



# A Review – Some Approaches to Combat Salt Stress in Wheat Crop

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## **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

## **Article Information**

DOI: 10.9734/IJPSS/2022/v34i242655

## **Open Peer Review History:**

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/95385>

**Review Article**

**Received: 18/10/2022**

**Accepted: 27/12/2022**

**Published: 28/12/2022**

## **ABSTRACT**

Wheat constitutes a central position for ensuring food and nutritional security; however, rapidly rising soil and water salinity pose a serious threat to its production globally. Salinity stress is a universal dilemma that is happening due to climate change. It affects hectares of arable land. Main focus regarding improving salinity tolerance in plants has been given to Na<sup>+</sup> exclusion/Na<sup>+</sup> compartmentalization and enhanced ROS system. Besides this, ameliorative activity of phytohormones, nutrients, amino acids and organic osmolytes has also been widely studied. Exploring traits in wild genotype aids search for better solutions. 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 genes. Wheat plants utilize a range of physiological biochemical and molecular mechanisms to adapt under salinity stress at the cell, tissue as well as whole plant levels to optimize the growth, and yield by off-setting the adverse effects of saline environment. Recently, various adaptation and management strategies have been developed to reduce the deleterious effects of salinity stress to maximize the production and nutritional quality of wheat. Thereby, this review highlights effects of salt tolerance, physiological mechanisms behind salt tolerance and transgenic wheat that are potential indicators of salinity stress tolerance.

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**Keywords:** *Salinity; transgenic wheat; genetic engineering; HKT gene.*

## 1. INTRODUCTION

“Recently, climate change and global warming have directly affected the crop’s yield and quality by intensifying the frequency and extent of numerous stresses. Wheat, rice, and maize are the most important staple crops globally and contribute a significant part of daily calorie and protein intake. Among these major cereals, wheat is ranked at the first position due to its domestication and contribution as the primary staple food crop globally. A variety of climate models predictable that wheat production might reduce by 6-7% because of stressful environments” [1]. “They are exposed to an array of abiotic stresses that severely affect their growth and grain yield and account for 50% losses in crop yield” [2]. “In addition, the constant rise in the human being population put pressure on worldwide food security as the world’s food demand needs to be raised by up to 65-70% by the year 2050. Salt stress affects 20% of global cultivable land and is increasing continuously owing to the change in climate and anthropogenic activities” [3]. About 35% of the world’s human population is directly dependent on wheat as a staple food. About 20% of calories and 55% of carbohydrates are provided by wheat across the globe. Various environmental stresses include drought, flooding, heat, chilling and salinity, among these environmental stress, salinity poses a significant threat to cereal crops [4]. Salinization (Soil Salinity) refers to the addition of soluble salts into the soil. Saline land is not appropriate for cropping [5]. There is an immense impact of salinity stress on plant growth (morphological as well as physiological), metabolism and productivity [6]. Root length, plant height, number of leaves, photosynthesis, water relations and chlorophyll contents are affected [7]. “Plant growth is influenced by salinity in osmotic phase and ionic phase. In osmotic (rapid) phase, there is inhibition of the young leaves. In ionic (slower) phase, results in premature senescence of older leaves and in toxicity symptoms (chlorosis, necrosis) in mature leaves due to high  $\text{Na}^+$  which affects plants by disrupting protein synthesis and interfering with enzyme activity” [8]. “Salt tolerance of wheat is greatly enhanced by potassium transporter (HKT) genes” [9]. “Plants overcome the salt stress by three major adaptations include osmotic stress tolerance, sodium ion or chloride ion exclusion and tolerance of tissue to accumulated sodium ion and chloride ion” [10].

For ensuring food security, one needs to adopt stratagem to surmount this specific threat. Undeniably, researchers have been exploiting a mixture of strategies such as molecular genetics and functional genomics to achieve enhanced crop productivity on salt affected soils [11]. “Conventional breeding, marker-assisted selection and genetic engineering are the biotic approaches that are used to develop salt-tolerant cultivars of various cereals and provide a great opportunity of molecular and physiological knowledge to improve the salinity tolerance in plants” [12]. “A number of barriers have been identified to the development of salt-tolerant cultivars. These include inadequate knowledge about the genetic makeup of crops, their physiological as well as biochemical behavior, broad divergence in environmental conditions” [13].

“Soil salinity is the 2<sup>nd</sup> most important feature that causes earth degradation after soil erosion, leads to decline in agricultural economic outputs for 10,000 years” [14]. Poor salinity management can because soil solicits of farming soils, where sodium in cationic form binds to anionic natured clay, leads to clay swelling and dispersal, consequently declining the crop productivity. Larger concentration of salinity confiscate approximate 1.6 million hectares of agriculture land worldwide every year and therefore ~50% of cultivable ground could be deteriorated by 2050. Soil salinity creates detrimental impacts on vital metabolic, biochemical, and physiological processes occurring within the plants leading to the deterioration of grain quality and quantity. The amount of changes in grain quality caused by salinity depends on the sternness of the stress. From physiological perspectives, grain quality is affected owing to the accumulation of salts in the root zone leading to osmotic stress induction, which vigorously disrupted cell ion homeostasis. Salt exposure causes high osmotic pressure stress at the initial stage, while consequently; cationic toxicity hampers growth kinetics, grain development, and quality, especially if the exposure times get prolonged. Soil salinity detrimentally alters a variety of phenotypic characteristics of wheat plants counting growth of seedling, height of plant, length of shoot, and root, roots number, leaves, surface area of leaf, dry and fresh weight, shoot/root ratio, and content of chlorophyll. Experimentally evaluate that the early maturity of

wheat because of salinity stress that reduced the height of crop and surface area of leaf and observed that plumule dimension was the mainly responsive during early growth stages. "Wheat (*Triticum aestivum*) is a cereal grain that is cultivated for its seed. It is the most common staple food used worldwide and provides calories to approximately 30% (4.5 billion) of the world population and 20% of the total protein requirements" [15]. "However, the wheat productivity per hectare is extremely lower than its production potential, which is due to many different factors/stresses and salt salinity is the most common one" [16]. "It is estimated that 60% of crop production lost is due to the salt stress in the environment. Salts in soil could increase naturally or may be introduced anthropogenically" [17]. "It could arise either by soil modifications, irrigation or by use of fertilizers. Very few salt tolerant bread wheat genotypes have been identified till date" [18]. "Some of them are Kharchia-65 (collected from Kharchi, in Pali District of Rajasthan, India) and KRL-210 (Developed at Central Soil Salinity Research Institute, Karnal, India)" [19].

Weathering of rocks discharges various soluble salts (chlorides of sodium, calcium and magnesium) [20]. "The most abundant is Sodium chloride and one of the most detrimental effects of salinity is the accumulation of sodium ion (Na<sup>+</sup>) in plant tissues" [21]. "Salt stress decreases wheat crop yield and damages plant metabolic processes through impairment of  $\Psi_w$  (water potential) of cells, uptake of essential elements, ion toxicity, cell membrane integrity and function" [22]. "The uptake of essential macronutrients such as Potassium and Calcium from soil is inhibited by higher concentration of sodium ion" [23]. So hereby in this review we will provide firstly the effect of salinity on nutrient uptake, seed germination, morphological and physiological characteristics etc and then an outline of the mechanisms of wheat salinity tolerance and present an outlook on prospective key research on this topic.

## 2. EFFECT OF SALINITY ON WHEAT

"One of the unfavorable effects of a saline environment, particularly high salt concentration in soil, causes a severe decline in the uptake of nutrients and water. Resultantly, high osmotic pressure intensifies cationic toxicity, imbalance of nutrients under water-deficit conditions. Salinity produces injury of photo-synthetically active leaves by causing chlorosis and triggering leaf

senescence in cereals" [24]. "It is essential to understand wheat's response at every phase of growth which can be helpful in improving or developing salt tolerant varieties" [25].

**"Effect of salinity in seed germination:** Elevated intensity of salt gradually amplified the time of sprouting but decreases the sprouting percentage. Thus, it affects the seedlings growth" [26]. "Previous studies have confirmed that in different wheat cultivars salinity stress suppressed and delayed the germination of seeds" [27].

**Morphological characteristics:** The morphological characteristic of wheat crop like 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) are influenced at salinity stress declined the plant leaf area [5]. This reduces the photosynthesis rate and resulted in low biomass production [28]. Under stress root is in fact the key organ since it has the probability to swing in order to avoid high salt conditions [29]. Therefore, increased salt concentrations poorly affect root length and its capability to sop up water [30].

**Effect of salinity on Physiological characteristic:** Wheat's bodily processes are disrupted by salinity through ionic and osmotic stress. The physiological processes consist of modification in plant development, mineral allocation and plasma lemma unevenness resulting from calcium ion dislocation by sodium ion [31]. High salt content in soil declines leaf  $\Psi_w$ , loss of  $\Psi_p$  (turgor pressure), stomata closure leading to decrease CO<sub>2</sub> exchange, elevation in oxidative stress, change in cell wall integrity and enhancement of toxic metabolites resulting in plant death [32]. Beside all this change in soil salinity also affects the photosynthetic activity and decreased yield. Chlorophyll, xanthophyll and carotenoid pigments are reduced due to salt stress [33].

**Effect of salinity on Biochemical characteristics:** ROS are usually produced in grana (photosynthesis), mitochondria (ETC) and peroxisome (glyoxylate cycle). Salinity stress further increases the formation of ROS [34]. Plants scavenge salinity induced ROS by overproduction of glutathione (GSH- reductase and synthetase), superoxide dismutase (SOD) and ascorbate peroxidase (APX) [35].

However, uninterrupted extreme creation of ROS retards the overall anti-oxidant defense system, leading to damaging effects on the ribonucleic acids, polypeptides and fatty acids [36]. Various metabolites like abscisic acid, glycine, proline, polyol and betaine are required to preserve the osmotic potential in the plant vacuoles against the ion toxicity gathered in the cell compartments. Thus, they help in protection of plant against salt stress [37]. Salinity stress enhanced the addition of Na<sup>+</sup> and Cl<sup>-</sup> contents and reduced the K<sup>+</sup> and Ca<sup>+</sup> contents in leaves and roots. The accumulation of Na<sup>+</sup> and Cl<sup>-</sup> ions aggravate toxicity posing harmful effects on vegetative and reproductive phases of growth in plants [38].

**Molecular and genetic level alteration:**

Assorted genes and proteins identified in diverse salinity-stress induced plants include Bnd22, Sal1, RAB 21 KDa, 27KDa protein, 25KDa protein P 150, fibronectin, vitronectin, Dehydrins, ABA- abscisic acid genes [39], Vacuolar acid invertase, proline induction and late embryo abundant proteins. Wheat tolerance to salinity is due ABA [40] in the roots. Numerous changes in 3A and 3D (homologous chromosomes) provoke stress tolerance [41]. Concentration of 26KDa protein and proline content was found to be significantly elevated in wheat. It proved that their production is induced by salt-responsive gene so as to guard the plant during salinity [5].

**Anatomical characteristics:** “Under salt-stressed conditions variations seen in plant anatomy includes decline in overall cell dimensions of stem, root & leaf parts, transformation in size & number of stomata, cuticle thickness, early deposition of lignin, change in number & diameter of xylem vessels and decrease in the leaf dermal cells expansion in wheat” [42]. “Likewise, there were many deviations observed amid ground and vascular tissue system that consist of a significant drop in cortex cell area, vascular bundle area and metaxylem vessels area” [43].

### 3. MECHANISM FOR SALT TOLERANCE

**“High-affinity potassium transporters (HKTs) in salt tolerance:** Different mechanisms for salt tolerance help in dipping Na<sup>+</sup> buildup in the cell cytoplasm by limiting Na<sup>+</sup> access into the cell, active transfer of Na<sup>+</sup> out of the cell, and

compartmentalizing Na<sup>+</sup> into the plant vacuole” [44]. “High-affinity potassium transporters (HKTs) are most active at level of plasma membrane. A major mechanism in salinity tolerance of wheat is Na<sup>+</sup> exclusion mediated by these HKT genes. Families of HKTs belonged to HKT/Trk/Ktr-type K<sup>+</sup> transporter superfamily and are discovered chiefly in microorganisms and plants” [5]. “They act as Na<sup>+</sup>/K<sup>+</sup> symporters as well as Na<sup>+</sup> selective uniporter. HKTs have two major subfamilies :-HKT1 and HKT2” [45]. “HKT1 are permeable to Na<sup>+</sup> only. HKT2 are permeable to both Na<sup>+</sup> and K<sup>+</sup>. Tetraploid wheat is less soil tolerant than bread wheat” [5]. “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” [46]. “Nax1 in chromosome 2AL co-segregated along with sodium transporter gene HKT1. It was found to regulate Na<sup>+</sup> unloading from xylem in roots and ultimately was shown as the functional candidate” [47]. “Phylogenetic analysis with Nax2, TmHKT1; exhibited that 5-A significantly decreased leaf sodium content and enhanced durum wheat grain production by 25%, when compared to lines without the Nax2 locus” [48]. “RNA silencing induced by mRNA interference resulted in buildup of Na<sup>+</sup> in leaves. This showed strongly that TaHKT1; 5-D should be the candidate gene of Kna1. AtHKT1 is controlled by small RNA as well DNA methylation. Transcription genes like AtAB14 and OsMYBc were found to control HKT genes in plants” [5]. This presented more candidate targets for decreasing salinity tolerance.

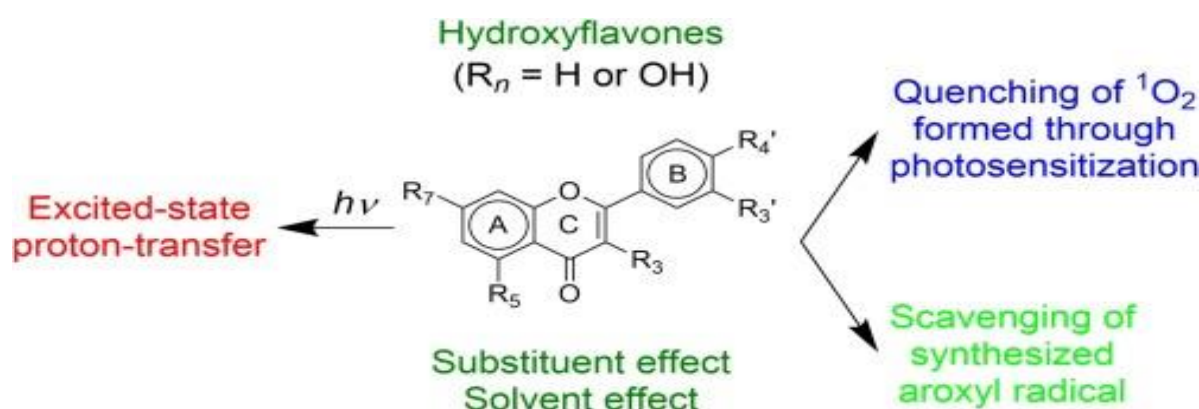
**ROS homeostasis in salt tolerance:** Reactive oxygen species (ROS) are free radicals that are produced during the stress. Under salinity stress, the level of ROS increases in the plant tissues as a result of irregularities in the electron transport chain and accumulation of photoreducing power. They cause chlorophyll degradation along with membrane-lipid peroxidation. The amplification in free-radicals causes the overproduction of MDA. Peroxidation of PUFA in plasmalemma produces MDA-Malondialdehyde [49]. Plants mount up numerous primary as well as secondary metabolites under various environmental stresses. The noteworthy modifications under abiotic stress are variation in amount of soluble sugars, phenolic compounds, chlorophyll and proline contents in leaves. Variation in K<sup>+</sup>/Na<sup>+</sup> and shoot-root biomass ratio is also observed. Of these total soluble sugar, which is a main

component of carbohydrate metabolism exhibits a close association amid photosynthesis and plant productivity [49]. Proline acts as an exceptional osmolytes. It further serves as anti-oxidative defense particle a metal chelator, and a signaling molecule [50]. Hence, proline checks the concentration of ROS and keeps it in normal range thereby preventing oxidative damage in plants. In addition to this, phenolic compounds neutralize the free radicals by quenching singlet oxygen and decomposing the peroxides (Fig. 1).

“On comparing SR3 with its wild type cv.jinan177 (JN177) wheat parent, it was found that ROS homeostasis was the chief biochemical basis for the salt tolerance of cv. SR3”[5]. “Mapping analysis localized a tolerance gene QTL on chromosome arm 5AL (ata position containing TaSRO1) encoding a polyADP ribose polymerase (PARP) domain protein. These PARP proteins have been suggested in modulation of redox homeostasis” [51]. Sequence deviation between the TaSRO1 alleles (present in both) was expected to influence the catalytic action that is noteworthy for DNA repair under oxidative stress. The transgenic constitutive expression of the allele from cv. JN17 augmented the intensity of salinity and ROS tolerance. But RNAi-induced knock-down of the gene in cv. SR3 conciliated the level of tolerance. Therefore, TaSRO1 was believed to be a strong candidate for the salt tolerance QTL in cv. SR3. Somatic hybridization brings in a smallest amount of exogenous chromatin material into a recipient genome, while causes genomic shock that provokes high frequencies of both point mutation and insertion and deletions encoding sequences. Thus, ROS homeostasis

was accomplished by a polygene effect. A Zinc finger transcription factor, TaCHP, was turned on in SR3 with much higher transcript abundance than in JN177. TaCHP facilitated salinity tolerance in wheat through improved leaf peroxidase (POD) activity and enhance ROS scavenging ability.

**Genes involved:** It is important to be acquainted with the environmental and growth signals during cereal cultivation. Plant growth regulators (PGRs) also called phytohormones are regulatory factors of both developmental processes as well stress response. Signalling pathways of PGRs are triggered during high concentrations of salt to adapt the stressful conditions. For example, TaAOC1 gene in wheat (encodes for enzyme cyclase that is involved in jasmonic acid (JA) synthesis) was found to be induced by high salinity [52]. “Constitutive expression of TaAOC1 in both wheat and *Arabidopsis* confined root growth, but boosted salt tolerance and JA content. Above data initially indicated different conduit of metabolic pathway that participated in a single process but was controlled by different mechanisms. Light is an affirmative factor for the growth and maturity of plants. TaGBF1 (G-box binding factor specific for blue light) was induced after exposure to salt. TaGBF1 caused salt sensitivity as well as supported blue light mediated photomorphogenesis” [53]. “This confirmed that TaGBF1 was a common part of the blue light and salt stress responsive signaling pathways. Genetic analysis proposed the role of TaGBF1 in response to salt depended on AB15, which is a key component of ABA signaling pathway” [5].



**Fig. 1. Among singlet-oxygen quenching, free-radical scavenging and excited-state proton-transfer in hydroxyflavones**

**Table 1. Transgenic wheat**

<b>Sr. No.</b>	<b>Genes</b>	<b>Source</b>	<b>Host plant</b>	<b>Reported transgenic plant performance during salt stress</b>	<b>References</b>
1	Na <sup>+</sup> /H <sup>+</sup> antiporter ( <i>AtNHX1</i> )	<i>Arabidopsis</i>	<i>Brassica napus</i> Wheat	Enhanced salt tolerance with higher accumulation in leaves but not in fruits. Maintenance of seed yield and seed oil quality under high salinity	[59]
2	<i>mtl D</i>	<i>E. coli</i>	Wheat	Improved growth performance of mannitol accumulating mature leaves	[60]
3	Myo-inositol O-methyltransferase	<i>Mesembryanthemum crystallinum</i>	Wheat	Maintenance of photosynthetic efficiency	[61]
4	Na <sup>+</sup> /H <sup>+</sup> antiporter ( <i>nhaA</i> )	<i>Salicornia brachiata</i>	Wheat	Altered Na <sup>+</sup> and K <sup>+</sup> accumulation Increased proline content	[62]
5	Vacuolar H <sup>+</sup> pyrophosphatase (vacuolar H <sup>+</sup> -PPase) Choline dehydrogenase ( <i>betA</i> )	<i>E. coli</i>	Wheat	Improved in photosynthesis rate	[63]
6	<i>AP2/ERF</i>	Cotton	Wheat	Improved in yield	[64]
7	<i>phy A</i>	<i>Asperigillus japonicas</i>	Wheat	Improved biomass	[65]
8	<i>Hv BADH 1</i>	Q1	Wheat	Increased in proline & soluble sugar content	[66]
9	<i>TabZIP15</i>	Wheat	Wheat	Improved salt tolerance by providing additional protection to cell membrane	[67]
10	<i>TaASR1-D</i>	Wheat	Wheat	Involved in glycolysis and gluconeogenesis pathways Reduce accumulation of ROS and oxidative damage to cell membrane	[68]

**Cellular signaling:** Cereals react directly and exclusively to the addition of sodium ion within few seconds. The extracellular Na<sup>+</sup> is sensed at plasmalemma, whereas intracellular must first cross the cell membrane. Therefore, carrier protein at cell membrane must act as sensor or straight away upstream of the sensor. Rise in Na<sup>+</sup> around the roots leads to rise in cytosolic free calcium ion. The signaling pathway involves calcineurin B-like protein (CBL4), also known as SOS3. There is physiological elevation in cytoplasmic Ca<sup>2+</sup> that aids the dimerization of CBL4/SOS3 and interaction with CBL-interacting protein kinase (CIPK24/SOS2). The CBL4/CIPK24 (SOS3/SOS2) complex is targeted to the cell membrane via a myristoyl fatty acid chain that is covalently bound to CBL4. This enables the phosphorylation with activation of the membrane bound Na<sup>+</sup>/H<sup>+</sup> antiporter (SOS1) and that help in maintaining ion homeostasis under salt stress conditions [54].

#### 4. TRANSGENIC WHEAT

“With the development of rDNA techniques in the 1980s; work began on creating the first transgenic wheat, coincident with the third Green Revolution. Three most important cereals in the world (corn, rice and wheat), wheat was the last to be transformed by biolistic methods in 1992, and by *Agrobacterium* methods in 1997. In year 2013, 34 field trials of GM wheat took place in Europe and 419 in the US” [55]. “As of 2020, no GM wheat is grown commercially, although many field tests have been conducted, with one wheat variety, Bioceres HB4, obtaining regulatory approval from the Argentinian government” [56]. New breeding technologies such as genome editing allow precise DNA manipulation, but their potential is limited by low regeneration efficiencies in tissue culture and the lack of transformable genotypes. [57] reported “33% transformation efficiency in hexaploid spring wheat cultivar “Fielder”, using a robust, reproducible *Agrobacterium-tumefaciens*-mediated transformation method. Several genes have been transferred in plants to increase salinity tolerance, which are involved in synthesis of stress mitigating compounds, regulatory proteins, antioxidant enzymes and signaling pathways protein, ion transporter etc” [58] (Table 1).

#### 5. CONCLUSION

Among abiotic stresses, salinity stress especially in the arid and semi-arid regions of the world are

one of has emerged as one of the most important threats to the sustainability of wheat production. It reduces germination, seedling growth as well as reproductive growth by disrupting numerous vital physiological and metabolic processes which lead to sharp decline in yield and quality depending on frequency and extent of saline environment. Although salinity tolerant plants employ several physiological and biochemical mechanisms to adapt under salinity stress, there is a lack of robust salinity tolerant wheat cultivars globally. Growth and productivity in cereals is adversely affected by Salinity, causing phenotypic, physiological, biochemical, and molecular changes in a cell. Genetic engineering has enabled development of wheat genotypes with improved salinity tolerance. The resulting transgenic wheat produces a group of generations that comprises of moderate to high-stress tolerant transgenic lines. Screening methods to identify high-stress tolerant germplasm *in-vitro*, greenhouse, or field conditions must be developed. This might contribute towards the identification of promising lines for field conditions. However, field level manifestation of transgenic lines and their yield under salinity stress conditions is challenging plus expensive and still we are at our infancy stage in understanding salinity stress tolerance in cereals. Therefore, various approaches such as conventional breeding, marker assisted selection and genetic engineering can be developed during different stages of plant growth for selecting salinity tolerant wheat.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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