose is vital in symbioses since it increases plant output and growth while also allowing for greater adaptability to severe environments. According to an updated study, applying trehalose topically to wheat (*Triticum aestivum* L.) can help it recover from decreased D1 protein production and prevent it against heat-induced photo-inhibition by lowering ROS generation and raising the gene transcript level of associated antioxidative enzymes(Luo *et al.,* 2018). External trehalose induces sugar metabolic changes, sustains enzymes, protein aggregation, and lipid membranes, and shields plants from dehydration by allowing them to retain moisture (Mohamed *et al.,* 2018).

### 3.2.3 Mannitol

Arabidopsis genes are transcribed in a vast number of plants to improve their resilience to salinity strain. Tobacco is an illustration of how heterologous expression of the *A. thaliana* gene *AtTPS1* improves salt tolerance. Because of mannitol's protective function, the mannitol gene from *E. coli* was ectopically amplified in wheat, resulting in increased salt stress resilience (Abebe *et al.,* 2003). The celery mannose-6-phosphate reductase (M6RP) gene that produces mannitol, was also transferred to transgenic Arabidopsis plants in another research (Sickler *et al.,* 2007).

### 3.2.4 Glycine betaine (GB)

Plants' glycine betaine synthesis pathways are triggered in response to dehydration stress, and quaternary ammonium compound is the most prevalent in plants. Glycine betaine, proline betaine, and Choline-O-sulphate are all examples of QAC. GB is one of them, and it helps plants regulate their osmotic pressure. Biosynthesis and accretion of this osmolyte are all observed in diverse taxa including Mammalian, bacterial, marine invertebrates, hemophilic archaea bacteria, and plants. The chloroplast is high in GB, which helps to maintain photosynthetic efficiency by adjusting osmotic equilibrium and protecting thylakoid membranes (Robinson and Jones 1986).GB concentrations are not consistent they vary greatly between organs and plant species. Plants having lower GB concentrations are subjected to salt stress, they begin to synthesize GB at a higher rate. As a result, plants are recognized as natural GB accumulators (Storey and Jones 1977). Most salt-tolerant plants gain a large amount of GB, according to Rhodes. The physiological roles of GB in higher plants like spinach, sugar beet, barley, and maize have been extensively studied (Rhodes and Hanson 1993). Cloning genes that transcribe GB synthesized enzymes resulted in transgenic plants. Finding a favorable GB content based on the kind of crop is critical for the best possible stress tolerance performance (Chen and Murata 2008). Like, broadleaved species such as bean, tomato, and grape are more sensitive to higher concentrations than cereals (Mäkelä *et al.,* 2000). As a result, the appropriate GB concentration should be employed to maximize the benefits of Osmotic adjustment in various plant species (Roychoudhury and Banerjee 2016). Salinity tolerance of different plants can be improved by enhancing the membrane stability in the stimulus of the stress of salt by using enzyme protectants and an osmolyte like Glycine betaine. Usually, GB gathers in low concentration in plants but in certain cases, it accumulated at a high level

in plants and causes osmotic pressure. The stability of the quaternary structures of different enzymes and intricated proteins can be improved by lowering the concentration of GB. Genetically engineered maize having the beta locus of *E. coli* that is enabled to encode choline dehydrogenase showed high-level GB under stress conditions (Welin-Klintström *et al.,* 1999). Beta expression produced enzyme choline dehydrogenase (CDH), carried out choline oxidation into betaine aldehyde followed by oxidation of betaine aldehyde and result in glycine betaine by using the similarly specified enzyme. This showed the reduced membrane damage, caused an increased activity of enzymes and better resistance and tolerance to numerous stresses by comparing with wild-type plants. L2 and L3 transgenic lines comprised elevated levels of GB and ratios of K+/Na+ were also more than the WT (Liang *et al.,* 2013). The results showed high GB accumulation protects membrane integrity along with protecting the macromolecules to lose their stability(ALLA *et al.,* 2019, Mamedov *et al.,* 1993). It helps to sustain average metabolism and to maintain K+ ion in cells under salt stress (NaCl). GB buildup soothes as well as holds macromolecules action by refraining the ROS-induced harm by saving intricated proteins from harm and enhancing membrane integrity (Sakamoto and Murata 2001).

### 3.2.5 Proline

Proline is known as a significant osmolyte present in plant cells having high solubility of water, lower molecular weight (Delauney and Verma 1993). Plant cells gather soluble osmoprotectants and lessen the osmotic stress effect due to c salt stress. So, the proline content can also be used for the plant to resist stress tolerance (Hayat *et al.,* 2012). Proline keeps osmotic stability and has a substantial osmoprotective role. In the laboratory, a wheat gene known as Ta-UnP was augmented and it showed noteworthy progress towards salt tolerance in transgenic rice and Arabidopsis. Proline content due to salinity stress in transgenic Arabidopsis thaliana was noticeably amplified, to maintain the osmotic potential and protect cells under stress environments (Liang *et al.,* 2013). In one of the studies, tobacco plants were designed for both salinity and drought resistance features through increasing

proline manufacturing(Hong *et al.,* 2000). In previous experiments, it was shown that transgenic plants grew faster and had higher chlorophyll levels when exposed to salt stress. In the case of petunia accumulation, a higher amount of proline allowed the plant to withstand dryness for 14 days. Under stressful conditions of 200 mM NaCl, proline concentration was found to be four times higher in recombinant pigeon pea (Surekha *et al.,* 2014, Yamada *et al.,* 2005).

In proline biosynthesis, two enzymes, i.e., P5CR (pyrroline-5-carboxylate reductase) and 5PCS (pyrroline-5-carboxylate synthase) have a significant role. P5CR is also called the rate-limiting enzyme in this biosynthesis (Liang *et al.,* 2013). Proline also has a contribution for maintaining sub-cellular structure, like membranes, proteins, etc., and in osmotic adjustment (Ashraf *et al.,* 2007). In a saline atmosphere, proline is among the best-known osmoprotectants. In wheat plants under drought and salinity stress environment, proline buildup generally happens in the cytosol and here it gives to the cytoplasmic osmotic adjustment. This situation is commonly thought that it shields against salt damage and in cytosol maintain osmotic balance (Mahboob *et al.,* 2016).

## 4. Effect of ectopic osmolarity on plant growth

Specific proteins formed throughout stress or unfavorable conditions act as osmotic agents .The quantity of polyols, polysaccharides, and proline in plants increases due to salinity in the soil around the roots of the plants rises, favoring the osmotic potential in the cell (Pollard and Jones 1979). However, when the water level is decreased, the membranes and enzyme structures change, reducing the integrity of the membranes and reducing enzyme activity (Genard *et al.,* 1991, Schwab and Gaff 1990). Osmoprotectants with high concentrations sustain osmotic balance. It also shields enzyme activity and membrane. Osmoprotectors use a different process to maintain and protect the cell. There is also evidence that when plants are given various osmoprotectants, these solutes change the functioning and morphological properties of plant organs, such as the leaf and pod (Garcia *et al.,* 1997). Fig 2 demonstrates the induction of osmotic stress produced due to saline conditions.
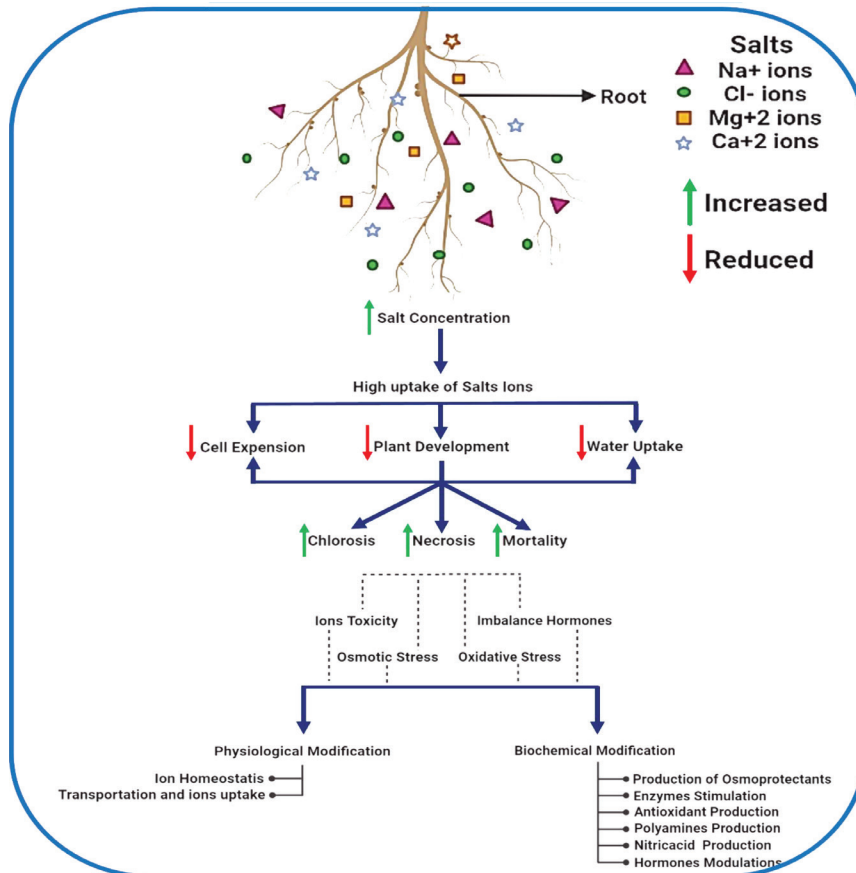
Figure 2. Illustration of osmotic stress produced due to high salt concentration outside the roots results in series of physiological and biochemical modification in plants

## 5. Role of transcription factors in salinity stress response

Gene transcriptional regulation is the fundamental process for the basic functioning of cells when they respond to specific stimuli as well as tissue specific gene expression (Latchman 1997). Plant metabolic routes undergo transcriptional control, and genetic expression related to these routes is frequently controlled via hormonal, phenotypic, or developmental processes. Recent extensive analysis of genomic expression have disclosed the true dimension of systematized metabolic regulation in legume plants (Broun 2004). Stress responsive TFs have gained so much consideration not only for modulating the genome expression patterns but also perform a prominent role in different environmental stress conditions i.e., salt toxicity or salinity stress (Läuchli and Grattan 2007). Fig. 3 summarizes the incidence of TFs in response to salinity stress.

### 5.1 MYB

*MYB*-TFs family is a renowned TF family in vascular plants and more than 200 *MYB* TFs are present in *O. sativa* and Arabidopsis that play critical roles in various metabolic processes under normal as well as unfavorable conditions (Zhang *et al.,* 2011, Zhao *et al.,* 2018). *ZmMYBC1* was the initial *MYB* gene identified in *Zea mays* regulating the biosynthesis of an essential flavonoid known as anthocyanin (Salih *et al.,* 2016). MYB domain constitutes R1, R2 and R3 DNA binding repeats (Rs), each having a length of 50-53 amino acid sequence at N-terminal and also possess distinct conserved sequence of TAACNA/G (Ma and Constabel 2019). When two wheat transgenic inbred lines were analyzed via quantitative RT-PCR for salinity tolerance then four *MYB* genes were upregulated out of which *TaMYBsdu1* showed significantly higher expression in salt stress condition which turned out to be a remarkable *MYB* gene to confer adaptation in wheat under high salt concentration (Rahaie *et al.,* 2010).
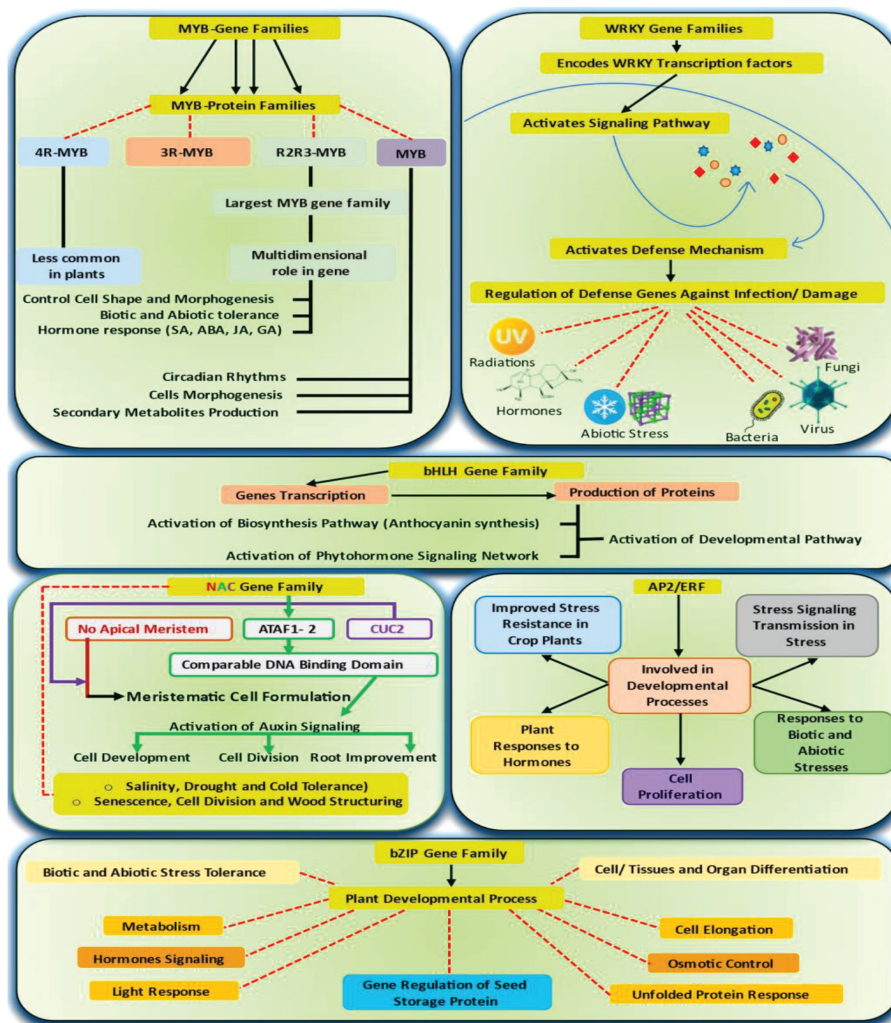
Figure 3. Illustration on brief role of different plant transcriptional gene families under salinity stress.

Cai and co-workers investigated that *TaMYB3R1* gene comprising of three conserved repeats cloned from *T. aestivum* showed the transcript abundance to be continuously increasing up to seventy-two hours and its expression was markedly increased by high salt concentration (Cai *et al.,* 2011). Exogenous expression of an R2R3 type transactivating *TaMYB73* in Arabidopsis was demonstrated by cDNA microarray to enhance endurance against high salt stress. ABA, GA and NaCl application induced the activity of promoter containing stress responsive elements of this particular *MYB* gene improving LiCl, NaCl and KCl resilience in Arabidopsis. This report implied that wheat *MYB73* is a unique *MYB* TF which show prominent transcriptional activity under salinity stress by modulating stress modulating genes that increase ionic tolerance (He *et al.,* 2012). When a strawberry *FvMYB24* gene encoding an R2R3-type MYB

TF was cloned in Arabidopsis , it showed endurance against high salt stress and resulted in high chlorophyll content, higher sprouting rate and extended root hairs than the WT plants (Wang *et al.,* 2021). Improved root characteristics also help salinity stress tolerance when micronutrients were applied

## 5.2 WRKY

*WRKY* TFs are pivotal factors of cellular transduction mechanisms in the implementation of defense responses to various environmental pressures in plants (Rushton *et al.,* 2010, Wani *et al.,* 2021). These are characterized by having a W-box cis-acting element i.e., TTGACT/C and initial discovery of *WRKY* TFs as a protein that binds with DNA from *Ipomoea batatas* L. has augmented the examination as seventy-nine *IbWRKY* genes were detected (Ishiguro and Nakamura 1994, Qin *et al.,* 2020). *FtWRKY46* TF from Tartary buckwheat (*Fagopyrum tataricum*), a small

grain having remarkable resistance capacity showed resistance against salt stress conducting a transcriptional activity to scavenge ROS as depicted by reporter gene expression in yeast one-hybrid assay (Lv *et al.,* 2020). *TaWRKY79*, which is a class II *WRKY* gene TF functions in ABA-dependent pathway that facilitates in boosting ionic tolerance as induced by ABA and NaCl with diminished ABA sensitivity (Qin *et al.,* 2013). The study established the role of both WRKY genes for improving drought tolerance in plants. Under salt stress, AtWRKY25 and AtWRKY33 have improved salt stress tolerance through SOS-pathway-independent manner. However, AtWRKY33 showed salt tolerance through ABA-dependent signaling pathway and *HbWRKY83* showed salt tolerance independently (Jiang and Deyholos 2009, Kang *et al.,* 2020).

## 5.3 bHLH

*bHLH* TFs were first reported in a study on R locus of *Z. mays* which contained 3 participants of this gene family, LC, P, and S encoding similar transcripts of each 2.5 kb in length. The recognized Lc protein, determined from the cDNA sequence analysis, was made up of 610 amino acids and homologous to the helix-loop-helix DNA-binding/dimerization motif present in L-myc gene product and cellular transcription modulating proteins necessary to accumulate at least 2 genes in the anthocyanin biosynthesis pathway(Ludwig *et al.,* 1989, Mol *et al.,* 1998). The distinguishing characteristic of *bHLH* TFs is sixty amino acids domain including 15 amino acid long DNA binding domain at N-terminal named as basic (b) region because of the 6 basic amino acids which binds to the G-box sequence i.e., 5´-CACGTG-3´ which is the fundamental part of this domain. Whereas the *HLH* part in the domain contributes in the formation 2 amphipathic α-helices with a coil of varying length (Cabrera and Alonso 1991, Van Doren *et al.,* 1992). The microarray-analysis of a salt tolerant *T. aestivum* mutant, RH8706-49, showed high expression pattern of a protein encoding gene having a conserved DUF662 domain. Later that gene was named as *TaSRG* (*Triticum aestivum* Salt Response Gene). RT-quantitative PCR analyses revealed that its appearance was affected by salinity, ABA, dehydration, low temperature, and other stress conditions. The upregulation of *TaSRG* in WT Arabidopsis plants caused an improved salinity tolerance compared with control plants. DUF662 domain is noticed in a group of hypothetical eukaryotic proteins and may be homologous to bHLH domain (He *et al.,* 2011). The separation, classification, and cloning of a *TabHLH1* gene encoding protein having a crucial role in salt resilience (Yang *et al.,* 2016). Sweet sorghum contains a bHLH TF encoding gene *SbbHLH85* that showed a distinguished behavior from its counterparts in other species as it performs a negative regulatory role in salinity tolerance. A phosphate transporter chaperone *viz.,* PHF1 was also detected to confer potential interaction and regulated the dissemination of phosphate, by examining a yeast two-hybrid library (Song *et al.,* 2021).

## 5.4 NAC

This family comprises of broad proteins group comprising *NAM*, *ATAF*, and *CUC*, hence referred collectively as *NAC* TFs containing extensive protein family (Guérin *et al.,* 2019, Lindemose *et al.,* 2014). Three *NAC* genes *viz.,* *AtNAC019*, *AtNAC055*, and *AtNAC072*, were observed to upregulate under dehydration and saline conditions, and overexpression of these genes in transgenic Arabidopsis plants exhibited improved salinity stress endurance compared to the wild type plants (Wang *et al.,* 2018). *NAC* conserved domain is positioned at the N-terminal, encompassing 150 to 160 amino acid residues and is allocated into 5 sub-domains (Ooka *et al.,* 2003). *TaNAC67* gene from durum wheat when expressed in Arabidopsis and exposed to various environmental stress conditions to evaluate modifications, unveiled tolerance induction against high temperature, saline conditions, and desiccation (Mao *et al.,* 2014). A soybean *NAC* TF *GmNAC06* overexpression associated with CRISPR Cas9 technology induced tolerance in treated plants accumulating GB and proline to lessen the negative impact of oxidative stress caused by ROS and correspondingly managed the sodium potassium ionic ratios to maintain homeostasis (Li *et al.,* 2021).

## 5.6 bZIP

Arabidopsis contains seventy-eight basic region/leucine zipper TF family members divided into thirteen subfamilies (Dröge-Laser *et al.,* 2018). The distinctive principal sequence of *bZIP* domain which forms adjacent amphipathic α-helices have 2 main regions, basic region at N-terminus which is sixteen amino acid long nucleus targeting sequence followed by a conserved DNA binding motif i.e., N-x7-R/K whilst C-terminus is distinguished by the occurrence of leucine rich residue or other

corresponding hydrophobic residues (Jakoby *et al.,* 2002). *TabZIP60*2 was isolated and mapped from 3 homologous genomes of hexaploidy wheat. Its analysis determined that *TabZIP60* protein is positioned within the nuclear region and developed tolerance to water scarcity, saline soil, chilling stresses and strengthened response to ABA in plant growth by binding to ABA-responsive *cis*-elements (Zhang *et al.,* 2015). In a study conducted to identify bZIP TFs of *Jatropha curcas* as a result of which fifty *JcbZIP* genes were determined while four putative TFs i.e., *JcbZIP*s *34*, *36*, *49* and *50* were shown to play a key role in tolerance associated genes under both saline condition and water shortage (Wang *et al.,* 2021).

### 5.6 AP2/ERF

The major function of AP2/ERF (DREB/ERF) TFs is to control a vast number of stress-related genes (Agarwal *et al.,* 2006, Hussain *et al.,* 2011). Abiotic stimuli responses are influenced by these genes. In Arabidopsis, DRE cis components have been found, and the DREB gene has roughly 40 conserved regions. These are present in 20 different plant species. Multiple stress events can activate a single DREB gene (Lata and Prasad 2011). Plants were transfected with a total of 20 distinct DREB transcription factors, as well as the CaMV35S constitutive promoter or the rd29A stress-inducible promoter (Khan 2011, Lata and Prasad 2011). Because Stress tolerance to abiotic variables is aided by DREB transcription factors, which can endure recurrent abiotic shocks. When DRE/CRT cis-acting elements bind to promoter regions, these elements are activated, and they can be exploited to create genetic variation (Kasuga *et al.,* 2004).

LEA proteins, osmoprotectants, phospholipase C, protease inhibitors, cold acclimation proteins, glucose transporter proteins, and transcription factors are all encoded by several of these downstream genes. In saline environments, they demonstrated improved seedling growth (Khan 2011, Lata and Prasad 2011, Nakashima *et al.,* 2009, Tran *et al.,* 2004). Higher salinity causes osmotic pressure, and increased salt consumption mixed with osmotic stress leads to an increase in reactive oxygen species (Boo and Jung 1999, Hasegawa *et al.,* 2000). These proteins aid abiotic tolerance by binding to DRE/CRT cis-acting regions in a variety of strain-related genes. These factors experience abiotic stress tolerance when they bind to DRE/CRT cis-acting sites in

the promoter regions of various stress-related genes like they are key plant stress tolerance factors (Agarwal *et al.,* 2006, Hussain *et al.,* 2011, Singroha *et al.,* 2022). T349 possesses an osmotic equilibrium due to the upregulation of methionine synthase, which increases transgenic wheat salt tolerance. Methionine synthase is a protein that participates in the production of methionine. A methyl group is transferred from 5-methyltetrahydrofolate to homocysteine. This process, also known as the lone carbon source, occurs in the active methyl cycle. That methionine is transformed to S-adenosylmethionine with the aid of S-adenosylmethionine synthetase (SAM). SAM provides the methyl group for a variety of metabolites in high salinity environments, including methylated polyols, glycine betaine, and polyamines. Glycine betaine and methylated polyols are two compatible solutes that concentrate in the cytosol (Bohnert *et al.,* 1995, Nomura *et al.,* 1998). As a consequence, during salt stress, the osmotic balance was maintained. This shows that through modulating the osmotic balance, upregulating methionine synthase in T349 increases transgenic wheat's capacity to tolerate salinity. Under salt stress, both Glyceraldehyde-3-phosphate dehydrogenase (GPD) and methionine synthase were increased in T349 cells. GDP plays a key role in glycolysis and gluconeogenesis. When GPD activity is boosted, carbon is metabolized away from glycerol. This would result in glycolysis and ATP production, as well as the production of suitable osmolytes.

## Conclusion

This paper provides information on physiological, molecular, and biochemical characteristic changes induced in wheat under high saline conditions. The relative susceptibility of wheat to salinization has been described, and the uptake and transport of NaCl[+] has been examined in terms of phytotoxicity and interaction with mineral nutrients. Various methods for enhancing salt tolerance are currently known. Upregulation of stress tolerant genes under high salt concentration by utilizing natural osmoprotectants, modulating hormones and overexpressing the stress related TFs are just a few of the key molecular factors that can be used for genetic engineering of wheat plants to induce resilience against unfavorable environmental conditions.

## Compliance with ethical standards

NA

## Conflict of interest

No

## Author contributions

MA: Conceptualization, Data curation, AA: Conceptualization, Writing & updating the manuscript for publication, WJ: Writing, TSB: Conceptualization, Writing, ME: Conceptualization, AG: Conceptualization, Supervision, and Validation. All the listed authors read and approved the manuscript.

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