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REVIEW ARTICLE

Biotechnological Advances in Designing Salt-Tolerant Crops

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ABSTRACT

Extreme climate variations and population increases pose considerable obstacles to global food security. In the near future, addressing the issues of hunger and a growing population involves breeding and/or genetically engineering crops to withstand abiotic stress while achieving higher yields. Plants respond to salt levels through strategies such as maintaining ion balance, regulating osmotic pressure, activating antioxidant defense mechanisms, and signalling through phytohormones, all of which help to counteract ion toxicity and osmotic stress. Despite ongoing initiatives, progress in breeding and rigorously selecting crops that are tolerant to salt has been minimal. Additionally, the untapped genetic diversity present in crop landraces and their wild counterparts remains largely uncharted. Exploring new genes from the wild relatives of crops offers an exciting chance to discover better salt-tolerant haplotypes. Biotechnology methods for precision breeding have a strong potential to speed up the creation of salt-resistant cultivars. This review intends to examine unique salt-tolerant genes and the use of contemporary biotechnological techniques to improve salinity tolerance in crops.

Keywords

• Biotechnological • Genetic diversity • Ion toxicity • Osmotic stress • Plants • Salt stress

INTRODUCTION

The rapid increase in the global population, which is expected to reach 10 billion by 2050, along with the challenges posed by climate change, soil desertification, and other environmental pressures, poses significant

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threats to global food security. Among the various environmental stressors, salt stress stands out as one of the most critical abiotic factors adversely affecting crop growth and yield. Currently, more than 1125 million hectares of arable land worldwide are impacted by salinization (Liu *et al*., 2020). Plants affected by salt stress typically exhibit stunted root development, wilting leaves, and reduced height (Van Zelm *et al*., 2020), all of which contribute to diminished agricultural output and substantial financial losses for farmers. Among various environmental stressors, salt stress is one of the most significant abiotic factors that negatively impacts crop growth and yield. Salt stress not only decreases crop yields but also diminishes the quality of crops. For instance, it has been shown to reduce the levels of free amino acids, proteins, sucrose, and starch in mature soybean seeds (Do *et al*., 2018). Similarly, in maize (*Zea mays*), salt stress caused a reduction in leaf dry weight by approximately 11% and root dry weight by 15-30% when exposed to 100 mM NaCl (Hessini *et al*., 2019). The adverse effects of salt stress on plant growth and productivity are driven by various mechanisms, including osmotic stress, ion toxicity, reactive oxygen species (ROS)-induced damage, and imbalances in Na^+/K^+ ions (Gong *et al*., 2020). Initially, high salinity in soil leads to osmotic stress and ion toxicity, which in turn cause nutrient deficiencies and oxidative damage (Roy *et al*., 2014). These negative impacts significantly impair plant growth and yield and, in severe cases, can result in plant death. Salt-affected plants often show stunted root development, leaf wilting, and reduced height (Van Zelm *et al*., 2020), all of which contribute to lower agricultural output and considerable financial losses for farmers.

Most crop plants are glycophytes, and improving salt tolerance is crucial for increasing the utilization of saline-alkaline land and sustaining global agricultural productivity. Researchers have utilized the genetic diversity found in various crop species to develop salt-tolerant varieties. Through advanced breeding techniques, salt-tolerant cultivars have been created and brought to market. In India and Pakistan, several salt-tolerant wheat varieties, which were developed using traditional breeding methods, have also been successfully commercialized (Munns *et al*., 2012).

A number of quantitative trait loci (QTLs) linked to salt tolerance in rice have been identified, with some playing a significant role. A recent study examined 935 salt-tolerant QTLs in rice, recommending 63 of these as critical genomic regions for improving salt tolerance (Singh *et al*., 2021). One prominent locus, SKC1, was the first cloned gene associated with salt tolerance, connected to shoot K^+ concentration (Ren *et al*., 2005). This locus was subsequently identified as OsHKT1;5, which encodes a high-affinity potassium transporter protein. OsHKT1;5 is responsible for regulating the movement of K^+ and Na^+ from roots to shoots, thereby helping to maintain the K^+/Na^+ balance in the shoots (Kobayashi *et al*., 2017). Despite advancements in identifying salt-tolerant genes, several obstacles impede their wider application in crop production: (1) Salt tolerance has not traditionally been a primary breeding goal, which limits the resources allocated to this trait; (2) The genetic diversity of mainstream crop cultivars is relatively low, which restricts the discovery and application of new salt-tolerant alleles; (3) There is frequently a trade-off between salt tolerance and high yield, as stress-tolerant varieties often exhibit lower yields under normal growing conditions; (4) Limited progress has been made in developing salt-tolerant varieties for many crops, underscoring the necessity for new salt-tolerant genes; (5) To address these challenges, it is essential to integrate superior haplotypes that provide both salt tolerance and high yield.

This review seeks to investigate the molecular mechanisms that govern ion uptake, transport, and accumulation within plant tissues. It highlights the essential role of genetic diversity in identifying new salt-tolerant genes and examines recent progress in biotechnological strategies designed to improve salinity tolerance in crops. These strategies encompass genome-wide association studies (GWAS), the combined use of multi-omics approaches, genome editing technologies, and the de novo domestication of crop wild relatives (CWRs).

Strategies to Resist Salinity Stress

Due to expansion in global population, food requirement has also increased, yet agricultural production is not expanding fast enough to provide it. As a result, several attempts were made to increase salinity resistance of plants through conventional breeding with plant omics technology. For combating soil salination, two broad approaches are used. Regulation of salinity through irrigation with drainage systems, as well as ecosystem engineering efforts is the first approach, and the second is to improve plant-dependent salt resistance through traditional as well as sophisticated breeding with genomics technologies.

Plants used three methods to develop salt tolerance: GMOs, PGPR (Plant Growth-Promoting Rhizobacteria) usage, and breeding. Hereditary salinity tolerance is a result of breeding and genetically modified organisms (GMOs); salinity tolerance is counteracted by PGPR (Bashan *et al*., 2014). To improve crop plant salt tolerance processes, a combination of molecular methods, biotechnological strategies, and plant breeding approaches could be used. Every technique to foster salt tolerance in plants has benefits and drawbacks.

In order to limit hyper-osmolarity and restore the ionic surroundings of homeostatic cells, plants must coordinate various processes in response to a salinity constraint. Salinity tolerance is the plant genotype capacity to withstand saline environment while lessening yield loss. At genetic and physiological levels, various genes regulate intricate salinity stress mechanism. Nonetheless, the main salt tolerance criterion should be plant yield, which eventually requires producing a cost-effective harvestable yield. Salinity resistance is a quantitatively hereditary feature that is strongly regulated by a variety of gene actions that are impacted through the environment, genotypes, as well as their exchanges (G x E) (Arzani, 2008).

MAS (Marker-assisted selection) have not produced desired progress as well as breeding, despite the essentially dynamic presence of salt resistance in the genetic nature. Instead, direct phenotype selection is the main cause of these outcomes. Even greater challenges are ahead for genomic investigations of salt resistance in polyploid plants like oats, cotton, maize, as well as alfalfa. Modern approaches in crop physiology, genetics, as well as genomics have led to novel insights into salt resistance, resulting in the deeper knowledge of gene networks and innovative strategies that participate in eradicating hunger (Rahman *et al*., 2019, Lee *et al*., 2011).

Understanding the cellular plants biology allows in knowing salinity resistance traits and application of functional and structural genomics tools to find QTL genes connected to particular characteristics (Saradadevi *et al*., 2021a). Transgenic production primarily aims to improve genes; QTLs could be engaged in molecular breeding for improving crops. As a result, the QTLs cover the MAS path (Ashraf and Foolad, 2013) and encourage transformation of gene (Negrao *et al*., 2011), allowing researchers as well as scientists to create extra tolerant plants by genetic engineering as well as gene transformation methods. Knowing salt resistance processes, as well as assessing salt stress-associated genes and its roles, would give a theoretical foundation for better perception of the stress signal network with pathways for crop development (Afzal *et al*., 2023).

Biotechnology-Dependent Sustainable Agriculture in Salinity Stress

Omics Strategies

Omics is a field of biology that studies variations in cells, genes, proteins, and molecules. Omics techniques, like transcriptomics, proteomics, genomics with metabolomics, aim for identifying as well as describing the genes, proteins, as well as metabolites of plants with their work, alterations, and interactions. By studying molecular characteristics of stress resistance in crops, multi-omics can help improve crop development (Raza *et al*., 2021a; Arif *et al*., 2020; Varshney *et al*., 2019a, 2019b, 2020; Raza *et al*., 2022). Crops alter its genes for management of salinity. This viewpoint suggests that combining data from various omics research is an interesting concept that provides a detailed information about plants in salinity at molecular level.

Genomic Resources

Plant breeding could be accelerated through utilizing genomic data, techniques, as well as tools including genetic markers in a process known as genomics-assisted breeding (GAB). It uses DNA markers linked to desired qualities to identify any plant (Varshney *et al*., 2020, 2021). Plant breeding can be accomplished much more efficiently and rapidly by using molecular or DNA markers, according to Varshney *et al*. (2020, 2021). Breeding operations use a number of markers, including microsatellites, single nucleotide polymorphisms (SNP), complex sequence repeats (SSR), RAPD, RFLP and AFLP. Salt tolerance was added to BC3F4 rice (japonica Italian varieties, recipient, Indica-donor) by using a technique called Saltol QTL, which identifies the genes that control salt resistance. Then, a marker-assisted backcrossing (MABC) system was employed for transferring these genes to original rice lines. (Mare *et al*., 2020).

Likewise, salinity-resistant lines having more than eighty percent of "Rassi", a modified rice type, except around the Salto QTL, were chosen at BC3F2 stage, while 8 of these varieties showed lesser harvest reduction (3 to 26% in comparison to control tests) (Bimpong *et al*., 2016). Muthu *et al*. (2020) used the MABC approach to include QTLs linked with salinity resistance in the improved White Ponni rice variety, which can endure a variety of stresses. The strong relationship between desirable qualities and indicators enables successful breeding, which can be accessed via gene mapping, quantitative trait loci (QTL) studies, or recombination studies.

Some research on QTL mapping, GWAS, and GS to enhance various crops in salty conditions is presented below.

QTL mapping

A QTL is a gene that controls major physical characteristic, and a marker that links it to that characteristic. Varshney *et al*. (2020, 2021) showed that QTL mapping analysis greatly enhanced the identification of important crop genes. Impact of salinity on rice development a common problem, was studied using genetically identical rice plants. Researchers combined and analyzed 935 previous studies on rice genetics to find out which traits affect how much salt rice can handle. The researchers found these QTLs by studying 13 different groups of rice that had different genes (BC2F5, BC1F9, BC2F8, BC3F4, BC3F2, BC4F4, BC3F5, DHs, BILs, F2:F4, F2, ILs, and RILs), of which mostly came from RILs. Singh *et al*. (2021) have found 63 areas in the genome which can be employed for improving salinity tolerance in crops.

QTL analysis identified 21 stable QTLs, and these QTLs were discovered to have phenotypic differences, including root length, root fresh weight, shoot length, shoot fresh weight, shoot dry weight, and root dry weight. A new substantial QTL of shoot length, qSL7, having 7.5 and 6.8% phenotypic variations when compared to K^+ as well as Na^+ levels, may help rice withstand salinity (Jahan *et al*., 2020). According to another investigation on salt-stressed rice, multi-environmental 7 novel QTLs for constituent phenotypes, comprising stress susceptibility index, spikelet degeneration, and spikelet sterility, were found. Genotype-environment interactions favourably altered two important quantitative trait loci qSSI-STE-2-1 and qDEG-S-2-1 (Chattopadhyay *et al*., 2021). A key QTL determining salt resistance in rice, F8 RILs linked to saltol, because of hybrid between the salt-resistant Pokkali with salt-sensitive IR29. In this hybrid, 64.3-80.2 percent overall phenotypic alteration providing salinity resistance in seedling phase were shared through three mutual QTLs that support less Na^+ intake, elevated K^+ intake, with Na^+/K^+ homeostasis inside shoots (Gregorio, 1997). The Nona Bokra/Koshihikari hybrid was the first to discover QTLs that contribute to salt tolerance for root K^+ level (qRKC-4) with total root Na^+ level (qRNTQ-1) (Lin *et al*., 2004). First main QTL to be cloned for salinity in rice was qSKC-1, which is situated within the Saltol gene and was previously identified by Lin *et al*. (2004). K^+/Na^+ homeostasis is regulated by the SKC1 gene (Os01g20160), which codes for an OsHKT-type Naá selective transporter.

Consequently, SKC1 controls the transportation of K^+ as well as Na^+ into shoots from roots, which in turn controls the K^+/Na^+ equilibrium inside the shoots (Ren *et al*., 2005). Similarly, with salinity treatment for 2 distinct developmental phases of rice, 14 QTLs were discovered to be antagonistic to yield-related and physiological characteristics. Further supporting the significance of cytoplasm-nuclear association in breeding methods is involvement of cytoplasmic influences in these quantitative trait loci (Haque *et al*., 2020). Few QTL studies have documented salt resistance at the reproductive phase in rice, and majority of rice QTL research have only examined tolerance in the seedling phase (Mohammadi *et al*., 2013, Hossain *et al*., 2015). These investigations have recognized many QTL for several biochemical, physiological with morphological variables. Commercial varieties resistant to salt would be produced via a new QTL called qGY-2, which was found to have a phenotypic variance of 45% for grain yield and other trait-related QTLs (Pundir *et al*., 2021).

As a result, QTL mapping has significantly enhanced many aspects of rice plants, such as their morphology, physiology, yield-related characteristics, and component variables, in addition to preserving salinity equilibrium. For fifteen agronomic traits in wheat, soil salinity-associated QTLs were identified; these yielded ninety stable quantitative trait loci with phenotypic variance of 2.34 to 32.43%. These QTLs were detected on the chromosomes of all three genomes, with the exception of 4D, 6B, and 7D. Additionally, it was demonstrated that QPh-4B is a Rht-B1 allele inside the QTL cluster. Furthermore, our study establishes the foundation for salt-tolerant QTL cloning in wheat and allele-specific PCR markers to facilitate MAS in salt-tolerant wheat breeding (Luo *et al*., 2021).

Using QTL mapping, biparental mapping of wheat salt-responsive characteristics was conducted throughout two distinct growth stages. Li *et al*. (2021) observed that roots exposed to salt stress had distinct expression patterns for 2 new candidate genes, TaRN1 and TaRN2, along with twenty-two loci that overlapped. Bread wheat possesses a novel QTL for salt resistance (QG (1–5) asl-7B) for K^+: Na^+ DW and (QK: Na.asl-2DS2), for Cl^- accumulation (QCl.asl-3A). This research could speed up the bread wheat breeding process for sub-traits and improve our knowledge of genetic mechanisms for salt resistance (Asif *et al*., 2021).

It was previously observed that TmHKT1;4-A2 and TmHKT1;5-A durum wheat genes are Na^+ transporters, which may improve resistance to damp and saline conditions. Due to their association with QTLs associated with salt tolerance, these two genes (HKT1;4 as well as HKT1;5) are referred to Na^+ exclusion genes Nax1 and Nax2 (Tounsi *et al*., 2016). These genes remove Na^+ from the xylem of roots, leaf sheaths, and diploid bread wheat (*Triticum monococcum* L.). Their purpose was to increase the salt tolerance of durum wheat (James *et al*., 2012). When cultivated in salinized soil, commercial durum wheat having TmHKT1;5-A gene inserted into the Nax2 locus seems to reduce Na^+ transport to leaves and increase grain production by up to 25%. Plant productivity and stress tolerance have increased when non-domesticated material has been switched to commercial genotypes using this method (Munns *et al*., 2012).

Additionally, it was found that barley accessions were experiencing salt stress, which resulted in the identification of six QTLs with significantly diminished phenotypic traits. To reduce the number of potential genes for the next generation of salt-resistant cultivars, chromosomes 1H and 3H were identified and examined (Mwando *et al*., 2021). After a thorough analysis, 28 QTLs for nine yield-related characteristics were discovered in chickpeas. Two salinity stress-related genomic areas, CaLG03 and CaLG06, were of special interest. According to Soren *et al*. (2020), these QTL regions contain potential kinase genes such as histidine kinases, sucrose non-fermenting related kinases, calcium-dependent protein kinases (CDPKs), and MAPKs. At the same time, some were linked to osmoregulation, which helps plants withstand salt stress and can be used to create high-yielding cultivars that are salinity stress-resistant.

Genome-Wide Association Studies

Genome-wide association studies (GWAS) use genetic markers to pinpoint genomic regions linked to biotic or abiotic stress-induced natural variation. GWAS uses genetic variations to identify genotype-phenotype associations in a large population (Varshney *et al*., 2020, 2021). The process involves fine-mapping QTLs associated with different phases of plant reactions to abiotic stressors, like salt stress. To find important QTLs and genes that shield cotton from salt stress, the GWAS method was applied to a sizable multi-parent advanced generation inter-cross (MAGIC) population. Nine of the 23 QTLs for pH, SDW, and salt tolerance were shown to be prevalent for drought stress, according to the data. About 53 potential candidate genes that allow MAB for abiotic stressors are found in these QTL regions (Abdelraheem *et al*., 2021).

Sodium transporters were found in cotton under salt stress, according to a GWAS study (NHX2, 8, NHX4, NHX6, and NHX7). There are now about twenty-five NHX genes known to exist. One of these, GbNHX7, interacts with the CBL-CIPK protein that is connected to the salt responsive pathway (Akram *et al*., 2020). There were 52 significant markers and 19 quantitative trait nucleotides (QTNs) in barley accessions. This study also identified four significant candidate genes in these sites that are essential for salinity resistance in germination stage (Mwando *et al*., 2020).

GWAS analysis was carried out using 2671 barley lines to look into the genetic pathways impacting the HKT1;5 genes' ability to withstand salt stress in barley. HKT1;5 gene, which moves Na^+ from the xylem to shoots and leaves, was discovered using the GWAS method. Nonetheless, new information on this barley gene can be obtained by knockdown studies employing cutting-edge CRISPR/Cas9 technology (Hazzouri *et al*., 2018). In order to progressively increase the salt resistance of wheat accessions, another study employed GWAS analysis. Several investigations have identified a novel QTL for salt tolerance, QSt.nwafu-6B, and introduced beneficial haplotypes. This work concentrated on improving genetic diversity to increase salinity tolerance (Yu *et al*., 2020). GWAS recognized 11 quantitative trait loci associated with various attributes by applying two distinct salt treatments to a sizable population of wheat accessions. Improved salt tolerance breeding in wheat was made possible by the discovery of three important salt-tolerant loci in biparental populations. Similarly, 27 SNP markers linked to salt tolerance were shown to be potential candidate genes in alfalfa through a GWAS study.

Additionally, enhanced salt tolerance from improved GS models led to better alfalfa breeding (Medina *et al*., 2020). Another study used GWAS analysis to gradually increase the salt resistance of wheat accessions. Numerous studies have revealed advantageous haplotypes with new QTL QSt.nwafu-6B for salt tolerance. The goal of this experiment was to increase salinity tolerance by increasing genetic diversity (Yu *et al*., 2020). By administering two different salt treatments to a large population of wheat accessions, GWAS discovered 11 QTLs linked to different traits. GWAS research employing a mixed linear model (MLM) on the first MAGIC Indica rice population subgroup revealed significant markers on chromosome 1 between 9.2 and 12Mb surrounding the Saltol QTL, qSKC1, and the previously established salt sensitivity QTL (Bandillo *et al*., 2013).

Numerous new candidate genes, together with transcriptional pathways for salt-associated phenotypes in salt stress, were discovered in a recent GWAS analysis of rice in Saltol QTLs. These results should help future rice breeding efforts (Nayyeripasand *et al*., 2021). Potatoes under salt stress were the subject of another GWAS investigation focusing on genes associated to potassium transport. The molecular elements of the K^+ transport method in the family Solanaceae were identified by the discovery of about 43 putative potassium channel and transporter genes (Azeem *et al*., 2021). Nine GmNHX genes were found in another study on tolerant and sensitive soybean varieties, and these genes directed 75 different miRNAs. Joshi *et al*. (2021) reported that every GmNHX gene contributed to the translocation of sodium into cells, providing insights on the development of salt stress resistance in breeding.

Genomic Selection

Genomic selection (GS) is an innovative molecular breeding strategy which can rapidly enhance plant breeding by rapidly selecting superior genotypes. This method evaluates the breeding merit of complex features utilizing several markers spread over entire genome. All QTL and genes in the GS architecture are in linkage disequilibrium with the fewest number of markers (Varshney *et al*., 2020, 2021; Crossa *et al*., 2017). The discovery of additional SNPs has improved whole-genome sequencing efficiency (Crossa *et al*., 2017). By taking into account important factors such statistical models, genetic architecture, the heritability of various targeted qualities, the availability of genotyping and phenotyping, breeding processes, and finance, breeding programs can increase GS efficiency (Crossa *et al*., 2017).

By improving selection precision in breeding operations, GS enhances genetic gain in conjunction with phenomics and machine learning techniques. All QTL and genes in the GS architecture are in linkage disequilibrium, with the fewest markers (Varshney *et al*., 2020, 2021; Crossa *et al*., 2017). More SNPs have been discovered, increasing the efficiency of whole-genome sequencing (Crossa *et al*., 2017). By considering crucial elements such statistical models, genetic architecture, the heritability of specific desired traits, the availability of genotyping and phenotyping, breeding procedures, and finance, breeding programs can increase GS efficiency (Crossa *et al*., 2017). A recent study that compared a new GS strategy for multiple characteristics to standard index selection found that the new approach is better suited for balancing multiple features (Moeinizade *et al*., 2020).

GS has several applications in crop plant development and improves breeding very rapidly (Annicchiarico *et al*., 2019; Cui *et al*., 2020). According to reported investigations, GS is used into modern crop breeding programs to speed up genetic gain in maize, rice and wheat (upto 70 percent GS experiments) (Gorjanc *et al*., 2018). Eight genomic prediction models for cassava (*Manihot esculenta* Crantz) were compared to MAS in terms of characteristics related to yield and starch pasting. The study discovered that while some aspects could be predicted, pasting qualities related to starch were less predictable. According to the findings, applying MAS and GS would increase the effectiveness of selection when it came to locating quality features in a big population (Phumichai *et al*., 2022).

In order to enhance genetic gains for complex traits with low heritability in crop research and production, like spring wheat, it is also recommended to combine GS with speed breeding utilizing recognized techniques (Watson *et al*., 2018, 2019). Traditional breeding could become a more precise and effective system with more genetic benefits and variants with the development of new technologies and optimization components (Merrick *et al*., 2022). Accelerated breeding, double-haploid technology, and creative field designs for product development are essential for optimizing a breeding program for GS. Recently, GS has been included to the wheat breeding program in an effort to increase genetic gain and produce new types. A two-part breeding approach has been employed to differentiate between population enhancement and product development. The GS recurrent selection technique maximizes population improvement with a shorter crossing cycle duration and more genetic gain.

In contrast to phenotypic selection (PS), genetic selection (GS) could be employed, at the discretion of the breeder, for any attribute at any stage of a breeding strategy. GS has been used to analyze genotypes simultaneously for many variables, such as quality, disease features, and yield components (Montesinos-Lopez *et al*., 2016). Along with genetic variance and prediction accuracy for low and highly heritable traits, this leads to the identification of genetic links across valued features, which in turn improves selection accuracy (Guo *et al*., 2020; Jiang *et al*., 2015; Jia and Jannink, 2012). Because genotyping prices have decreased, breeders are utilizing more GS instead of PS. Like QTL and GWAS, MAS/MAB (differentiating breeding methods) can find novel ways to better crops under salt stress thanks to developments in GS and machine learning in genomic prediction.

Transcriptomics

Transcriptomics, sometimes referred to as the functional genome of living organisms, encompasses the entire collection of transcripts, their abundance in a specific cell, and post-transcriptional changes (Rich-Griffin *et al*., 2020; Leebens-Mack *et al*., 2019; Hussain, 2018). The active processes that take place inside tissue cells are necessary for plants to function. A variety of technologies, such as microarrays, RNA sequencing (RNA-seq), and other sequencing techniques, can be used to conduct plant transcriptome investigations. Fortunately, advancements in RNA-seq technology have enabled us to examine these profound and wide-ranging transcriptional changes inside specific plant tissues. RNA-seq's enormous potential enables researchers to recognize and make use of the complex regulatory networks produced in plant tissue by developmental changes or environmental stimuli.

Researchers have challenges in RNA-seq or transcriptome analysis due to many constraints, including analyzing large and intricate datasets (Rich-Griffin *et al*., 2020; Leebens-Mack *et al*., 2019). Nonetheless, rare opportunities to produce comprehensive information regarding a tissue's response to salt stress might be extremely helpful to research. Because transcriptome analysis has so much potential, it is currently a commonly utilized method to study the various molecular pathways behind several stressors, including salt. Through RNA-seq analysis of the salt-tolerant mutant (M4-73-30) line and wild-type (WT-Zarjou) cultivar of barley, it was discovered that genes that modulate ion transporters, such as SOS1/SOS3/SOS2, NHX1, TPK1/KCO1, HAK, and HKT for the transportation of Ca^{2+}, Na^{+}, and K^{+}, enable salinity tolerance (Yousefirad *et al*., 2020). Numerous salinity-responsive transcription factors (TFs), such as WRKY, AP2/ERF, NAC, CTR/DRE, MAD, HSF, bZIP, and others, were identified by RNA-seq data. By depleting stored carbon and energy, the mutant lines' respiration and photosynthesis rates were significantly reduced, protecting the tissues from the harmful effects of salt. In order to maintain ion homeostasis, mutant lines also expressed more ion transporter genes and channel-related genes than WT.

RNA-seq analysis was employed to identify the genes in bread wheat that respond to salt stress (Ma *et al*., 2020). The genes discovered were connected to the metabolism of carbohydrates, chaperones, and ROS scavenging. Numerous genes that respond to stress, both early and late, were also discovered. An enrichment investigation revealed that the metabolism of pentose phosphate, secondary metabolites, and carbohydrates was greatly enriched with salt response (Ma *et al*., 2020). Furthermore, lower expression of several stress-related genes belonging to the TIR-NBS-LRR gene class that had not previously been connected to salinity was found in a recent study examining salt tolerance adaptive mechanisms in the model legume *Medicago truncatula* in the salt-sensitive cultivar TN6-18 (Filippou *et al*., 2021).

In a different study, wheat genotypes that were sensitive and tolerant at the ionic phase (Altay2000-tolerant and Bobur-susceptible) and osmotic stage (Zentos-tolerant and Syn86-susceptible) were assessed using RNA-seq analysis. In response to salt-associated osmotic stress, the tolerant genotype enhances salinity tolerance by upregulating genes related to cell wall production and Ca^{2+} binding. Increased oxidative stress and down-regulation of genes involved to photosynthesis and Ca^{2+} bindings are linked to an increase in photosynthetic reserve during the osmotic stage in the sensitive genotype.

The tolerant genotype is crucial for controlling ion homeostasis and Na^+ elimination, as evidenced by the precise up-regulation of ABC transporters and Na^+/Ca^{2+} transporters during the ionic phase (Duarte-Delgado *et al*., 2020). With a high industrial value, castor beans (*Ricinus communis* L.) are a crop of major economic importance. However, it is recognized that castor crop yield is decreased by salt stress (Lei *et al*., 2021). In order to further comprehension of the mechanism behind castor’s ability to withstand saline stress, two cultivars were employed: wild castor (Y) as well as farmed castor “Tongbi 5” (Z). Numerous DEGs were found through analysis, mostly belonging to the bHLH TF, NAC, WRKY, and ERF/AP2 families (Lei *et al*., 2021). Castor cultivars that were grown and those that were in the wild both had hormone-related DEGs. The majority of DEGs in cultivated cultivars are GA and JA, which belong to the PP2C TFs family. Alternative transcription factors (TFs) like WRKY or NAC may be in charge of adaptation or stress resistance, as evidenced by the down-regulation of the GA signal suppressor gene DELLA in cultivated crops against salinity (Lei *et al*., 2021). Previous researchers found that DELLA gene reduced stress by controlling the growth-to-defense ratio (Jusovic *et al*., 2018). As a result, variations in sensitivity to salt stress between species, genotypes as well as within species are expected. Arbuscular mycorrhizal fungi (AMF) are well-known multi-stress resistors that are commonly employed to promote plant development in difficult circumstances. Thus, the AMF-induced salt tolerance of the *Suaeda salsa* plant was mapped using the transcriptome approach (Diao *et al*., 2021). The AMF strain *Funneliformis mosseae* was employed in this investigation. The study discovered 1306 and 424 DEG in the shoot and root, respectively. In shoot tissue, the bulk of DEG responses to salt were linked to energy, carbohydrate, and photosynthetic metabolism. The metabolism of starch and sucrose was primarily associated with DEG in root tissue. It seems that via reducing stress-induced ROS in the chloroplasts, *Funneliformis mosseae* shields *Suaeda salsa* plants from salt stress (Diao *et al*., 2021). Auxin signalling system genes, on the other hand, showed higher expression and can make up for the lost carbon absorption, controlling healthy plant growth (Diao *et al*., 2021).

Proteomics

Proteomics is the study of how functional proteins are used, located, interact with other proteins, and function under stressed or natural environments. Proteomics enables more thorough investigation of post-transcriptional and protein-level alterations. Proteomics thus becomes an essential technique for identifying significant stress protein indicators that could aid in developing stress-tolerant crops (Kosova *et al*., 2018). Proteomics is becoming more advantageous, useful, and economical for scientific research centers around the world because to modern biotechnology technologies.

The proteome of salinized rice plants was examined by Frukh *et al*. (2020). Two types of rice were used by the researchers: cv. Vytilla-4 and cv. Jhelum, which is sensitive to salt. According to the study, rice plants exhibit a wide range of distinct protein expression patterns and responses to salt stress. Particularly, proteins involved with photosynthesis, such as ferredoxin, carboxylase small chain, and Chl a-b binding protein, are activated by salinity stress in cv. Vytilla. Conversely, PS-II CP47 was downregulated in the Jhelum strain. It has been demonstrated that cv. Vytilla has significantly greater levels of several carbon fixation-related proteins that are essential for energy metabolism, including cytochrome c oxidase subunit 5C, peroxisomal (S)-2-hydroxy-acid oxidase GLO5, and GLO1 (Frukh *et al*., 2020). This demonstrates unequivocally that these proteins may serve as helpful biomarkers for controlling a plant's response to salt stress without jeopardizing its growth.

Nearly all plants can mimic the effects of salt thanks to hormones (Fahad *et al*., 2015). The hormone-related proteins in hulless barley were examined under salt stress (Lai *et al*., 2020). This study examined the germination of two cultivars the salt-tolerant LK573 and the salt-sensitive landrace LK621 under salinity stress. Compared to the LK621, which germinated at a much lower rate, the LK573 cultivar germinated more readily in salt. In the salt-tolerant cultivar, 171 differentially expressed proteins (DEPs) were found. Four hours following the salt stress, a large number of proteins related to terpenoid-quinone production, ascorbate and aldarate metabolism, nitrogen metabolism, and ABC transporters were expressed.

The terpenoid-quinone and ABC transporter are under the significant influence of hormones, as was previously mentioned (Mendoza-Poudereux *et al*., 2015; Abualia *et al*., 2018; Borghi *et al*., 2015). In contrast to this, the majority of the proteins produced in LK621 were linked to the production of fatty acids and terpenoid backbones (Lai *et al*., 2020). This suggests that via interacting with plant hormones, these DEPs control the germination of barley seeds under salt stress. Due to its ability to thrive in a variety of challenging conditions, sorghum (*Sorghum bicolor* L.) is frequently considered a stress-resistant fodder crop (Chakrabarti *et al*., 2020). Investigating the protein profile of sorghum under salt stress could therefore be highly intriguing. The stress-responsive proteins of the two sorghum genotypes, G-46 and CSV 44 F, are examined using a proteomic analysis. Two salt-tolerant genotypes, G-46 and CSV 44 F, may produce significant amounts of proteins linked to their ability to withstand salt stress (Punia *et al*., 2020). As predicted, both genotypes showed dominant expression of the kafirin a, b, and c forms. They included a number of seed storage proteins, also known as kafirin-related proteins, that functioned as an energy source and varied according to the amount of salt present. Kafirin is a biomolecule that has already been shown to respond to a number of stressors (Labuschagne, 2018; Nida *et al*., 2021). Breeding programs could leverage functional studies of these proteins to identify regulatory networks in stressed sorghum and create agricultural genotypes that are high-yielding and stress-tolerant (Punia *et al*., 2020).

Proteome analysis was performed on wheat seedlings under salt stress in a different study (Zhu *et al*., 2021). This study looked at how salt stress affected the wheat plant's chloroplasts at the protein level. They have found and mapped 194 DEPs within the chloroplast. The Calvin cycle, nitrogen and carbon metabolism, transcription and translation triggered by post-salinity stress therapy, and (Zhu *et al*., 2021) have all been linked to many DEPs. In soil contaminated with 300 mM NaCl, Zhu *et al*. (2020) generated two salt-tolerant (T46 and T66) and salt-sensitive (N33 and N53) near-isogenic lines (NILs) of barley. Proteomics analysis revealed a group of proteins present in both sensitive and tolerant lines. The majority of the dominantly expressed proteins in tolerant lines were linked to photosynthesis, ATP production, and ROS scavenging.

All of the facts presented above showed how crucial chloroplasts and activities connected to photosynthesis are, since they are the main location of the ATP-mediated energy biosynthesis reaction. According to Munns *et al*. (2020), these ATP energy packets are crucial for preserving the ion balance, eliminating dangerous reactive oxygen species, and potentially altering the general course of growth. Thus, the identified proteins could potentially help researchers create usefully stress-resistant crops.

Metabolomics

Plants are susceptible to alterations brought on by stress on the transcript, protein, and biochemical levels. The metabolic processes of a plant are often the only ones that respond to stress, with no changes in transcriptional and protein expression (Raza, 2022; Razzaq *et al*., 2019; Raza *et al*., 2021b). The study of these biological components, also referred to as metabolites, is called metabolomics (Raza, 2022; Razzaq *et al*., 2019). By metabolomics one can investigate and understand the detailed alterations that stress-sensing plant cells go through. Due to their remarkable properties, which include differing structures and functions, the study of metabolites has gained popularity in contemporary scientific research (Raza, 2022; Razzaq *et al*., 2019). In order to provide future researchers with a starting point, we want to provide an overview of the metabolomics studies on plants under salt stress.

Under harsh environmental circumstances, foxtail millet outperforms other crops. The influence of salt stress on metabolic levels was assessed in young seedlings of foxtail millet in order to investigate how the plant reacts to stressors (Pan *et al*., 2020). For this study, two cultivars with varying degrees of tolerance were used: Yugu2 and An04. The metabolomics approach was used to identify a number of compounds in the Yugu2 cultivar. Salt stress boosted the expression of several metabolites linked to stress tolerance, including ascorbate, glutathione, and MDA, throughout the early growth stage. cinnamyl alcohol dehydrogenase, Cinnamoyl-CoA reductase, and 3-ketoacyl-CoA synthase, three essential metabolites of lignin synthesis, exhibited elevated accumulation following salt (Pan *et al*., 2020).

Because maize is naturally sensitive to salt, salt stress has a significant effect on the crop at every stage of reproduction. The response of maize plants to salt stress was investigated using the metabolomics technique (Yue *et al*., 2020a). 100 mM NaCl was used to treat the two genotypes, PH6WC (salt-tolerant) and PH4CV (salt-sensitive). Yue *et al*. (2020a) discovered that the PH6WC genotype induced more than twice as much of a set of metabolites (sugars, amino acids, delspray, organic acids, and alkaloids) as the control treatment.

In a different study, Abdel-Farid *et al*. (2020) subjected cucumbers (*Cucumis sativus* L.) and tomatoes (*Solanum lycopersicum* L.) to different salt stress concentrations (25, 50, 100, and 200 mM NaCl). Both tomatoes as well as cucumbers may express a number of significant chemicals that are beneficial in breeding efforts, and both are quite vulnerable to the detrimental effects of salinity. A metabolomics study was carried out to learn more about the metabolic response of tomatoes and cucumbers to salinity (Abdel-Farid *et al*., 2020).

Plants of cucumber and tomato showed a considerable rise in flavonoid concentrations when exposed to a 200 mM NaCl salt stress. Cucumber as well as tomato displayed enhanced percentages of 2 and 30% when compared to the control treatment (Abdel-Farid *et al*., 2020). Only tomatoes stored a large number of phenolic chemicals, while cucumbers showed little change. Similarly, under salt stress (200 mM), cucumber saponin level decreased, whereas tomato saponin content increased dramatically ((Abdel-Farid *et al*., 2020). Therefore, it is thought that cucumber and tomato may respond differently to salinity at the metabolic level.

The majority of plant growth activities are mainly controlled through TCA cycle metabolites. By linking all other metabolic pathways, the TCA cycle is the primary intermediary mechanism that guarantees healthy plant growth (Martínez-Reyes and Chandel, 2020). In a metabolomic experiment, tomato plants thus faced challenges from salt. According to Tang *et al*. (2020), the study found that tomato fruit, both green and mature, acquired a variety of metabolites, including carbohydrates and amino acids, when exposed to salt stress. The amount of metabolites associated with the TCA cycle has significantly increased. These metabolites may control how the tomato plant reacts to NaCl stress.

Therefore, it can be concluded that metabolomics is a cutting-edge method that could improve our understanding of metabolites and their variety. As a biomarker, these stress-responsive metabolites might be useful. Rewiring transcription factors (TFs) connected to proteins and metabolites is made simpler by an understanding of how plants respond to stress through metabolism. (Raza *et al*., 2023).

Transgenic Approaches

Genetic engineering techniques are used by transgenics to alter specific DNA segments (Kotula *et al*., 2020; Anwar and Kim, 2020). In transgenic breeding, a desired feature is improved by carefully introducing a gene of interest (a DNA fragment) from one plant into the genome of another host plant. In the face of diverse climatic conditions, transgenic plants with altered genetic composition increase food yield and sustainability. Transgenic Arabidopsis plants containing the MtDof32 gene and derived from *Medicago truncatula* showed tolerance to osmotic and salt stressors. Under stress, several phenotypic traits have been observed, including delayed flowering and restricted branching (Guo *et al*., 2021).

Due to improvements in osmolytes, stigmosterol, and membrane integrity, over-expressing Arabidopsis lines exhibited much greater stability against salt stress in comparison to wild-type. Due to the SGT gene's protective function through sterol regulation, plants that target it may be able to tolerate salinity better (Mishra *et al*., 2021). By transgressing the distinct RING-H2 type E3 ubiquitin ligase gene (IbATL38) of sweet potatoes into Arabidopsis, transgenic plants with enhanced salt tolerance were created. Reduced H2O2 levels and increased gene regulation of the ROS scavenging system are the results of overexpressing IbATL38 (Du *et al*., 2021). Hormone signaling and ROS scavenging in maize were positively regulated when ZmEREB20 was overexpressed in Arabidopsis. Additionally, higher rates of root hair survival and growth were observed, indicating the necessity of enhancing crop breeding for salt tolerance (Fu *et al*., 2021).

The overexpression of the APX gene has improved *Brassica juncea*'s antioxidative defense potential and increased its resilience to stress. The host plant has maintained ROS homeostasis under salt stress with little membrane damage (Saxena *et al*., 2020). In *Arabidopsis*, overexpression of the *Malus baccata* MbNAC25 gene enhanced the plant's resistance to drought, salt, and cold while preserving a high survival rate. Reactive oxygen species (ROS) can be more effectively scavenged by enhanced enzymatic processes associated with the homeostasis process (Han *et al*., 2020). Targeting rice OsMT-3a gene, overexpressing *Arabidopsis* lines demonstrated enhanced ROS scavenging capabilities, decreased H_2O_2 and NaÍ levels, and improved seedling development. The outcomes showed how important this gene is for enhancing plant stress tolerance, which would ultimately boost crop yield (Mekawy *et al*., 2020). Commercial citrus types that are tolerant of salt may be produced with the aid of transgenic citrus lines that are tolerant of the Arabidopsis AtCBF3 gene and exhibit a notable improvement in their enzymatic activity (Romero-Romero *et al*., 2020). Because they include foreign DNA, unlike CRISPR-based altered plants, scientists have concluded that these genetically modified plants for salt tolerance are classified as genetically modified organisms (GMOs) under GMO rules (Raza *et al*., 2023).

Conclusions and Future Prospects

Soil salinization poses a significant threat to global food production, requiring a thorough understanding of the mechanisms underpinning salinity tolerance. Recent studies have highlighted pivotal aspects of salinity responses, such as ion homeostasis, osmotic regulation, hormone metabolism, signal transduction, and transcriptional regulation. Comprehensive studies leveraging multi-omics datasets-including resequencing, transcriptomics, epigenomics, proteomics, metabolomics, and phenomics provide a robust approach for identifying salt response gene modules and elucidating transcriptional regulation mechanisms (Saradadevi *et al*., 2021a, Raza *et al*., 2023)

Emerging approaches in gene discovery and crop breeding, such as GWAS, multi-omics-driven molecular module analyses, genomic selection, and gene editing, represent innovative engineering tools that enable a deeper exploration of the underlying mechanisms. However, limited information about highly salt-tolerant species remains a significant hurdle to their large-scale utilization.

To overcome the limitation information about highly salt-tolerant species, genetic diversity-based breeding strategies hold promise for developing new crop varieties by utilizing the potential of highly salt-tolerant ancestors of crop species. These strategies include haplotype breeding and *de novo* domestication of wild relatives. The integration of haplotype-based breeding with marker-assisted selection (MAS) offers an accelerated pathway for selecting salt-tolerant germplasm. In the future, high-throughput phenotypic evaluation of CWRs, novel gene discovery, and the development of molecular markers will expand opportunities for improving salt tolerance in crops. *De novo* domestication of wild relatives, facilitated by precise genome editing technologies, represents a promising avenue.

Soil salinization presents a considerable risk to global food production, necessitating a comprehensive understanding of the mechanisms that support salinity tolerance. Recent research has underscored critical elements of salinity responses, including ion homeostasis, osmotic regulation, hormone metabolism, signal transduction, and transcriptional regulation. In-depth studies utilizing multi-omics datasets such as resequencing, transcriptomics, epigenomics, proteomics, metabolomics, and phenomics offer a solid framework for identifying gene modules related to salt response and clarifying the mechanisms of transcriptional regulation (Saradadevi *et al*., 2021b; Raza *et al*., 2023).

Innovative methods in gene discovery and crop breeding, including GWAS, multi-omics-driven molecular module analyses, genomic selection, and gene editing, serve as advanced engineering tools that facilitate a more profound investigation into the underlying mechanisms. Nevertheless, the scarcity of information regarding highly salt-tolerant species continues to be a major obstacle to their widespread application.

To address the challenge of limited information on highly salt-tolerant species, breeding strategies based on genetic diversity show promise for creating new crop varieties by tapping into the potential of highly salt-tolerant ancestors of crop species. These strategies encompass haplotype breeding and the de novo domestication of wild relatives. The combination of haplotype-based breeding with marker-assisted selection (MAS) provides a faster route for selecting salt-tolerant germplasm. Looking ahead, high-throughput phenotypic evaluation of CWRs, the discovery of novel genes, and the creation of molecular markers will enhance opportunities for improving salt tolerance in crops. The de novo domestication of wild relatives, supported by precise genome editing technologies, represents a promising path forward.

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