

## Salinity stress adaptation in finger millet – a mini review

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Abiotic stressors like salt and drought are two examples that may harm crop production. Therefore, increasing production and creating stress-tolerant cultivars requires crop innovation in response to these stresses. Whole genome sequence (WGS) data releases are used for crop trait development. Tolerance to abiotic stress cannot be induced through single-gene engineering transformation. Plants like finger millet can be mined for undiscovered genes because they already have the gene in their genomes, but it is dormant. Therefore, abiotic stress-tolerant genes can be expressed in the same plant by RNA sequencing and transcriptomics by creating abiotic stress for agricultural development. New genes can have their profiles refined with the aid of this transcriptome research.

*Key words: Abiotic stress, finger millet, whole genome sequence, RNA sequencing, transcriptomics, gene profiling*

Abiotic stress is one of the biggest environmental risks to crop development and productivity which includes things like drought and salinity. Consequently, the introduction of stress-tolerant crop types and crop improvement in the face of these harsh conditions will increase productivity. Releases of genomic data, such as whole genome sequence (WGS), are used to enhance crop traits. The expression of tolerance to stress cannot be induced by engineering a single gene transformation for abiotic stress. Finger millet and other crops like it contain genes for fresh genomic data, but they are dormant until triggered to express them. Abiotic stress can be induced in the same plant by RNA sequencing and transcriptomics, allowing for the expression of abiotic stress-tolerant genes. This transcriptome study can aid in the profiling of uncharacterised genes.

The monocotyledonous *Poaceae* family includes finger millet, which is a member of the *Chloridoideae* subfamily (Dida *et al.*, 2008). Compared to rice and other popular cereals, sorghum, and wheat, this annual herbaceous cereal crop provides rich proteins and minerals, making it popular among the impoverished in Africa and Asia. It's crucial to the health of children's diets and the prosperity of farmers' economies. The grain is high in beneficial amino acids including iron, calcium, phosphorus, cysteine, tryptophan, tyrosine, and methionine, making it a healthy alternative to other cereals (Latha *et al.*, 2005, Gupta *et al.*, 2017). Its high calcium (Ca) concentration has earned it widespread recognition, and its numerous health benefits include effects on blood sugar, cholesterol, and ulcers. The flour made from the grain is a nutritious option for kids' baked goods like cakes and breads Ceasar and Ignacimuthu (2011); Mgonja *et al.* (2007). Finger millet grains can be kept for a long time in storage, and they may grow well in marginal agroecological zones where other crops struggle. Climate change, specifically a rise in temperature and a decrease in the availability of irrigation water has a significant effect on agricultural output. Due to rapid population growth and the consequences of global climatic change, the agricultural industry is under intense pressure to increase food production while decreasing the quantity of land used to do so. Most people live in developing countries, which are also predicted to suffer

the biggest loss in food output. A fundamental issue is whether or not we can impartially, healthily, and sustainably feed the projected 9 billion people on Earth in 2050. (Beddington, 2010). A person may be getting enough calories from his diet, yet still not enough of the vitamins, minerals, and trace elements he needs. The health benefits of finger millet are shown in Figure 1.

## SALINITY STRESS

One of the abiotic variables that hinders finger millet development is the soil's salinity. The crop quality and growth are severely impacted by this essential environmental stress (Hema *et al.*, 2014). Osmotic stress brought on by salinity causes physiological changes including membrane rupture, dietary imbalance, "impairment of the body's capacity to detoxify reactive oxygen species (ROS), reductions in photosynthetic activity and stomatal aperture, and changes in the antioxidant enzymes are just a few of the effects that may occur (Rahnama *et al.*, 2010; Shahzad *et al.*, 2021). Salinity stress has a considerable influence on the development of seeds and roots, ions and relative water content, photosynthetic pigments, proline content, membrane peroxidation, reducing sugars, and total protein levels (Kumar *et al.*, 2016; Sarabi *et al.*, 2017; Dugasa *et al.*, 2020; Mukami *et al.*, 2020)". Due to its antioxidant, antidiabetic, antimicrobial, aldose reductase enzyme inhibitory, wound healing, antiulcerative, and anticancer properties, Predictions state that by 2050, More than half of the world's arable land may have been negatively impacted by salt. Because of its ability to respond to a variety of abiotic stress conditions, finger millet is considered a promising crop for the identification of genes and pathways involved in adaptation to extreme environmental settings. (Sood *et al.*, 2016).

### **Plant salinity tolerance mechanism**

Plants are classified as halophytes (those that can survive in salt water) or glycophytes (those that can't) based on their specialized adaptations (which cannot endure salinity and finally die). Since glycophytes make up the vast majority of cultivated plants and are particularly vulnerable to salty conditions, this is one of the most significant negative pressures on worldwide crop output

(Munns and Tester, 2008). Ion toxicity caused by osmotic stress from salty soil is known to stunt plant growth (James *et al.*, 2011). There is an initial decrease in the roots' capacity to take in water and an increase in water loss through the leaves as a result of salinity stress. One of the most harmful impacts of salt stress is the buildup of Na<sup>+</sup> and Cl<sup>-</sup> ions in plant tissues growing in media with high NaCl concentrations. When sodium levels are too high, plants cannot absorb potassium ions, which are essential for plant viability (James *et al.*, 2011). Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), hydroxyl radicals, and reactive oxygen species (ROS) are the plant responses to salinity stress (OH). Proteins, nucleic acids, membrane lipids, and chlorophyll are just a few of the biological elements that reactive oxygen species have the potential to oxidatively harm. Enzymatic and non-enzymatic defensive mechanisms are used by plants to protect themselves from oxidative stress. Plants adjust the pace at which various gene products are synthesized in response to environmental stresses like salt (RNA or protein). As a consequence of salt stress, several genes and transcription factors are upregulated in various plant species (Chakraborty *et al.*, 2012). Species and genotypes have a role in how well an organism can handle salt.

#### **Biochemical response to salinity stress**

Plants are split into two groups, the halophytes (which can tolerate salt) and the glycophytes (which cannot withstand salt and finally die). Since glycophytes, the most common type of cultivated plant, cannot withstand salinity stress, it has become one of the most significant negative pressures on crop production worldwide (Munns and Tester, 2008). Osmotic stress, which results in ion toxicity, is a known plant growth inhibitor, and so is soil salinity (James *et al.*, 2011). "Increased salt buildup in soil and plants causes salinity stress, which first reduces the roots' ability to take in water and increases water loss through the leaves. The accumulation of Na<sup>+</sup> and Cl<sup>-</sup> ions in plant tissues growing in a medium containing elevated NaCl concentrations is one of the most detrimental effects of salinity stress. The mortality of plants is caused by an excess of sodium because it stops them from taking in potassium, an essential nutrient (James *et al.*, 2011). Hydroxyl radicals, hydrogen peroxide, and reactive oxygen species are all produced by plants in response to salinity

stress (OH). Reactive oxygen species via oxidative stress cause harm to membrane lipids, proteins, nucleic acids, and chlorophyll, among other biological components. Both enzymatic and non-enzymatic defences against oxidative damage are present in plants. In response to salt stress, plants use several strategies to regulate gene expression, including or down-regulation of the synthesis of certain gene products (RNA or protein). Many plant species upregulate a huge number of genes and transcription factors in response to salt stress, each of which has a somewhat different function (Chakraborty *et al.*, 2012). There are genetic variations in salt tolerance in addition to species- and genotype-specific variances.

#### **Genetic improvement for abiotic stress tolerance**

Plants are divided into halophytes (which may survive in salty environments) and glycophytes (which require a high level of moisture to survive) (which cannot endure salinity and finally die). Because most cultivated plants are glycophytes and are sensitive to salt, salinity stress has emerged as one of the most important factors lowering agricultural yield globally (Munns and Tester, 2008). Inhibiting plant growth via osmotic stress and subsequently ion toxicity, soil salinity has been widely documented (James *et al.*, 2011). Salt accumulates in the soil and on the plants, reducing the plants' ability to take in water, and increasing the rate at which they lose water via their leaves. One of the most harmful impacts of salt stress is the buildup of Na<sup>+</sup> and Cl<sup>-</sup> ions in plant tissues growing in media with high NaCl concentrations. An excessive amount of Na<sup>+</sup> prevents the absorption of the crucial nutrient K<sup>+</sup>, which results in plant death (James *et al.*, 2011). Reactive oxygen species (ROS), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and hydroxyl radicals are all produced by plants as a defense mechanism against salinity stress (OH). Lipids in cell membranes, proteins, nucleic acids, and chlorophyll can all be damaged by oxidation when exposed to reactive oxygen species. Plants use both non-enzymatic and enzymatic defensive mechanisms to combat oxidative stress". It is known as gene expression monitoring (RNA or protein) when plants use a variety of strategies to either boost or reduce the synthesis of certain gene products when exposed to high levels of salt. In response to salt stress, several plant species upregulate

numerous genes and transcription factors, each of which plays a distinct function (Chakraborty *et al.*, 2012). The rate at which organisms can adapt to settings with high salt content varies according to the genotype and species.

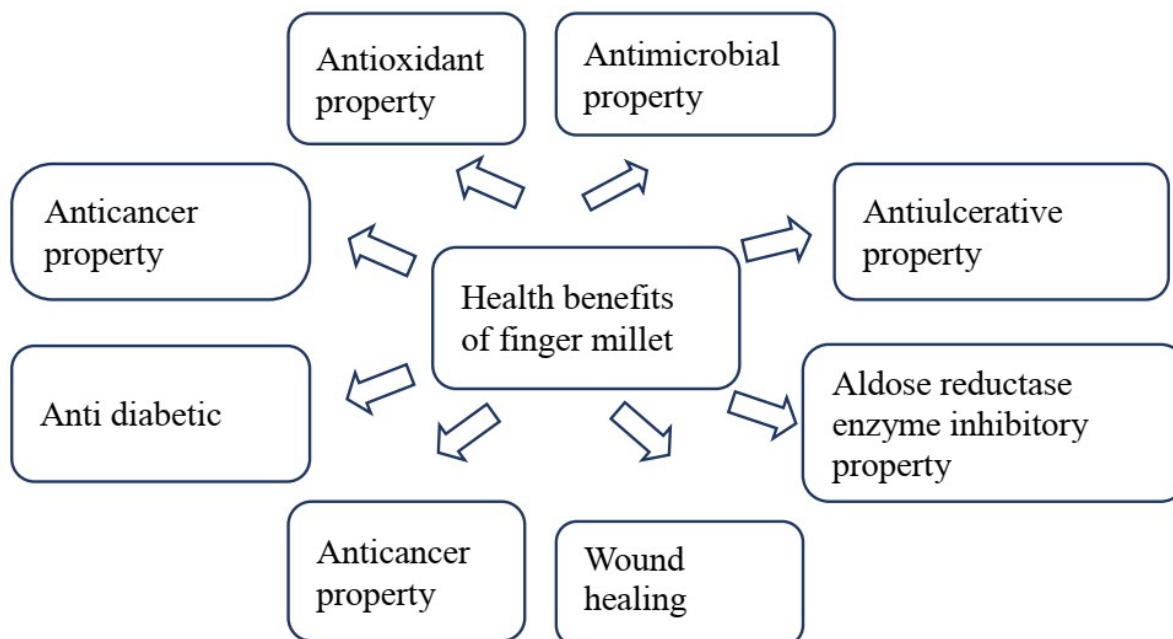
### **Phytohormones' role in stress resistance**

Plants are divided into halophytes (which can survive salinity) and glycophytes (which can't) based on their morphological and physiological adaptations (which cannot withstand salt and finally die). Since the bulk of farmed plants are glycophytes and cannot withstand saline stress, it has become one of the most severe negative pressures on agricultural output worldwide (Munns and Tester, 2008). "It is well recognized that salt in the soil inhibits plant development due to osmotic stress and consequent ion toxicity (James *et al.*, 2011). Early on, salt accumulation in the soil and plants reduces plants' capacity to absorb water via their roots, while water loss through the leaves rises. The accumulation of Na<sup>+</sup> and Cl<sup>-</sup> ions in plant tissues growing in a medium with high NaCl concentrations is one of the most detrimental effects of salt stress. Potassium ions, which are essential for plant vitality, can't be absorbed by plants when there are high salt concentrations (James *et al.*, 2011). Plants also release hydroxyl radicals (OH), reactive oxygen species

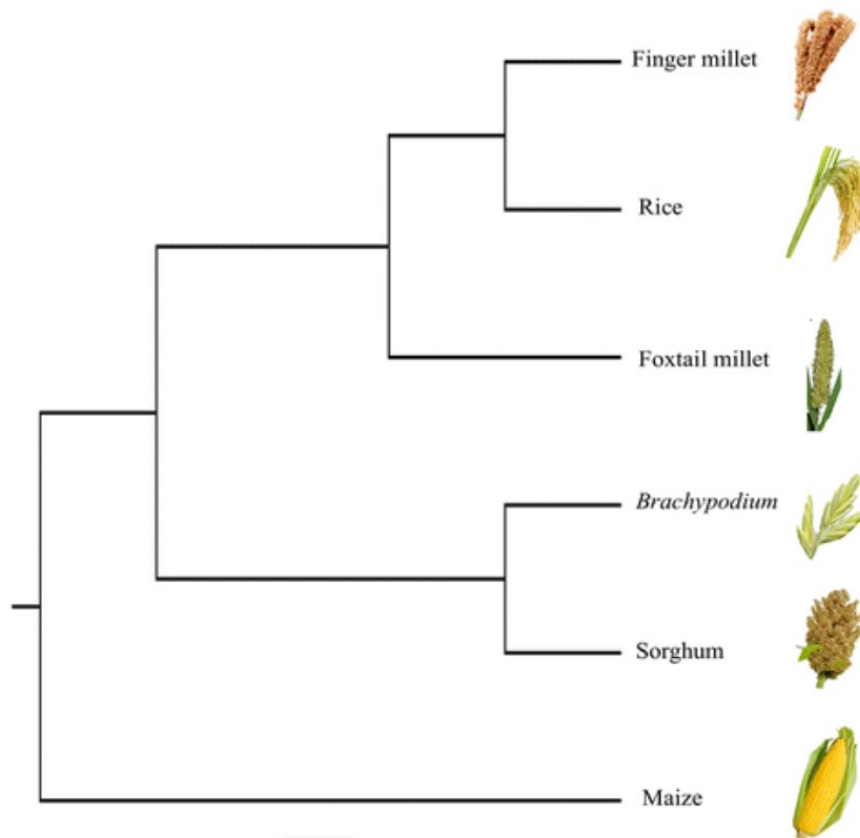
(ROS), and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in response to salt stress (OH). Proteins, nucleic acids, lipids, and chlorophyll in membranes may all be ruined by reactive oxygen species (ROS). To stop oxidative damage, plants contain both enzymatic and non-enzymatic defense mechanisms". In response to environmental challenges like salt (RNA or protein), plants use a variety of methods to regulate the pace of gene synthesis. Numerous plant species experience increases in gene expression and transcription factors in response to salt stress, and these responses have a range of functions (Chakraborty *et al.*, 2012). The pace at which organisms can adapt to high salt concentrations varies depending on the species and genotype.

### **ANNOTATION**

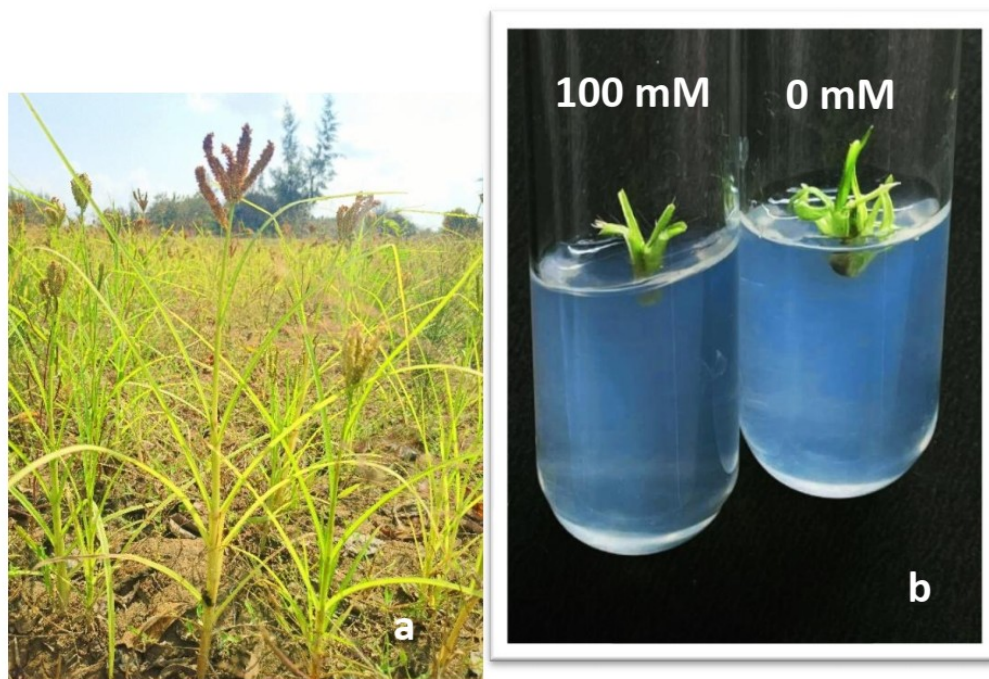
To functionally annotate the transcript sequences, it is crucial to compare the unknown transcript readings to a reference genome that is quite similar to them to demonstrate the molecular processes changed during the stress (Ward *et al.* 2012). Comparative transcriptome mapping of finger millet, rice, and foxtail millet (all members of the *Poaceae* family). The data in Figure 2 for finger millet were analyzed using this reference genome.



**Figure. 1.** Health benefits of finger millet (Adopted from Dinesh *et al.*, 2016)



**Figure 3.** Six species of Poaceae have their phylogenetic relationships revealed using single copy ortholog genes. (Adopted from Hittalmani *et al.*, 2017)



**Figure 4.** (a) Finger millet grown in agricultural field, Villupuram, Tamil Nadu.

(b) Multiple shoot formation observed at 0 mM and 100 mM concentration of NaCl supplemented with BAP

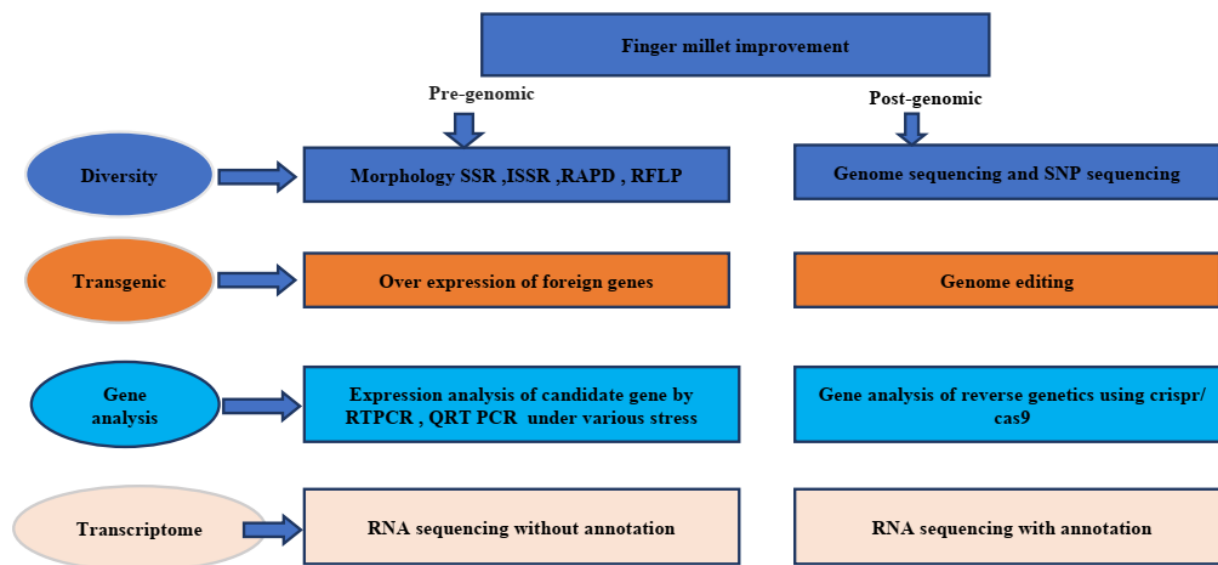


Figure 5. Finger millet improvement in the pre-genomic and post genomic eras

## IN VITRO REGENERATION UNDER SALINITY STRESS

We have also generated salinity stress at 0 and 100 millimoles of sodium chloride, respectively. The seedlings' shoots have emerged after germination. "This investigation has been carried out by the Department of Plant Biology and Biotechnology at Loyola College in Chennai, India. We are grateful that this research used finger millet from the Tamil Nadu Agricultural University (TNAU) in Coimbatore, India, shown in Figure 3. VL 400 and KM 252 were shown to be particularly prone to NaCl, but GPU 28, GPU 67, ML 365, Udurumallige, PYR1, and GPU 48 were found to be less susceptible were found to be less susceptible were unaffected by salt accumulation at 150 Mm or 200 Mm (Divya *et al.*, 2022).

## WHOLE GENOME SEQUENCE

The long-awaited whole genome sequence (WGS) of the finger millet genotype ML-365 has been successfully acquired by researchers using Illumina and Sequencing by Oligonucleotide Ligation and Detection (SOLID) sequencing technology" (a genotype with good properties that is drought tolerant and resistant to blast disease). This

work resulted in the generation of 21 GB of mate-pair data and approximately 45 GB of paired-end data. The N50 length of the assembled genome was 23.73 Kb, and the average scaffold length was 2275 bp. In total, there were 525,759 scaffolds (200 bp) in the assembly. Transcriptomes were successfully sequenced and constructed from both In this research, genotype ML-365 plants were well-watered (WW) (53,300 unigenes) and low moisture stressed (LMS). Using protein-protein homology modeling, researchers were able to identify 11,125 genes in plants that were likely to share homology with TFs from 56 different families. Involvement in calcium transport and accumulation was shown to be mediated by 330 genes, whereas roughly 1766 genes were found to be R-genes for different illnesses. In comparison to other Poaceae species, there was more co-linearity between the finger millet WGS and foxtail millet and rice. "This study also shows how closely linked the genome sizes of the coracana and African subspecies of *E. coracana* are. The availability of WGSs of ML-365 and PR-202 allows for the development of finger millet. These WGSs may be used for many different types of research, including marker-assisted breeding projects, next-generation sequencing-based allele discovery, building linkage and association

maps, identifying candidate genes for agronomically important characteristics, and characterizing candidate genes' functional properties".

## TRANSCRIPTOME ANALYSIS

Examining how plant gene expression varies in response to abiotic stress has been done using transcriptomics or during any developmental changes to find genes that affect plant growth, development, and tolerance to external stress. A deeper comprehension of these factors may help in plant breeding and culture as well as targeted selection of superior varieties. To gather plant genomic information rapidly, with great coverage, efficiency, and throughput. Overexpression of foreign genes, Genome editing, Expression study of candidate gene by RT-PCR and QRT-PCR under different stress conditions; CRISPR/cas9-based study of reversed-genes (Clustered regularly interspaced short palindromic repeats) Pre-genomic RNA sequencing, RNA sequencing with annotation, and RNA sequencing without annotation. It has been used in a variety of post-genomic contexts (figure 4), including the identification of plant developmental pathways, the manufacture of secondary metabolites, and the mining of novel functional genes (Huang *et al.*, 2014), Understanding the intricate mechanisms that control gene expression throughout development and under stress requires transcriptome analysis. For genome-wide transcriptome studies, microarrays and RNA-seq have emerged as the de facto standard methods of choice (Kogenaru *et al.* 2012). In crops for which there is no genomic sequence data, RNA-seq has lately emerged as a prominent approach to transcriptome study.

## CONCLUSION AND FUTURE PERSPECTIVES

Recent studies have demonstrated that finger millet is vulnerable to salinity stress while being a nutrient-rich and drought-resistant crop. Some genotypes can independently express the abiotic stress-tolerant genes, but it is still important to reevaluate the germplasm resources to find the most promising alleles. Allele mining refers to the process of searching through several genotypes for evidence of allelic variation in a known gene. This overview explains how finger millet's

biochemical and molecular pathways can shift as a result of salt stress. Characterizing the genes, proteins, metabolites, and the mechanisms that finger millet uses to tolerate salt will shed light on how this trait is expressed. These expressions may be increased or downregulated depending on the trait. The crop can respond to numerous environmental elements thanks to ongoing research into the systems. CRISPR/Cas9 genome editing, high-throughput phenotyping, genomic selection, and speed breeding are all approaches to crop development that can help with the trait prediction required for salt management.

## CONFLICT OF INTERESTS

The authors declare that they have no potential conflicts of interest.

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