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REVIEW ARTICLE

Smart reprograming of plants against salinity stress using modern biotechnological tools

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ABSTRACT

Climate change gives rise to numerous environmental stresses, including soil salinity. Salinity/salt stress is the second biggest abiotic factor affecting agricultural productivity worldwide by damaging numerous physiological, biochemical, and molecular processes. In particular, salinity affects plant growth, development, and productivity. Salinity responses include modulation of ion homeostasis, antioxidant defense system induction, and biosynthesis of numerous phytohormones and osmoprotectants to protect plants from osmotic stress by decreasing ion toxicity and augmented reactive oxygen species scavenging. As most crop plants are sensitive to salinity, improving salt tolerance is crucial in sustaining global agricultural productivity. In response to salinity, plants trigger stress-related genes, proteins, and the accumulation of metabolites to cope with the adverse consequence of salinity. Therefore, this review presents an overview of salinity stress in crop plants. We highlight advances in modern biotechnological tools, such as omics (genomics, transcriptomics, proteomics, and metabolomics) approaches and different genome editing tools (ZFN, TALEN, and CRISPR/Cas system) for improving salinity tolerance in plants and accomplish the goal of "zero hunger," a worldwide sustainable development goal proposed by the FAO.

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Introduction

In the last few decades, the world population has increased enormously and is expected to reach ~10 billion by 2050 [1]. It is a matter of great concern to fulfill the present and future global food demand, which seems impossible with current agricultural production from already shrinking arable land due to urbanization and land degradation [1]. The food for the extra mouths will have to come from the marginal areas; hence, strong efforts and practically effective strategies are needed to enhance crop productivity, especially in the marginal areas in the face of ever-changing climate and various other biotic and abiotic stresses [2]. Among numerous abiotic stresses, salinity/salt stress is the major abiotic constraint threatening global food security by decreasing agricultural productivity and a major hurdle in accomplishing the "zero hunger" goal proposed by FAO-UN [3,4]. Millions of people in extreme, rural areas lead stressful lives under hunger and poverty. The number of malnourished people, i.e., facing chronic food poverty, has risen to nearly 821 million in 2017, from around 804 million in 2016 [5]. Approximately 1.125 billion hectares of agricultural land

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and more than 52% (4.03 billion) of the population are affected by salinity (Figure 1) [4,6]. Consequently, poor agricultural land directly leads to food shortage affected by several environmental factors, including salinity stress, which ultimately hinders achieving the "zero hunger" goal (sustainable development goal: SDG2), to "end hunger, attain food security, better nutrition and help sustainable agriculture," by 2030 [5]. In short, salinity stress impairs plant growth and development, physiological, biochemical, and molecular mechanisms, ultimately reducing overall plant productivity [3,4].

For instance, salinity drastically impacts overall plant growth and yield in the long run. Salinity negatively affects seed germination by disturbing the physiological activity of seeds, causing an overall reduction in plant: biomass, yield, leaf area, stem, root, and shoot length [3]. In quinoa (*Chenopodium quinoa*), salinity caused a 49 and 47% decrease in the shoot and root lengths, respectively, in the "A7" genotype. In contrast, in the "Vikinga" genotype, more than 60% of the reduction was observed in shoot and root lengths [7]. In the "A7" genotype, dry weights of root and shoots were reduced to 49%, while in the case of "Vikinga," the reduction percentage was up to 59 and 71%, respectively. The relative water content (RWC) in leaves was also reduced to 33 and 46% in "A7" and "Vikinga," respectively [7]. In maize (*Zea mays*), leaf growth (dry weight) was reduced by 11 and 7%, whereas the reduction in root growth was 30 and 15% at 100 mM NaCl stress level [8]. In Libyan hard wheat (*Triticum durum Desf.*), plant height and dry weight were reduced by 33 and 16%, respectively, while the number of tillers and harvest index were reduced by 27 and 38%, respectively [9]. A significant reduction of 32.6% in wheat grain yield was observed due to higher salinity levels [10]. Salinity caused a yield reduction of up to 50% at EC 7.2 dS/m in rice (*Oryza sativa*) [11]. In another study, the yield of "Pokkali" rice varieties was reduced by 20–82% under salinity [12]. In cotton (*Gossypium hirsutum*), the number of bolls was also reduced due to salinity stress leading to an overall yield reduction [13].

Plants' adaptive response to salinity is extremely complex and regulated by various intricate signaling networks linked to multiple stress-related sub-traits. Plants' salinity response also depends on the growth stage as their tolerance or sensitivity to salt-stress changes substantially based on the plant development stage. Rice is relatively tolerant at germination but becomes: very sensitive during the early seedling stage (1–3 weeks), tolerant during active tillering, and most sensitive at panicle initiation to flowering and fertilization, affecting the overall grain yield, and lastly, more tolerant at maturity [14]. The functional and structural key to each trait and its components lies in a unique genetic code that could be manipulated to modify their functions [15,16]. Moreover, with the recent advancement in sequencing technology, the genomic sequence of many crops, such as rice [17], wheat [18], etc., is available. Furthermore, some salt-tolerant halophytes, such as *Thellungiella parvula* [19], *Thellungiella salsuginea* [20], *Eutrema salsugineum* [21], *Oryza coarctata* [22], etc., have also been sequenced. The next step is to manipulate this sequence to develop stress-resilient future crop varieties. In this regard, omics approaches, such as genomics, transcriptomics, proteomics, and metabolomics have emerged as excellent platforms for identifying stress-responsive: key genes, proteins, metabolites, and mapping complex signaling pathways [23–27].

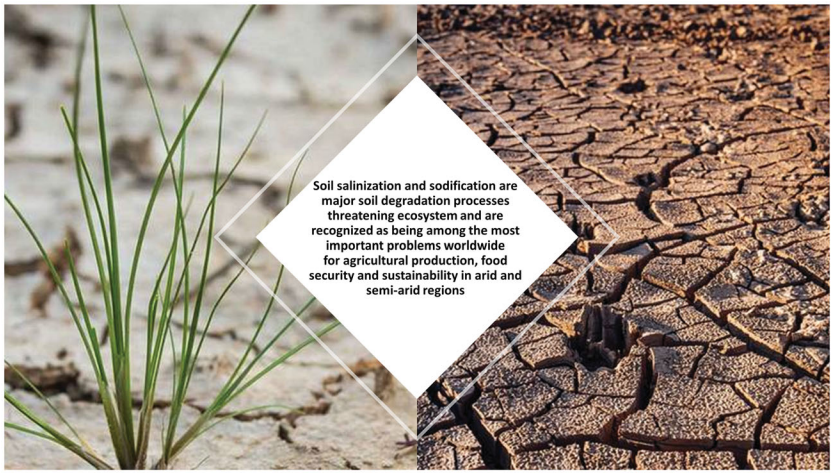

Figure 1. Soil salinity is one of the utmost important threats globally to sustainable agricultural production and food security in arid and semi-arid areas. Source: FAO http://www.fao.org/global-soil-partnership/areas-of-work/soil-salinity/en/.

In summary, the systemic integration of multi-omics datasets is required to identify the functions of novel players (genes, proteins, and metabolites) underlying plant responses and tolerance to salinity, which will further help explore the mechanisms regulating complex physiological, biochemical, and phenotypic traits under salinity [28]. Manipulation of genes controlling important traits either involves genetic engineering or site-directed genome editing technologies [29,30]. The first green revolution (GR1) was composed of multiple innovations related to big effect single genes to fulfill the hunger gap, so the second one (GR2) is likely to build on multiple genome editing interventions with similar characteristics to GR1 to overcome the drawbacks of GR1 [31].

Plant genetic engineering either involves integrating foreign genes into a plant genome (transgenic technology) or a few base pairs addition/deletion within intrinsic genes to develop plants with desirable traits [30]. This technique has led to the rapid development of plants with enhanced yield, stress tolerance, and high nutritional values [32]. Considering the innovations made in recent years, biotechnological-assisted breeding for enhanced tolerance *via* gene transfer and the development of transgenic plants is believed to be a tremendous and affordable technique compared with conventional breeding and management strategies, such as hybrid development and agronomic practices. Perhaps one of the most significant outcomes of biotechnological approaches is to utilize molecular tools for breeding programs. Detecting closely correlated molecular markers with the objective gene and mapping it on the chromosome is a vital aim for cloning the genes and marker-assisted selection [33,34]. Therefore, this review examines the recent progress in several biotechnological tools, i.e., omics approaches (including genomics, transcriptomics, proteomics, and metabolomics) and various genome editing tools for engineering salinity tolerance in different crop plants. We propose that a set of biotechnological tools would help to contribute to the achievement of a "zero hunger" goal to feed the ever-growing world population.

Plant responses to salinity stress: an overview

Plants are co-evolved with innate adaptation mechanisms to cope with different stresses. Depending upon their capacity to grow and survive under salinity, plants are classified as glycophytes or halophytes [6,35]. The measure of all soluble salt in soil water is called soil salinity. The main soluble mineral salts are the cations, i.e., sodium (Na^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^+), and the anions, i.e., chloride (Cl^-) [36,37], whereas sodicity is the calculation of Na^+ in soil water, compared with Ca^{2+} and Mg^{2+} ions. It is expressed either as sodium adsorption ratio (SAR) or as the exchangeable sodium percentage (ESP). The soil is termed sodic when the SAR of the soil equals or is >13 (mmol L^{-1}), or the ESP equals or is >15 [36,37]. To maintain growth and production, plant response to salinity differs either in the short-term or ultimately in the long-term at biochemical, cellular, molecular, and physiological levels (Figure 2).

Occurrence of salinity, its sources, and accumulation in plants

The initial cellular responses to salt, sodium import, and sodium sensing are arguably the least understood, and they remain a black box in salt-induced signaling pathways. Salt can enter the root through non-selective cation channels (NSCCs), which transport sodium across the plasma membrane [38]. NSCCs are regulated by different salt-induced signals, such as calcium, 3,5-cyclic guanosine monophosphate (cGMP), and ROS. Other channels and transporters may also contribute, but their actual role in sodium import in planta is debated. The hypothesized action and regulation of sodium import have recently been critically assessed [39]. Despite recent advances, the mechanisms by which plants perceive salt is another open question. It has been proposed that plants sense osmotic changes rather than Na^+, while sodium-specific responses occur much later through the toxic effects of sodium (or chloride) in the leaves [40]. However, rapid salt-specific responses, such as sodium-specific calcium waves, were recently identified in roots [41]. Furthermore, the rapid and sodium-specific effect of salt on root growth direction (halotropism) predicts the presence of a root-based sodium sensor [42]. Sodium may be sensed intercellularly, extracellularly, or by ion transporters at the plasma membrane. Recently, significant progress has been made with the identification of Monocation-induced [Ca^{2+}] I Increases 1 (MOCA1) likely functioning in extracellular salt sensing, including, but not restricted to, Na^+ [43]. The *moca1* mutant lacks the early response calcium waves that occur in response to Na^+, K^+, or Li^+ ions. Functioning as a glucuronosyltransferase, *MOCA1* produces glycosyl inositol phosphorylceramide (GIPC) sphingolipids at the plasma membrane. These GIPCs can bind monovalent cations and, upon binding, are hypothesized to open a Ca^{2+} channel to induce downstream responses to salinity. In addition, salt-induced changes in the cell wall are perceived *via* FERONIA

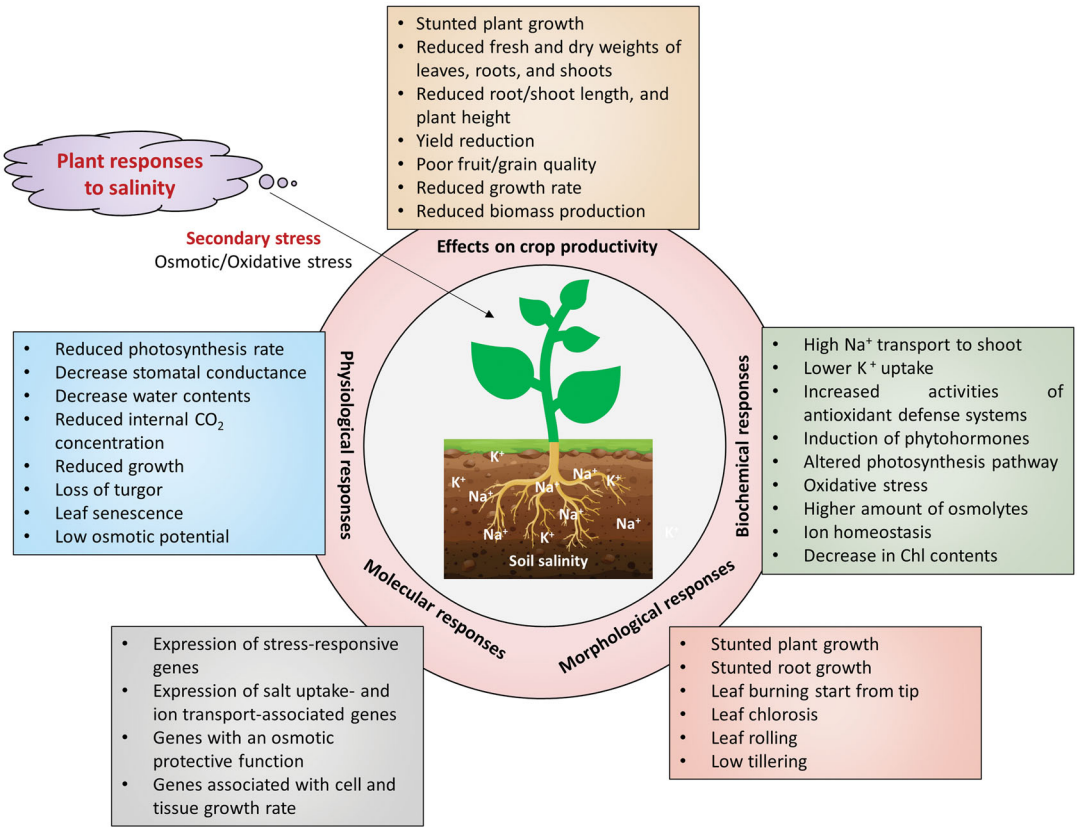

Figure 2. Major features of plant responses to salinity stress at a physiological, biochemical, morphological, molecular level and effects of salinity stress on crop productivity. In brief, the first signs related to salinity stress (from the early hours until a few days later) can be seen in the roots by suffering osmotic stress combined with the accumulation of phytotoxic ions (e.g., K^+ and Na^+). In the long duration, salinity stimulates ion toxicity owing to a nutrient discrepancy in the cytosol. Additionally, salinity manifests as oxidative stress at the subcellular level, arbitrated by ROS production. Overall, these responses participate in harmful consequences on plants and ultimately reduce plant productivity. To cope with these harmful consequences, plants modify their several physiological, biochemical, morphological, and molecular mechanisms. Read the text for detailed information about plant responses to salinity stress.

(FER), a receptor-like kinase (RLK) [44]. However, downstream signaling of this receptor happens several hours after salt application, and not during early salt-induced signaling responses. It is likely that no single sodium sensor exists, but rather that different aspects of salt stress are sensed and integrated through different signaling routes. The accumulated salt is further processed by ion transporters which are crucial for maintaining the balance [3,4].

Role of ion transporters in salinity response and tolerance

Sustaining cellular ion homeostasis is a crucial characteristic of salt-tolerant plants. To deny the cellular damage and nutrient deficiency, a desirable K^+:Na^+ ratio in the cytoplasm can be obtained by reducing cytoplasmic Na^+ and increasing cytoplasmic K^+ [45,46]. There are major ion transporters regulating the homeostasis of these ions. Among them, *HKT1* is a key player in fine-tuning the plant response to salinity stress *via* ion homeostasis [47]. *HKT1* restrict the accumulation of Na^+ in the shoot tissue thus mitigating Na^+ toxicity in the leaves. Arabidopsis *HKT1* is strongly expressed in root stellar cells and leaf vascular tissues [47]. The *hkt1* mutants displayed sensitivity to salt stress by accumulating more Na^+ in shoots and less Na^+ in roots, indicating that *HKT1* controls the distribution of Na^+ between root and shoot [48]. The salt overly sensitive (SOS) pathway in plants is a salt-responsive pathway that acts as a guard of the cell to sweep out Na^+ ions. Several reports have stated that, in roots, the SOS proteins may have novel roles in addition to their functions in sodium homeostasis. SOS3 plays a critical role in the plastic development of lateral roots through modulation of auxin gradients and maxima in roots under mild salt conditions [48]. The SOS proteins also play a role in the dynamics of the cytoskeleton under stress. The transcriptional levels of *SOS1*, *SOS2*, and *SOS3* increased significantly over time in the *atbzip62* upon NaCl

application, while they were downregulated in the wild type [48]. Recent studies report that *NHX1* and *NHX2* mediate K^+ uptake into vacuoles; Arabidopsis *nhx1* and *nhx2* null mutants display no changes in salt sensitivity and Na^+ sequestration in the vacuoles [49,50]. It would be interesting to determine whether the potassium transport activity of *NHX1* and *NHX2* is mediated by Na^+ concentration changes in the cytosol or whether *NHX1* and *NHX2* exchangers may primarily mediate K^+/H^+ exchange; however, at certain Na^+ concentrations they may have Na^+/H^+ exchanger activity [49].

Long- and short-term salinity response

According to literature, plant responses to short- and long-term salinity stress are different. For example, citrus plants displayed normal growth phenotypes under short-term and under long-term salinity stress. However, a decrease in photosynthesis activity was observed under prolonged salinity stress [51]. Several other studies reported the long-term response of plants to salinity [52,53]. These changes are directly controlled by phytohormones, such as abscisic acid (ABA). The overexpression of *OsSAPK10* considerably attenuated the rice tolerance to salinity stress by triggering the transcription of *EXPANSIN* genes and ABA signaling activity. The induction in *EXPANSIN* genes positively regulates the cell division and elongation under prolonged saline conditions [54]. Therefore, it can be suggested that ABA and other hormones promoted the immunity of plants under long-term salt stress by activating the transcription of cell elongation and division genes. The plant's short-term response to salinity stress is generally controlled by the immediate changes occurring in biochemical reactions. For instance, watermelon seedlings were exposed to short-term salinity stress (300 mM NaCl) [55]. A sharp decline was observed in the photosystem II, whereas an increased level of free amino acids was observed in the stressed plants [55]. Similar results were achieved in tomato plants when subjected to short-term salinity stress [56].

Role of aquaporins in salinity responses and tolerance

Major intrinsic proteins (MIPs) are a kind of membrane channel protein found in all kingdoms of life, including bacteria, archaea, protozoa, yeast, and plants [57,58]. It is primarily responsible for water homeostasis and transport, as well as the transport of a variety of low-molecular-weight solutes, such as glycerol, urea, ammonia (NH_3), methyl ammonium, hydrogen peroxide, formamide, acetamide, lactic acid, CO_2, and metalloids, such as boron (B), silica (Si), arsenic (As), and antimony (Sb) [57,58]. Aquaporins are involved in the transport of tiny uncharged and cation molecules as well as water channels [59]. Salt exclusion from the cytoplasm, salt compartmentalization in vacuoles, and a decrease in the hydraulic conductivity of the membranes through aquaporins as they control the water movement through the soil-plant system are also reported as protective responses of plants under saline stress [60,61]. Plant MIP is one of the biggest superfamilies, having about three times the number of isoforms compared to animal MIP family members. MIPs are thought to play key roles in plant life due to their many isoforms, however, the activities of several subfamilies and individuals remain unclear. Plasma membrane intrinsic proteins (PIPs), tonoplast intrinsic proteins (TIPs), NOD26-like intrinsic proteins (NIPs), small basic intrinsic proteins (SIPs), GlpF-like intrinsic proteins (GIPs), hybrid intrinsic proteins (HIPs), and uncategorized X intrinsic proteins are all members of the membrane intrinsic protein (MIP) family (XIPs) [62].

The plasma membrane intrinsic proteins (PIPs) are a large subfamily of aquaporins that are exclusive to the plasma and thylakoid membranes. Under salt-stressed circumstances, overexpression of *MusaPIP2;6* improved salt tolerance, photosynthetic efficiency, and membrane damage in transgenic bananas [63]. The effects of salt-water stress on two tomato cultivars were detected in terms of transcript levels of the *LePIP1* and *LePIP2* genes, with the salt-sensitive tomato cultivar showing greater transcript accumulation than the salt-tolerant cultivar [64]. Except for *BrPIP1;1a* and *BrPIP1;1b*, all *Brassica BrPIP* transcript abundance was high under salt stress. The majority of the *BrPIP* transcript, on the other hand, displayed an initial downregulation and subsequent upregulation pattern, with the maximum expression occurring after 24 h of salt stress [65]. The downregulation of the two most highly expressed isoforms of *PIPs* (*CsPIP1;2* and *CsPIP2;4*), caused by osmotic and salt stress in cucumber seedlings resulted in a decrease in hydraulic conductivity of leaves, which could be attributed to downregulation of the two most highly expressed isoforms of *PIPs* [66].

Tonoplast intrinsic proteins (TIPs) are a subtype of MIPs that have a limited localization to the vacuolar membrane [67]. A halophyte (*T. salsuginea*) tonoplast AQP gene (*TsTIP1;2*) may be implicated in the survival mechanism of *T. salsuginea* under a variety of conditions, including drought and salinity [68]. Tomato *SlTIP2;2* produced in *Arabidopsis* transgenics may improve salt tolerance by interacting with related proteins SlTIP1;1 and SlTIP2;1 [69].

PeTIP4;1–1, a bamboo aquaporin family member, was engaged in shoot development and led to drought and salinity tolerance in transgenic *Arabidopsis* [70]. *Glycine max TIP2;1* heterologous expression in yeast and overexpression of *GmTIP2;1*, *GmTIP1;7*, and *GmTIP1;8* in *Arabidopsis* increased salt and drought stress tolerance. Moreover, *GmTIP2;1* also forms homodimers and interacts with GmTIP1;7 and GmTIP1;8 proteins [71]. NOD26-like intrinsic proteins (NIPs) are a distinct subfamily of MIPs, with nodulin 26 protein being the first archetypal described in soybean [72]. However, no functional investigations on their role in salt tolerance have been published.

Biotechnology-assisted sustainable agriculture under salinity stress

Omics approaches: the scientists' favorite tools

Omics is a biological knowledge domain about differences at the cellular, DNA, protein, and metabolite levels. Omics approaches, including genomics, transcriptomics, proteomics, and metabolomics, aim to map the genomes and characterize their functional roles, modifications, and biological processes in plants. Multi-omics provide molecular insights to achieve stress-tolerant crop production (Figure 3) [27,28,33,34,73]. Plant response to salinity stress depends on the regulation of genes (up-regulation or down-regulation). In this framework, the integration of datasets obtained from multi-omics studies is an interesting idea that deals with the in-depth insight with a comprehensive understanding of the molecular level of salinity-stressed plants. In the subsequent sections, we have documented the importance of omics tools in identifying the stress-responsive: genes, proteins, metabolites, mechanisms, and metabolic pathways.

Genomic resources

Genomics-assisted breeding (GAB) is an approach that deploys: genomic resources, tools, and technologies, including molecular markers, to accelerate the plant breeding approach that uses DNA markers associated with desirable traits to select any plant [34,74]. Molecular or DNA markers are used as a powerful tool for improving plant breeding efficiency [34,74]. Some important markers used in breeding programs are restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), microsatellites, or simple sequence repeat (SSR), and the recent one is single nucleotide polymorphism (SNP). For instance, BC3F4 rice (Indica-donor, japonica Italian varieties, recipient) lines have been developed by introgression of salt tolerance especially using *Saltol* QTL, followed by a marker-assisted backcrossing (MABC) scheme [75]. Similarly, salt-tolerant lines with over 80% "Rassi," an adapted rice variety, alleles except in the region around *Salto* QTL were selected in the BC3F2 stage, and eight introgression lines had less yield loss (3–26% relative to control trials) [76] MABC breeding approach was also used in pyramiding QTLs controlling tolerance, introgressed in rice variety (Improved White Ponni) against various stresses, including salt stress [77]. The strong linkage between desirable traits and markers promises efficient breeding that can be evaluated using quantitative trait loci (QTL) analysis, gene mapping, or recombination analysis. Some studies related to QTL mapping, genome-wide association study (GWAS), and genomic selection (GS) to improve diverse crop species under saline environments *via* MAB are documented in the subsequent section.

Quantitative trait locus mapping. Quantitative trait locus (QTL) is a terminology used to identify genes controlling: important phenotypic traits, molecular markers, and markers' association with these traits. The QTL mapping analysis improved important genes of crops to a greater extent [34,74]. The following are some case studies identifying novel QTLs in crops under salinity stress, while some recent examples are presented in Supplementary Table 1.

Salinity stress, as a major growth-limiting factor in rice, was studied among the recombinant inbred lines (RILs) population. A recent meta-QTL study analyzed 935 QTLs reported in rice over the last two decades for various contributing traits to salt tolerance. These QTLs deduced from 13 different genetic background mapping population (BC1F9, BC2F5, BC2F8, BC3F2, BC3F4, BC3F5, BC4F4, BILs, DHs, F2, F2:F4, ILs, and RILs) with majority of them from RILs. Based on these studies, 63 meta-QTLs as the most potential genomic regions are recommended to enhance the degree of salt tolerance [14]. QTL analysis exhibited a variation in phenotypes, including shoot length (SL), root length (RL), shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), and root dry weight (RDW) and found 21 stable QTLs. A novel major QTL for shoot length *qSL7*, with a phenotypic variation of 7.5 and 6.8%, was identified against K^+ and Na^+ concentrations and can provide new avenues for salinity tolerance in rice [78]. Another study in rice under salinity stress identified seven novel multi-environmental QTLs for component traits, such as spikelet degeneration, stress susceptibility index, and spikelet sterility. Two major QTLs (*qDEG-S-2-1* and *qSSI-STE-2-1*) were positively influenced by genotype × environment interactions [12].

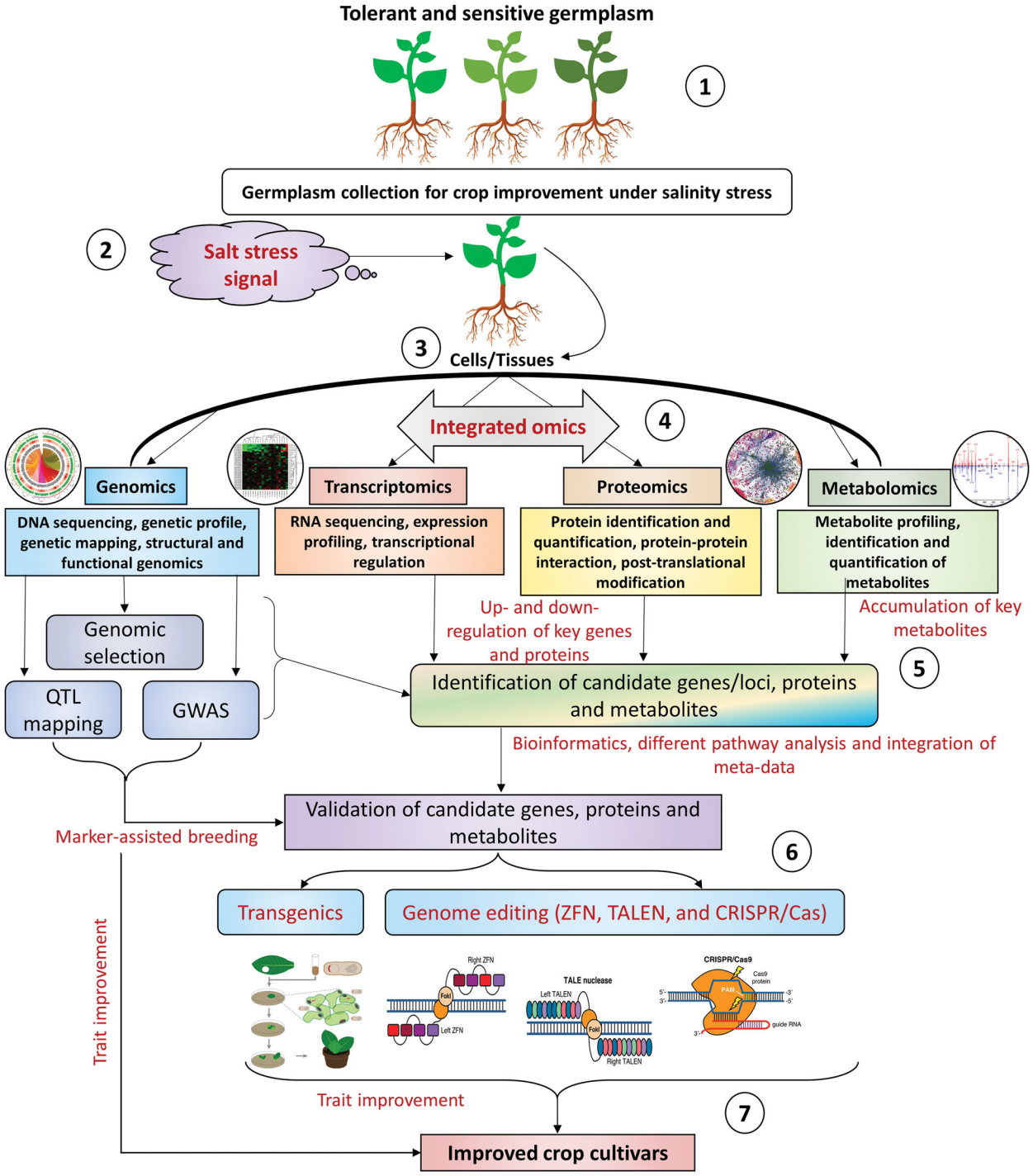

Figure 3. An overview of omics approaches for crop improvement under salinity stress. Mainly, the entire scheme comprises seven key steps, i.e., germplasm collections, plant exposure to salinity, sample collection, single or/and multi-omics analysis, identification of key regulators by bioinformatics, and validation of key regulators *via* genome editing or transgenics. The omics approaches (primarily genomics alone or the integration examination of multi-omics) can provide huge datasets to improve numerous plant traits *via* the biological systems. Furthermore, combined omics investigation can be completed by merging two, three, or multi-omics approaches in one experiment with the same stress and tissue to acquire wide-ranging omics datasets. On the other hand, functional validation can be carried out using genome editing and transgenic technologies for advancing sustainable agricultural production.

Saltol, a major QTL governing salinity tolerance in rice, was mapped in F8 RILs of a cross between IR29 (salt-sensitive) and Pokkali (salt-tolerant) that included three common QTLs for maintaining: low Na^+ uptake, high K^+ uptake, and Na^+/K^+ homeostasis in shoots with 64.3–80.2% of total phenotypic variation

conferring seedling-stage salinity tolerance [79]. QTLs for root Na^+ total quantity (*qRNTQ-1*) and root K^+ concentration (*qRKC-4*) underpinning salt tolerance were first reported in the cross Nona Bokra/Koshihikari [80]. The *qSKC-1*, a major QTL localized within the *Saltol* locus reported previously [80], was cloned as the first for salinity in rice. SKC1 gene (*Os01g20160*) controlling K^+/Na^+ homeostasis encodes an OsHKT-type Na^+ selective transporter and is preferentially expressed in parenchyma cells surrounding the xylem vessels. Thus, SKC1 affects K^+ and Na^+ translocation between roots and shoots and thereby regulates K^+/Na^+ balance in the shoots [81]. Similarly, 14 QTLs were found against physiological and yield-related traits under salt treatment for two different developmental stages in rice. In addition, the cytoplasmic effect was involved in these QTLs, highlighting the importance of cytoplasm-nuclear interaction for breeding programs [82]. Most of the QTL studies in rice are limited to seedling stage tolerance, and only a few reported the reproductive stage salinity tolerance, reporting several QTLs for different physiological, biochemical, and morphological traits [83,84]. A novel QTL *qGY-2*, identified for grain yield with 45% phenotypic variance, including other trait-related QTLs, would develop commercial salt-tolerant varieties [85]. Thus, QTL mapping has significantly improved a vast range of rice plant traits, including: morphological, physiological, yield-related traits, component traits, and maintaining homeostasis against salinity stress.

Soil salinity-related QTLs were identified in wheat for 15 agronomic traits and identified 90 stable QTLs with a phenotypic variation of 2.34–32.43%. These QTLs were present on all chromosomes of three genomes except 4D, 6B, and 7D. Moreover, *QPh-4B* was confirmed as an allele of *Rht-B1* in the QTL cluster. This study also provides the basis for salt-tolerant QTL cloning in wheat, allele-specific PCR markers that would help MAS for salt-tolerant wheat breeding [86]. Biparental QTL mapping has been conducted against salt-responsive traits in wheat at two different growth stages. Two novel candidate genes (*TaRN1* and *TaRN2*) and 22 overlapping loci were identified with different expression patterns in roots against salt stress [87]. Novel QTL for salt tolerance has been identified in bread wheat as a shoot ion-independent tolerance (*QG*(*1-5*). *asl-7B*), (*QCl.asl-3A*) for Cl^- accumulation, and (*QK*: *Na.asl-2DS2*) for K^+: Na^+ DW. This study may help understand genetic mechanisms for salt tolerance and speed up breeding for sub-traits in bread wheat [88]. Earlier, *TmHKT1;4-A2* and *TmHKT1;5-A* genes were reported as the Na^+ transporters in durum wheat exhibiting the potential to improve tolerance under a combination of waterlogged and saline conditions. Subsequently, these two important (*HKT1;4* and *HKT1;5)* genes were termed as Na^+ exclusion genes *Nax1* and *Nax2* due to their correspondence with *Nax1* and *Nax2* QTL associated with salt tolerance [89]. These genes are responsible for the removal of Na^+ from the xylem in roots and leaf sheaths, and the removal of Na^+ from the xylem in the roots, respectively found in diploid bread wheat (*Triticum monococcum* L.) and were introduced into durum wheat to improve salt tolerance [90]. Later, the introgression of the *TmHKT1;5-A* gene in the *Nax2* locus of commercial durum wheat has reportedly reduced the transport of Na^+ to leaves and has improved grain yield up to 25% when grown under saline soil. The incorporation of this approach from non-domesticated germplasm to commercial genotypes has enhanced plant productivity and stress tolerance [91].

Salinity stress was also observed in barley accessions and identified six QTLs with significantly reduced phenotypic traits. Chromosomes 1H and 3H were identified to be studied for narrowing down the candidate genes for further development of salt-resistant varieties [92]. In chickpea, a generalized study identified 28 QTLs for nine yield-related traits, majorly on two genomic regions, *CaLG03* and *CaLG06*, for salinity stress. The putative genes found in these QTL regions encode kinases, i.e., calcium-dependent protein kinases (CDPKs), MAPKs, histidine kinases (HKs), sucrose non-fermenting related kinases (SnRK1) [93]. Simultaneously, some were also involved in osmoregulation, helping the plants cope with salinity stress and be further used in breeding high-yielding salinity stress-tolerant varieties [93].

Genome-wide association studies. Genome-wide association studies (GWAS) are a powerful approach for uncovering the genomic regions related to natural variation using genetic markers and are caused by biotic or abiotic stresses. GWAS identifies genotype-phenotype association by genetic variants in a large population [34,74]. It involves fine mapping of QTLs linked with plant responses to abiotic stresses, including salinity stress at different stages (Supplementary Table 2).

A large multi-parent advanced generation inter-cross (MAGIC) population was used to identify major QTLs and genes against salinity stress in cotton using the GWAS approach. Results depicted 23 QTLs for salt tolerance, PH, and SDW, out of which 9 QTLs were common for drought stress. About 53 putative candidate genes were narrowed down in these QTL regions facilitating MAB for abiotic stresses [94]. A GWAS study of cotton has been studied under salt stress revealing (*NHX2*,

NHX4, *NHX6*, and *NHX7*) as sodium transporters. Almost 25 *NHX* genes were identified, in which *GbNHX7* interacts with the CBL-CIPK protein involved in the salt-responsive pathway [95]. Nineteen quantitative trait nucleotides (QTNs) with 52 significant markers were identified in barley accessions. This research further identified 4 major candidate genes within these regions involved in salt tolerance at the germination stage [96]. A GWAS study was conducted in barley for *HKT1;5* genes against salt stress using 2671 barley lines to identify the molecular mechanisms for salinity tolerance. By GWAS approach, the mapping results identified *HKT1;5* gene responsible for evacuating Na^+ from the xylem and its distribution and transportation to other parts, i.e., shoots and leaves. However, new insights for this gene in barley can be revealed by knockdown experiments with the latest technology (CRISPR/Cas9) [97]. In another study, GWAS analysis was conducted in wheat accessions to improve salt tolerance's progress over time. Different experiments revealed the introduction of favorable haplotypes and a novel QTL, *QSt.nwafu-6B*, for salinity tolerance. This study emphasized broadening genetic diversity for increased salinity tolerance [98].

GWAS in a large population of wheat accessions under two different salt treatments evaluated 11 QTLs related to diverse traits. Three major salt-tolerant loci were identified in bi-parental populations, eventually improving salt tolerance breeding in wheat. Likewise, a GWAS study in alfalfa reported 27 SNP markers associated with salt tolerance and potential candidate genes. Moreover, optimized GS models improved alfalfa breeding with enhanced salt tolerance [99]. In rice, the GWAS study identified 19 novel marker-trait associations (MTA) close to candidate genes related to transcription factors, membrane transporters, and signal transducers playing a role in saline tolerance. Other than this, grain yield, salt injury, and physiological parameters were measured under saline stress at the reproductive stage, unraveling genomic regions and functions of these candidate genes [100]. GWAS studies on the first MAGIC indica rice population subset using a mixed linear model (MLM) detected significant markers on chromosome 1 between 9.2 and 12 Mb near the previously reported QTLs on salt sensitivity, *qSKC1*, and near the *Saltol* QTL [101]. Recent GWAS analysis of rice in *Saltol* QTLs identified many novel candidate genes, including transcriptional factors for salt-related traits under salinity stress, which would help future rice breeding programs [102]. Another GWAS analysis was conducted on potassium transport-related genes in potatoes under salinity stress. About 43 putative genes were identified as potassium channels and transporters, revealing the potassium transport system's molecular entities in the Solanaceae family [103]. Another study on soybean varieties (tolerant/sensitive) exhibited nine *GmNHX* genes, which further directed 75 different miRNAs. All *GmNHX* genes were involved in sodium transport across the cells and provided information for breeding salinity stress tolerance [104].

Genomic selection. Genomic selection (GS) is a novel method of molecular breeding and is a powerful and promising tool to improve plant breeding by quickly selecting superior genotypes. This approach uses many markers over the whole genome and predicts the breeding value of complex traits. In GS layout, all QTL and genes have a linkage disequilibrium with the minimum number of markers [34,74,105]. This approach has become efficient due to numerous identified SNPs by whole-genome sequencing [105]. The efficiency of GS in breeding programs can be enhanced by keeping in view some important factors, such as statistical models, genetic architecture, the heritability of several targeted traits, assurance of genotyping and phenotyping availability, breeding methods, and it's budget [105]. GS, together with phenomics and machine learning models, enhance genetic gain with the help of increased selection accuracy in the breeding programs. These tools may explore better genetic diversity in crops, such as rice, wheat, common bean, chickpea, and groundnut for various traits [106].

The index selection and classical index selection have been used for crop improvement in their respective way. However, the index selection approach is used for multi-trait GS. A new GS approach for multiple traits has been proposed in a recent study by comparing it with conventional index selection and suggests this approach is more suitable for balancing multiple traits [107]. GS, having a great pace for enhancing breeding, has many applications in crop plant improvement [108,109]. Reported studies, including rice, maize, and wheat (up to 70% studies of GS), exhibit the integration of GS into recent crop breeding programs for rapid increment in genetic gain [110]. In cassava (*Manihot esculenta* Crantz), eight genomic predictive models were compared along with MAS against yield-related and starch pasting property-related traits. The results highlighted the predictive ability for various traits while starch-related pasting property-related traits had lower predictability. Results indicated that the implementation of MAS and GS would enhance selection efficiency for selecting quality traits in a large population [111].

Integration of GS and speed breeding using standard protocol is also encouraged to enhance genetic gains

for complex traits with low heritability to enhance crop research and production, such as in spring wheat [112,113]. The plethora of new technologies and optimizing components can change traditional breeding into a precise and efficient system with increased genetic gains and improved varieties [114]. Optimizing a breeding program for GS requires the integration of speed breeding, double-haploid technologies, and the implementation of new field designs for product development (PD). GS has been recently integrated into the wheat breeding program to develop new varieties through attaining greater genetic gain. A two-part breeding strategy has also been introduced for differentiating product development and population improvement. GS recurrent selection scheme is used to optimize population improvement strategy with reduced crossing cycle time and improved genetic gain [115]. Contrary to phenotypic selection (PS), GS can be used for any trait at any stage in a breeding program based on the breeder's choice. GS has been conducted for multiple traits at the same time to evaluate the genotypes, such as yield components, quality, and disease traits [116]. This leads to the identification of genetic correlation among preferred traits, thus increasing prediction accuracy for low and highly heritable traits and eventually enhancing selection accuracy coupled with genetic variance [117–119]. With the decrease in genotyping costs, the breeders have started implementing GS practically as compared to PS. Like QTL and GWAS, the advancement in GS and machine learning in genomic prediction can open new windows in discriminating breeding programs (MAS/MAB) for crop improvement under salinity stress.

Transcriptomics

Transcriptomics comprises the functional genome of living organisms dealing with: the whole set of transcripts, their plethora in a specific cell, and post-transcriptional amendments [120–122]. Plant function largely depends on the intensive activities that are happening inside a tissue cell. Plant transcriptomic experiments can be carried out using various technologies, such as RNA sequencing (RNA-seq), microarray, and other sequencing methods. Fortunately, advancements in RNA-seq technologies allow us to study these intensive and large-scale transcription changes inside specific plant tissues. RNA-seq, with its incredible potential, helps researchers to unlock and exploit the complex regulatory networks caused by environmental conditions or developmental changes in plant tissue [120,121]. Several limitations related to RNA-seq or transcriptomic analysis, such as analyzing large and complex datasets, throw challenges at researchers [120,121]. However, unique opportunities to generate knowledge about a tissue-specific response to salt stress in unprecedented detail can facilitate the research by many folds. Based on its great potential, transcriptomic analysis is now a widely used approach to understand the multifaceted molecular mechanism underlying different stresses, including salinity (Table 1). Below recent studies have been compiled and discussed the utilization of transcriptomic analysis in understanding salinity stress.

The RNA-seq study of salt-tolerant mutant (M4-73-30) line and wild-type (WT-Zarjou) cultivar of barley indicates specific genes facilitating salinity tolerance by the modulation of ion transporters, such as *SOS1/SOS3/SOS2*, *NHX1*, *TPK1/KCO1*, *HAK*, and *HKT* for Ca^{+}, Na^{+}, and K^{+} transportation [123]. RNA-seq data identified numerous salinity-responsive transcription factors (TFs), such as *WRKY*, *AP2/ERF*, *NAC*, *CTR/DRE*, *MAD*, *HSF*, *bZIP*, etc. The photosynthesis and respiration rate were significantly decreased in the mutant lines and preserved the tissues from the adverse effect of salinity by consuming stored energy and carbon. Moreover, ion transporters' expression and channel-related transcripts were increased to sustain the ion homeostasis in mutant lines than WT [123]. In bread wheat, 73 401 genes were identified in response to salinity stress *via* RNA-seq analysis [25]. The identified genes were involved in ROS scavenging, chaperons, and carbohydrate metabolism, and several early and late-stress responsive genes were also detected. Enrichment analysis showed that: carbohydrate metabolism, secondary metabolites, and pentose phosphate pathways were highly enriched in salinity response [25]. In addition, another recent report exploring salt tolerance adaptive mechanisms in the model legume *Medicago truncatula* through global transcriptomic profiling using microarray analysis in the salt-sensitive cultivar TN6-18 identified a lower expression of many genes related to stress signaling, not previously linked to salinity, and corresponding to the TIR-NBS-LRR gene class [135].

In another study, RNA-seq analysis was carried out at the osmotic stage (Zentos-tolerant and Syn86-susceptible) and ionic phase (Altay2000-tolerant and Bobur-susceptible) between tolerant and sensitive genotypes of wheat [136]. In response to salt-associated osmotic stress, the initial up-regulation of Ca^{+}-binding and cell wall synthesis genes was detected in the tolerant genotype and considered key players in enhancing salinity tolerance. Alternatively, the down-regulation of photosynthesis-associated and Ca^{+}-binding genes and the augmented oxidative stress in the susceptible genotype are connected with the better photosynthesis reserve at the osmotic stage.

The precise up-regulation of some ABC transporters and Na^+/Ca^{2+} transporters in the tolerant genotype at the ionic phase specifies their contribution to regulations of Na^+ elimination and ion homeostasis [136].

The castor bean (*Ricinus communis* L.) is an economically important crop with great industrial value. However, the productivity of castor crops is usually hampered by salinity stress [137]. To understand the mechanism underlying salinity stress tolerance in castor, two cultivars, namely wild castor: Y, and cultivated castor "Tongbi 5": Z were used. The study yielded many DEGs consisting mainly of ERF/AP2, NAC, WRKY, and bHLH TFs families [137]. Hormone-related DEGs were also noted in both the wild and cultivated castor cultivars. The predominant DEG observed in the cultivated cultivars belongs to the PP2C TFs family, GA, and JA. The GA signaling gene *DELLA* (GA signal suppressor) was down-regulated in cultivated cultivar in response to salinity, indicating that stress resistance or adaptation could be because of the upregulation of other TFs, such as WRKY or NAC [137]. As previously reported, the *DELLA* gene mitigated the stress by governing the trade-of between defense and growth [138]. Therefore, it can be assumed that response to salt stress varies among species, genotypes, and within the species.

Arbuscular mycorrhizal fungi (AMF) is a well-identified multi-stress resistor and is generally used to enhance plant growth under unfriendly conditions. In this regard, the AMF-induced salinity tolerance was mapped by employing the transcriptomic approach in *Suaeda salsa* plant [139]. The AMF strain *Funneliformis mosseae* was used in this study. The research revealed 1306 and 424 DEG in shoot and root, respectively. The majority of the DEG responses to salinity in shoot tissue were involved in photosynthesis, carbohydrate, and energy metabolism. DEG in root tissue was mostly annotated to sucrose and starch metabolism [139]. It suggests that *Funneliformis mosseae* protects *Suaeda salsa* plants from salinity stress by suppressing the stress-induced ROS in the chloroplast [139]. In contrast, genes from the auxin signaling pathway displayed up-regulated expression and could compensate for the lost carbon assimilation, thus maintaining normal plant growth [139].

Proteomics

Proteomics deals with functional proteins': role, structure, function, localization, connections with other proteins, and their implementation in stress responses or natural conditions. Proteomics allows us to study changes at protein level/post-transcriptional changes in greater detail. Therefore, proteomics becomes an indispensable approach in identifying key stress protein markers that could be useful in generating stress-resilient crops [140]. Currently, thanks to sophisticated biotechnological tools, proteomics has become more feasible, affordable, and beneficial to research labs worldwide in the field of science (see Table 1 for key examples).

The proteome of rice plants subjected to salinity was profiled in a recent study [141]. Two rice cultivars, namely cv. Vytilla-4 (highly salt-tolerant) and cv. Jhelum (salt-sensitive) was used. The study unfolded an array of different proteins expression and responses to salt stress in rice plants [141]. In particular, proteins involved in photosynthesis, such as Chl *a-b* binding protein, carboxylase small chain, and ferredoxin triggered under salinity stress in cv. Vytilla [141]. On the contrary, the PS-II CP47 was suppressed in the cv. Jhelum. Other proteins related to carbon fixations that are crucial for energy metabolism (Cytochrome *c* oxidase subunit 5C, peroxisomal (S)-2-hydroxy-acid oxidase GLO1, and peroxisomal (S)-2-hydroxy-acid oxidase GLO5) were substantially increased in cv. Vytilla [141]. This clearly indicates that these proteins could perform as potential biomarkers in regulating plant response to salinity without compromising the growth. Hormones play a prominent role in mimicking the salinity in almost all plants [142]. Proteins related to hormones were profiled in the hulless barley under salinity stress [143]. Two cultivars (salt-sensitive landrace lk621, and the salt-tolerant lk573) were tested for germination under salinity stress in this study. The lk573 cultivar germinated properly under saline conditions compared to the crippled germination rate in lk621. A total of 171 differentially expressed proteins (DEPs) were detected in the salt-tolerant cultivar [143]. Among them, proteins involved in nitrogen metabolism, ascorbate and aldarate metabolism, ABC transporters, and other terpenoid-quinone biosynthesis were expressed at 4 h after salinity stress [143]. As previously reported, ABC transporter and terpenoid-quinone are tightly regulated by hormones [144–146]. On the other hand, in lk621, most proteins expressed were associated with terpenoid backbone biosynthesis and fatty acid biosynthesis [143]. It suggests that these DEPs work in coordination with plant hormones to modulate barley seed germination under salinity stress.

Sorghum (*Sorghum bicolor* L.) is naturally considered a stress-resistant fodder crop and performs relatively well under numerous harsh environments [147]. Therefore, it could be of great interest to study the protein profile of sorghum under salt stress. In line with this, a proteomic analysis was performed to examine the stress-responsive proteins in two sorghum

Table 1. Some recently conducted transcriptomics, proteomics, and metabolomics studies under salinity stress in different crop plants.

Plant specie	Stress conditions	Tissue	Approach	Key findings	References
Transcriptomics					
Barley (*Hordeum vulgare*)	300 mM NaCl; 6 h	Roots and shoots	RNA-seq	• 7116 DEGs • Salinity stress-responsive different TFs were identified from WRKY, ERF, AP2/EREBP, NAC, CTR/DRE, AP2/ERF, MAD, MIKC, HSF, and bZIP • As a key mechanism, photosynthesis and respiration were reduced in the mutant and maintained the plants' tissues under salinity by consuming stored energy and carbon • The expression of ion transporters and channels-related genes were up-regulated to maintain the ion homeostasis	[123]
Tomato (*Solanum chilense*)	500 mM NaCl; 21 d	Leaf	RNA-seq	• 265 158 DEGs • 134 566 DEGs up-regulated and 130 592 DEGs down-regulated • Several DEGs were involved in Ca^{2+}, auxin, and ethylene-mediated signaling networks and were identified as key genes against salinity • Genes encoding proline and arginine metabolism, ROS scavenging systems, transporters, osmotic regulation, defense, and stress response, and homeostasis were significantly induced and up-regulated under salinity	[26]
Wheat (*Triticum aestivum*)	150 mM NaCl; 24 h	Roots and shoots	RNA-seq	• 8 DEGs • Most of the identified genes were involved in ROS scavenging, chaperons, and carbohydrate metabolism • Early stress-responsive genes (LOXs, BGLU, OPR2, CAD, UDPG, RPs, GLUD, and PAL) • Late stress-responsive genes (6-PGDH, CPODs, GSTs, BGLUs, SAM, PODs, and OXO) • Carbohydrate metabolism, secondary metabolites, and pentose phosphate pathways are highlighted as enriched under salt stress	[25]
Zoysia macrostachya	30 mM NaCl; 24 h	Leaf	RNA-seq	• 8703 DEGs • 4903 DEGs up-regulated and 3800 DEGs down-regulated • Identified genes were involved in the hormone signal transduction, ion homeostasis, and ROS scavenging	[124]
Oats (*Avena sativa*)	150 and 300 mM NaCl; 24 h	Roots	RNA-seq	• 3915/13 492, 16 076/23 707, 4898/3414, and 34 040/14 757 DEGs in BY2/BY5 genotypes • The expression of several Na^{+}/K^{+} transporter genes was up-regulated under both NaCl level • Identified DEGs were enriched in starch and sucrose metabolism, galactose metabolism, and glycolysis/gluconeogenesis pathways	[125]
Cucumber (*Cucumis sativus*)	100 mM NaCl; 72 h	Seeds	RNA-seq	• 1420 DEGs • Common enriched pathways were porphyrin and chlorophyll metabolism, photosynthesis, linoleic acid metabolism, glyoxylate and dicarboxylate metabolism, fatty acid degradation, carbon metabolism, carbon fixation in photosynthetic organisms, biosynthesis of unsaturated fatty acids, biosynthesis of secondary metabolites, and alpha-linolenic acid metabolism • Identified genes were involved in signal transduction of plant hormone, photosynthesis, and argine and proline metabolism	[126]
Proteomics					
Wheat (*Triticum aestivum*)	150 mM NaCl; 24 h	Roots and shoots	iTRAQ	• 180 DEPs • Identified DEPs were involved in ethylene-dependent salt response	[25]

(*continued*)

Table 1. Continued.

Plant specie	Stress conditions	Tissue	Approach	Key findings	References
				• The majority of the proteins are enriched in ribosome of the translation process, pyrimidine metabolism, purine metabolism, pentose phosphate pathway, cyanoamino acid metabolism, and pyruvate metabolism • Mainly, nucleoside diphosphate kinases, transaldolases, beta-glucosidases, phosphoenlpyruvate carboxylases, and SODs were significantly up-regulated under salt stress	
Barley (*Hordeum vulgare*)	300 mM NaCl; 2, 4, and 6 d	Leaves and roots	MALDI TOF-TOF	• 53 and 51 DEPs in leaves and roots • Identified DEPs related to photosynthesis, ROS scavenging, and ATP synthase were significantly up-regulated	[127]
Alfalfa (*Medicago sativa*)	50, 100, 200 and 400 mM NaCl; 14 d	Leaf	LC-MS/MS	• 226 DEPs • 118 DEPs were involved in glutathione metabolism and oxidation-reduction pathways, and these antioxidant-related metabolisms were pointedly up-regulated • TCA and CBB cycle, and ROS metabolism were found to be key pathways for improving salinity tolerance	[128]
Beet (*Beta vulgaris*)	300 mM NaCl; 3 weeks	Leaves	NanoLC–MS/MS	• 82 DEPs • 54 DEPs up-regulated and 28 down-regulated • Identified DEPs were involved in lipid metabolism, cell wall modification, ATP biosynthesis, and signaling • Several stress-related proteins, such as lipid transfer protein, chaperone proteins, heat shock proteins, and inorganic pyrophosphatase 2 were significantly up-regulated under salt stress	[129]
Cucumber (*Cucumis sativus*)	200 mM NaCl; 7 d	Leaves	MALDI-TOF/TOF-MS	• 61 DEPs • Identified DEPs associated with plant-pathogen interaction, sulfur-containing metabolism, cell defense, and signal transduction pathways • Key proteins were cysteine synthase 1, glutathione *S*-transferase U25-like, protein disulfide-isomerase, and peroxidase 2	[130]
Wheat (*Triticum aestivum*)	200 mM NaCl; 24, 48, 72 and 96 h	Leaves	MALDI-TOF/TOF MS	• 194 DEPs • Identified DEPs were involved in the light-dependent reaction • Several DEPs were significantly up-regulated that relate to the Calvin cycle, transcription and translation, amino acid, carbon, and nitrogen metabolisms • Further, DEPs associated with plastoglobule development, protein folding and proteolysis, hormone, and vitamin synthesis, were also significantly up-regulated under salinity	[131]
Metabolomics					
Bean (*Phaseolus vulgaris*)	125 mM NaCl; 3 d	Roots and leaf	GC-CMS	• 79 DAMs • Mainly, lysine, valine, and isoleucine metabolites were strongly induced by salinity stress • Salinity stress boosted amino acids and carbohydrate metabolisms	[132]
Tomato (*Solanum Lycopersicon*)	60 mM NaCl; 45 d	Pericarp	GC-TOF-MS	• 114 DAMs • Identified DAMs including alkylamines, amino acids, carbohydrates, fatty acids, organic acids, and nucleotides metabolites were significantly accumulated • Mainly, L-tryptophan, L-valine, L-aspartic acid, trehalose, D-galactose, chlorogenic acid, alpha-tocopherol, and glycolic acid were induced by salt stress	[23]

(*continued*)

Table 1. Continued.

Plant specie	Stress conditions	Tissue	Approach	Key findings	References
				• Identified metabolites were involved in alanine, aspartate, and glutamate metabolism, pentose and glucuronate interconversions, arginine biosynthesis, TCA cycle, ascorbate and aldarate metabolism, and beta-alanine metabolisms	
Barley (*Hordeum vulgare*)	150 mM NaCl; 14 d	Seeds	GC-MS	• 14 DAMs • Under salinity, amino acids, sugars, sugar alcohols, sugar acids, and other derivatives acted as osmolytes • Identified DAMs were involved in amino acids metabolism, sugar metabolism, and TCA cycle pathways	[133]
Rimth Saltbush (*Haloxylon salicornicum*)	400 mM NaCl; 21 d	Shoots	GC-QTOF–MS and HPLC-DAD	• 47 DAMs • Most of the DAMs are belongs to amino acids, organic acids, amines, sugar alcohols, sugars, fatty acids, alkaloids, and phytohormones • In response to salinity, several amino acids were down-regulated and carbohydrates were up-regulated • Enrichment analysis showed that amino sugar and nucleotide sugar metabolism, TCA cycle, starch, and sucrose metabolism, phenylalanine metabolism, cysteine, methionine, glycine, serine, and threonine metabolism, etc. were significantly enriched by salinity stress	[24]
Potato (*Solanum tuberosum*)	20 mM LiCl; 45 d	Shoots	GC-MS	• 50 DAMs • Mainly, sugars, terpenes, alkanes, fatty acids, amines, and organic acids were induced by salt stress	[134]
Oats (*Avena sativa*)	150 and 300 mM NaCl; 24 h	Roots	GC-TOF-MS	• 201 DAMs • Several vital DAMs were accumulated under salt stress, i.e., sucrose, sophorose, isomaltose, melibiose, and 3, 6-Anhydro-D-galactose • Identified DAMs were involved in amino acids, carbohydrates, and organic acids metabolisms and were also significantly accumulated	[125]

DEGs: differentially expressed genes; DEPs: differentially expressed proteins; DAMs: differentially accumulated metabolites; ROS: reactive oxygen species; TCA cycle: the citric acid cycle.

genotypes (G-46 and CSV 44 F). Both the G-46 and CSV 44 F are salt-tolerant genotypes and could yield key proteins related to salt stress tolerance [148]. As expected, α, β, and γ forms of kafirin were detected and expressed dominantly in both genotypes. Among them, α kafirin-related proteins (seed storage proteins): acted as an energy source, were abundant in numbers and varied under different salinity levels [148]. Previously, it was documented that kafirin is a multi-stress responsive biomolecule [149,150]. Functional analysis of these proteins would be helpful to identify regulatory networks in sorghum under stress and can be further utilized in breeding programs to develop high-yielding and stress-tolerant sorghum genotypes [148]. In another study, the proteomic profiling of wheat seedlings subjected to salinity stress was performed [131]. The focus of this study was to analyze the changes that occur at the protein level in the chloroplast of the wheat plant after being treated with salt stress. A total of 194 DEPs were mapped inside the chloroplast. Many DEPs were attributed to transcription and translation, Calvin cycle, carbon, and nitrogen metabolism and were induced post-salinity stress treatment [131]. In barley, two near-isogenic lines (NILs), salt-tolerant (T46 and T66), and salt-sensitive (N33 and N53) were grown in soil adulterated with 300 mM NaCl [127]. Proteomics analysis was conducted, which yielded a set of proteins recorded in tolerant and sensitive lines. The dominantly expressed proteins in tolerant lines mostly belonged to photosynthetic, ROS scavenging, and ATP biosynthesis-related activities [127].

All the above evidence was pointing to the crucial importance of chloroplast and photosynthesis-related activities, as the ATP-mediated energy biosynthesis reaction mainly occurs in chloroplast. These ATP energy packets are critical in maintaining the normal ion homeostasis, scavenging harmful ROS, and perhaps determining the fate of overall growth [151]. Thus, the

summarized proteins could help the research community to generate stress-resistant crops functionally.

Metabolomics

Stress can inflict changes in a plant at a: transcript, protein, and biochemical level. Often, the plant responds to stress only at the biochemical level without altering its transcriptional and protein expression [152–154]. These biochemical molecules are also called metabolites—the study of metabolites is called metabolomics [152,153]. Metabolomics allows us to study and explore the in-depth changes in plant cells after sensing stress. They possess different structures and functions, and because of these striking characteristics, metabolites study has become a hot trend in the current scientific research [152,153]. Our focus is to skim the metabolomics studies on salt-stressed plants to provide a platform for future beginner researchers (Table 1).

Foxtail millet performs better than other crops in adverse ecological conditions. To study how foxtail millet responds to stress conditions, young seedlings were subjected to salinity to evaluate the impact of salt stress on metabolic levels [155]. Two cultivars Yugu2 and An04 were used in this study, and both have different tolerance levels [155]. By employing the metabolomics approach, different metabolites were observed in the Yugu2 cultivar. The prominent metabolites involved in stress resistance, i.e., MDA, glutathione, and ascorbate, were up-regulated under salt stress at the early growth stage. Other stress-responsive metabolites, such as cinnamoyl-CoA reductase (*CCR*), cinnamyl alcohol dehydrogenase (*CAD*), and 3-ketoacyl-CoA synthase (*KCS*) are key lignin biosynthesis metabolites that showed induced accumulation after salinity [155]. Maize is a relatively salt-sensitive crop and is greatly affected when salt stress occurs at reproductive stages. The metabolomics technique was used to identify maize plant response to salt stress [156]. Two genotypes PH6WC (salt-tolerant) and PH4CV (salt-sensitive) were treated with 100 mM NaCl. The result revealed that a group of metabolites (sugars, amino acid, delspray, organic acids, and alkaloids) induced more than 2-fold to control treatment in the PH6WC genotype [156].

In another study, tomato (*Solanum lycopersicum* L.) and cucumber (*Cucumis sativus* L.) were subjected to varying degrees of salt stress (25, 50, 100, and 200 mM NaCl) [157]. Both cucumber and tomato are extremely sensitive to salinity stress and could express some key metabolites useful in breeding programs. For this reason, a metabolomics study was carried out to understand the metabolic response of cucumber and tomato toward salt stress [157]. Flavonoid contents were sharply increased in cucumber and tomato plants under 200 mM NaCl salt stress. The increment percentages of 2 and 30% were recorded in cucumber and tomato compared to their control treatment [157]. The phenolic compounds were accumulated greatly only in tomatoes, whereas no changes were observed in cucumbers. Likewise, saponin content was down-regulated in cucumber under salt stress (200 mM), which inversely increased significantly in tomatoes [157]. It can be suggested that cucumber and tomato plants exhibit different responsive natures to salinity on a metabolic level.

The metabolites present in the TCA cycle are mainly involved in regulating most plant developmental processes. TCA cycle is the main intermediate pathway that wires all other metabolic pathways and ensures proper plant growth [158]. In agreement with this, a metabolomic experiment was conducted by subjecting tomato plants to salinity stress. The research revealed various metabolites, such as carbohydrates and amino acids accumulated in green and mature tomato fruit under salinity stress [23]. Additionally, TCA cycle-related metabolites' content was increased significantly and could be a potential regulator in tomato response to NaCl stress [23].

Altogether, metabolomics is a unique technique that could help us to understand the growing world of metabolites and their changes. Further, these stress-responsive metabolites could also be used as a potential biomarker. Another benefit of metabolomics is that it facilitates the researchers to rewire the TFs related to proteins and metabolites and give a clear and detailed image of plant response to stress at multiple levels.

Genome editing tools: the promising future

Genome editing technologies are: rapid, site-directed, sequence-specific, and provide desired modifications at genomic loci to develop multi-stress-resistant plants with improved traits [159]. These technologies are based on the artificial sequence-specific nucleases (SSNs) to induce the DNA double-strand breaks (DSBs), which are repaired by one of two main major pathways: Non-homologues end joining (NHEJ), which is an error-prone mechanism, and induce random indels at the targeted site, and the homologs recombination (HR) which results into more precise and specific modifications at DSB site [30,32]. At present, three different types of SSNs are being used as genome editing tools, including zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats/CRISPR-

associated proteins (CRISPR/Cas), have multiple editing functions, such as deletions, mutations, insertions, inversions, duplication, and translocation of genes in a wide range of organisms and cell types [30,32]. The application of these tools has been successively applied in different crops to enhance abiotic stress tolerance, such as salt tolerance in rice [29,160,161]. However, CRISPR/Cas has some advantages over ZFN and TALEN. The below sections highlight each of these genome editing tools' roles in improving plants' salinity stress tolerance.

TALEN

TALEN is the chimeric protein consisting of repeat variable di-residues (RVDs) mediated DNA binding domain fused with *Fok*I endonucleases [162]. The DNA binding domain elements consist of highly conserved 16–20 tandem repeats of about 33–35 amino acids, which are derivatives of transcription activator-like effectors (TALEs), secreted by *Xanthomonas* spp. through the type III secretion system as a natural host response [163]. The binding specificity of TALEN is strictly determined by two RVDs present at positions 12 and 13 in each repeat which modulate the binding to one of four different types of nucleotides at the targeted DNA sequence [162,163]. Similar to ZFN, for targeted genome modification, the FokI domain's dimerization is required, which causes the DSBs by cutting the subsequent spacer DNA between dimeric effector binding elements (EBEs). These DSBs are either repaired by NHEJ or HR mechanism to induce the insertions or deletions [164]. This composition of TALEN, a single base recognition between DNA-binding repeats and TALE provides more flexibility as compared to ZFN protein which needs a triplet bps for binding. The DNA binding domain of TALENs had overcome the obstacle of engineering and recognizing new target sites faced by using ZFN [165,166]. To date, TALEN has been applied in some plants to develop resistance against diverse biotic and abiotic stresses and agronomic trait improvement [167–169]. Knock-out mutagenesis by TALEN for *TaMLO* gene in bread wheat identified resistant plants against multiple stresses (including salinity) and are heritable [170]. The genome of *Arabidopsis* was modified by targeting five genes, namely: *ADH1*, *TT4*, *MAPKKK1*, *DSK2B*, and *NATA2*, with seven engineered TALENs and found to have higher mutagenesis frequencies in transgenic plants [171]. So far, TALEN has not been vastly utilized in improving tolerance against salinity stress in plants. Therefore, this tool can be used for engineering salinity tolerance in different crop plants shortly.

ZFN

ZFNs are engineered restriction enzymes used to bind and cleave the targeted DNA sequences. The 4–6 arrays of zinc finger proteins are subsequently fused to a type IIS restriction enzyme *Fok*I which is a non-specific cleavage domain, each recognizing the 3 bp of DNA [163]. For targeted mutagenesis, dimerization of two *Fok*I nucleases is needed, requiring two ZFN monomers' proper orientation with precise spacing around them [172,173]. Heterod imerization of *Fok*I between ZFN monomers has been achieved to increase the precision, and specificity and to avoid off-site cleavage activity [174]. Efficiently directed mutations for targeting specific genes were made possible by ZFN. The genes against biotic and abiotic stresses reportedly modify loci by ZFN-mediated gene targeting mutations in plants [175]. ABA-INSENSITIVE4 (ABI4) gene encodes the ERF/AP2 TF family in *Arabidopsis thaliana*. ZFN-based mutagenesis was carried out, and the mutant plants showed the ABA accumulation and tolerance to salinity along with other various abiotic stresses [176]. Knock-in mutations were identified in maize in which the *IPK1* gene was first knocked out, and further biotic and abiotic resistant genes were introduced by ZFN [177]. Similarly, resistance genes were incorporated into endochitinase genes in *Nicotiana tabacum via* HR-mediated ZFN [178]. However, ZFNs do not have target flexibility due to the inadequacy of recognizing all DNA triplets as compared to TALENs and CRISPR-Cas9 for advancement in genome editing. Limitations caused by ZFN's off-target effects urged researchers to work on other approaches for genome editing with enhanced specificity [173]. Likewise, more investigations are required in deciphering the ZFN potential in engineering salinity tolerance in crop plants.

CRISPR/Cas system

Although ZFN and TALEN have successively increased genome editing precision and efficiency in targeting different genomic sites, they required re-engineering and re-designing new sets of effector proteins [159,179]. The difficulty in: protein engineering, cloning, and protein/DNA precipitation partially limited these tools from being adopted by the scientific community. In this scenario, CRISPR is very flexible and easy to use [30,32,159]. Its DNA targeting efficiency and endonuclease activity are directed by 20–28 bp guide RNA sequence [30,32,159]. CRISPR acts as a source of adaptive immunity in 40% of bacteria and 90% of archaea by degrading the invader plasmid DNA [180]. To expand the targeting efficiency, researchers are actively developing novel ways to increase the targeting

specificity of the CRISPR-Cas9 system. Depending upon the recognition and targeting of the ssDNA, dsDNA, and RNA, there are reported variants of Cas protein, such as Cas9 [181], Cas12 and Cas13 [182], and Cas14 [183] with programmed efficiency. The new CRISPR systems, i.e., CRISPRa and CRISPRi using dCas9 followed by CRISPR-Act 3.0, provide a powerful toolkit for activating gene expression and repression in plants [184]. Multiplexed gene activation in rice, Arabidopsis, and tomato has been reported using RNA-guided CRISPR activation (CRISPRa) system, thus resulting in metabolic engineering in mentioned plants and improved targeting scope [185].

CRISPR/Cas technology is now revolutionizing diverse fields of medical research, biotechnology, and agriculture. CRISPR-Cas is no longer just a gene-editing tool; the application areas of catalytically impaired inactive Cas9, including gene regulation, epigenetic editing, chromatin engineering, and imaging, now exceed the gene-editing functionality of Cas9 [159]. Over the past few years, CRISPR-Cas-directed genome editing has played a significant role in enhancing salinity tolerance in plants (Table 2). The basic strategy for using CRISPR technology for salinity tolerance includes: the selection of target gene(s), and designing and synthesizing sgRNA using available online resources. This designed sgRNA, along with the best suitable Cas variant (Cas9 or Cas12), would be cloned into a plant binary vector and transformed into target plant species *via Agrobacterium*-mediated transformation. Transformed plants would be screened for the presence of sgRNA and Cas9/Cas12 variant and then screened for targeted mutations, i.e., salinity tolerance. A well-established CRISPR-Cas9 system with no target effects for site-directed modifications has been reported to enhance salinity tolerance. The truncated-gRNAs (tru-gRNAs) based system focused on modifying functional gene OPEN STOMATA 2 (*OST2*) produced mutants against salt stress in *Arabidopsis*. The mutants expressed modified stomatal closing in response to abiotic stresses, i.e., salinity and drought stress [195].

In rice, NAC TF coding gene *OsNAC041* was targeted through CRISPR-Cas9 to determine its function under salt stress [29]. The mutant seedlings showed retarded growth compared to WT seedlings that remained alive under 150 mM L^{-1} NaCl treatment. Mutation in the *OsNAC041* gene disrupted the membrane protection system by decreasing activities of sediment oxygen demand (SOD), photochemical oxygen demand (POD), chloramphenicol acetyltransferase (CAT), and a significant increase in ROS accumulation and MDA content, thereby weakening salt tolerance. This study provided evidence that *OsNAC041* plays an important role in salinity in rice [29]. In another study, the function of Auxin Response Factors 4 (*ARF4*) in tomatoes was determined using CRISPR-Cas9. The down-regulation of the *SlARF4* gene resulted in better root development and low stomatal conductance under 150 mM NaCl stress treatment. CRISPR mutant plants (*arf4-cr*) showed an increased ABA level, coupled with up-regulation of *Cu/ZnSOD* and *mdhar* genes resulting in better growth under salinity conditions [190].

Histone deacetylase (HDAC) inhibitors (HDI) play a significant role in various biological processes, including epigenetic regulation and abiotic stress responses in plants. Seven out of 12 HDACs have a distinct role in conferring resistance against salt stress. Transcriptome analysis depicted that down-regulation of the *HDA19* gene, a member of the class I HDAC through CRISPR/Cas9, enhanced the salinity tolerance in wild-type *Arabidopsis thaliana*, dysfunction of HDA5/14/15/18 showed the antagonistic response [196]. Inositol trisphosphate 5/6 kinases (ITPKs) are involved in the bioavailability of phosphate and minerals and the stress signaling process in plants [189]. CRISPR/Cas9 was used to create the *HvITPK* mutant barely plant with single bp insertion and deletion mutagenesis. *HvITPK1* gene is involved in the phosphorylation of inositol phosphate to inositol hexakisphosphate (IP6) and confers the resistance to plants against soil salinization [189]. The *HvITPK* mutant plants with insertion were more tolerant than deletion mutants when grown at 50, 100, and 200 mM NaCl media. The expression of all ITPKs was induced in roots in response to salinity [189]. In another study, CRISPR/Cas system was used for functional analysis of soybean accessions. All these germplasms revealed a higher tolerance against soil salinization [197]. *OsmiRNA535*, a member of the miR156/miR529/miR535, negatively regulates the salt stress response in rice [194]. Moreover, *OSmiR535* is also involved in regulating plant growth and development, determining panicle architecture and grain length, and other abiotic stress management. CRISPR/Cas9 was used to down-regulate its expression by inducing the 5 bp deletion in a coding sequence. Mutant *OSmiR535* plants showed maximum tolerance and normal growth under saline conditions [194]. Rice *OsNCEB3* gene was activated and overexpressed using the CRISPRa (activation) system, and the resulting plants displayed the over-accumulation of ABA and increased tolerance to salt stress [193]. In rice, *OsRR22* TF is involved in the metabolism and signal transduction of cytokinin. The CRISPRi system was used to disrupt the *OsRR22* gene, and disrupted plants showed improved tolerance to salinity [160]. As

Table 2. CRISPR/Cas-mediated salt tolerance in different crop plants.

Crop	Stress conditions	Gene	Impact on plant system	References
Rice (*Oryza sativa*)	150 mM NaCl; 4 d	*FLN2*	• *FLN2* gene was knocked out • Involved in carbon transport in leaf • Responsible for sugar metabolism, sucrose biosynthesis, and Rubisco activity • Accounts for seedling growth of plant's response to salinity	[186]
Tomato (*Solanum lycopersicum*)	150 mM NaCl; 4 d	*SlHyPRP1*	• Negative regulator of salt tolerance • Multiplexed editing of *SlHyPRP1* for deletions of its functional motif(s) • Precision breeding	[187]
Cotton (*Gossypium hirsutum*) and *Arabidopsis thaliana*	150 mM NaCl; 2 weeks	*GhHB12*	• Down-regulation of *GhHB12* increases salt tolerance • Response to salinity through ABA content and regulation of *ABI2*, *DREB2A*, *RD29A*, *RD22*, *RD26*, *RD28*, *SOS1*, *SOS2*, *NHX1*, and *HKT1* genes	[188]
Barley (*Hordeum vulgare*)	50, 100, and 200 mM NaCl; 2 and 3 d	*HvITPK1*	• Involved in phosphate storage through phosphorylation of inositol phosphate to inositol hexakisphosphate (IP6) • Positive regulator of salinity stress • Salt-specific response induced in roots	[189]
Tomato (*Solanum lycopersicum*)	100, and 150 mM NaCl; 24 h	*SlARF4*	• *SlARF4* was down-regulated • Involved in root development and density • Increases soluble sugars and Chl content • Regulate the salt stress through stomatal conductance and ABA content • Activity coupled with *Cu/ZnSOD* and *mdhar* genes	[190]
Rice (*Oryza sativa*)	0.75% NaCl; 14 d	*OsRR22*	• Negative regulator of salinity tolerance • Salt stress was induced through six mutations that were heritable to T2 plants • Transgene free plants	[160]
Pumpkin (*Cucurbita*)	75 mM NaCl; 24 h	*RBOHD*	• Salinity-related activity is coupled with *GRF12*, *AHA1*, *HAK5* • Mediate the H_2O_2 signaling, which regulates K^+ uptake in the root under salt stress • Involved in RBOHD-mediated transcriptional and post-translational activation of plasma membrane H^+-ATPase	[191]
Rice (*Oryza sativa*)	200 mM NaCl; 24 h	*OsRAV2*	• *OsRAV2* is transcriptionally regulated by salt stress • Serial 5′ deletions and site-specific mutations at the promoter site were performed • GT-1 element relative to the putative translation start site is essential for the salt induction of P-OsRAV2	[192]
Rice (*Oryza sativa*)	200 mM NaCl; 4 d	*OsDST*	• Mutant showed reduced stomatal density accompanied by an increase in leaf water retention • Regulate the functions of *SPCH1*, *MUTE*, and *ICE1* genes • High level of tolerance to NaCl stress	[161]
Rice (*Oryza sativa*)	150 mM NaCl; 15 d	*OsNAC041*	• Promote the antioxidant activity (*SOD*, *POD*, and *CAT*) activities • ROS scavenging • Coupled with six different pathways, i.e., MAPK signaling, plant hormone signal transduction, peroxisome, eukaryotic-type ABC transporters, and photosynthesis	[29]
Rice (*Oryza sativa*)	150 mM NaCl; 4 d	*OsNCED3*	• Mediates leaf senescence by regulating ABA biosynthesis, osmotic and H_2O_2 stress • Positively induced under salt stress • CRISPRa (activation)	[193]
Rice (*Oryza sativa*)	200 mM NaCl; 1 week	*OsmiR535*	• Negative regulator of salinity tolerance • Regulate the NaCl, ABA, osmotic, and PEG stress	[194]

CRISPR-Cas technology has multiplexing ability, this efficient strategy targeting multiple loci would be a dynamic approach for the modification of plant genomes for salinity tolerance and enhanced yield [198].

Salinity tolerance is a multigenic complex trait controlled by multiple pathways, and it is imperative to say that single gene integration through genome editing technologies can develop salt resilient plants. Despite all these findings, there is a huge gap and a need to introduce site-specific multiple modifications with no apparent off-target effects to minimize unintended yield losses. Extreme care is required while designing sgRNA to minimize off-target activity. However, recently various orthologues of Cas9, including *Francisella novicida* (FnCas9) [199], *Streptococcus thermophilus* (StCas9), *Staphylococcus aureus* (SaCas9), *Campylobacter jejuni* (CjCas9), *Neisseria meningitidis* (NmeCas9) [200], *Geobacillus thermodenitrificans* (GeoCas9) with better properties and performance have been identified. In the CRISPR system, Cas9 protein only cuts and binds to the DNA at specific gRNA-guided target sites without disrupting any other genes, thus does not involve any foreign DNA insertion mutation. Due to the absence of foreign DNA, genome-edited plants may consider as non-GMOs [201]. Recently, a new method has been reported for introducing Cas9 protein and gRNA into plant cells so that it does not involve foreign DNA [202]. The non-GMO plants with the above-mentioned strategy of CRISPR-Cas system without foreign DNA against salinity stress have been reported in different plants.

Transgenic approaches

Transgenics deal with altering DNA segments *via* genetic engineering techniques [203,204]. In transgenic breeding, a gene of interest (a DNA fragment) from one plant is incorporated into the genome of another host plant with great precision which ultimately improves the targeted trait [203,204]. Transgenic plants with modified genetic makeup lead to crop improvement and sustainability against various climatic challenges (see Supplementary Table 3 for some recent examples) [203,204]. Figure 4 shows the molecular mechanism of plant salinity tolerance and key genes that have been engineered and improved the salinity tolerance in transgenic plants.

Transgenic *Arabidopsis* plants using *Medicago truncatula* as a source plant for *MtDof32* gene exhibited tolerances against osmotic and salt stresses. Some altered phenotypic traits like reduced branching and delayed flowering have been observed under stress conditions [205]. Over-expressing *Arabidopsis* lines were significantly more stable against salt stress than WT plants by enhancing: osmolytes, stigmosterol, and membrane integrity. Plants targeting the *SGT* gene may improve salinity tolerance due to its defensive role through sterol modulation [206].

A novel RING-H2 type E3 ubiquitin ligase gene (*IbATL38*) from sweet potato was transgressed into *Arabidopsis*, resulting in enhanced salt-tolerant transgenic plants. Overexpression of *IbATL38* up-regulates the ROS scavenging system's genes and decreases H_2O_2 contents [207]. In maize, *ZmEREB20* resulted in positive regulation of molecular mechanisms, such as hormone signaling, and ROS scavenging when overexpressed in *Arabidopsis*. Enhanced root hair growth and survival rates were also observed, verifying to improve crop breeding of salt resistance [208]. The overexpression of the *APX* gene in *Brassica juncea* has improved stress tolerance by strengthening anti-oxidative defense potential. The host plant has maintained ROS homeostasis with lesser membrane damage under salinity stress [209]. In *Arabidopsis*, the overexpression of the *MbNAC25* gene from *Malus baccata* enhanced: salinity, drought, and cold tolerance under stress with a high survival rate. Different enzymatic activities involved in the homeostasis mechanism have been improved, thus enhancing ROS scavenging capability [210]. Improved ROS scavenging ability, seedling growth, and lower levels of H_2O_2 and Na^+ were observed in overexpressing *Arabidopsis* lines targeting the *OsMT-3a* gene of rice. Results showed that this gene's importance for developing plant stress tolerance would eventually enhance crop production [211]. Tolerant transgenic lines of citrus with *Arabidopsis AtCBF3* gene exhibiting significant improvement of enzymatic activities may contribute to developing salt-tolerant commercial citrus variety [212]. Contrary to CRISPR-based edited plants, these genetically modified plants for salinity tolerance are considered as GMOs by a group of a scientific community under GMO legislation as they have foreign inserted DNA.

Conclusion and future outlooks

Salinity stress is the second biggest yield-limiting abiotic factor that poses a significant threat to sustainable agricultural production globally and counteracts accomplishing a goal of "zero hunger." This review proposes that recent advances in various biotechnological approaches could be considered a safer process for generating saline tolerant future plants to achieve "zero hunger." Increasing soil salinity significantly disturbs the:

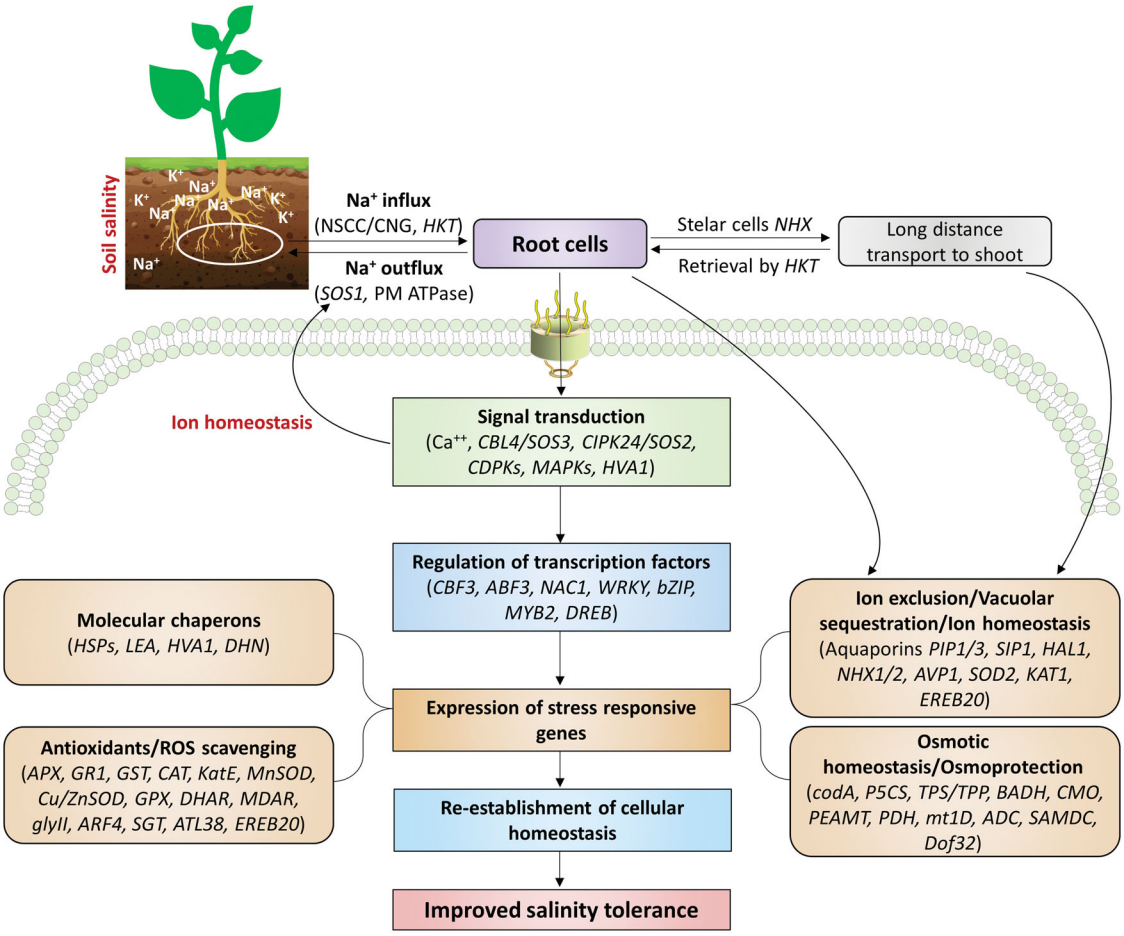

Figure 4. General molecular signaling pathways of salinity tolerance in plants. Soil salinity starts impacting the plants' aerial tissues *via* Na^+ influx and outflux (signal perception) from the root cells. Plants sense the early occurrence of stress *via* receptors/sensors cascades and signal transduction by secondary messengers, including Ca^{++}, *CBLs*, *SOS3*, *MAPKs*, *CDPKs*, and *CIPKs*. These signals cause differential regulation of transcription factors and stress-responsive genes. The regulation of these transcription factors and genes causes the reestablishment of cellular homeostasis by adjusting physiological, biochemical, and molecular responses, consequently improving salinity tolerance in plants. Boxes on the left and right side of the "expression of stress-responsive genes" indicate the role of candidate genes that have been genetically engineered and showed improved plant survival in transgenic plants against salinity stress.

morphological, physiological, biochemical, and molecular mechanisms (Figure 2). The normal functioning of these mechanisms is important for healthy plant growth and production under stress conditions. Under a saline environment, plants adjust themselves by modulating the stress-responsive genes/proteins (up- or/and down-regulation) and accumulating key metabolites to survive against stressful conditions. Hence, comprehensive studies are crucial to coping with salinity in the modern technological and rapidly climate-changing era.

Over the past few years, substantial advancement has been accomplished in exploiting state-of-the-art omics approaches, i.e., genomics, transcriptomics, proteomics, and metabolomics, evolving sustainable agricultural production under salinity. Notably, the integration of comprehensive omics or multi-omics datasets aids in identifying: stress-responsive genes, proteins, metabolites, and metabolic pathways that are highly correlated with plant phenotype under stress conditions. From the above-discussed examples, it can be noticed that several genes/proteins that are responsible for Na^+/K^+ movement, hormone signal transduction, ion homeostasis, ROS scavenging, etc., have been exclusively reported in improving salinity tolerance in plants. Likewise, many organic sugar compounds and amino acid-related metabolites and metabolic pathways have been identified, playing a significant role in improving salinity tolerance in various crop plants (Table 1).

Advanced studies that discriminate the molecular organization of interconnecting stress regulators are immensely important to underpin the salinity tolerance

in crop plants. In this line, genome editing using: ZFN, TALEN, and CRISPR/Cas systems have emerged as the most promising tools for genetic engineering of stress-responsive genes/regulators. Likewise, ever-green transgenics played a significant role in enlightening stress tolerance in the model (*Arabidopsis*) and the major crop plants (Supplementary Table 3). Several examples have been presented in their respective section highlighting the potential of genome editing and transgenics in improving salinity tolerance. Moreover, genetic engineering studies can be carried out on candidate genes (including transporters, sensors, and receptors) that are involved in mitigating the adverse effect of salinity stress and stress-responsive signaling pathways (see Figure 4 for key candidate genes). Similarly, the engineering of metabolic pathways can deliver new paths for advancing sustainable agriculture. The recent focus on speed breeding as a robust and time-saving method to boost crop productivity in a controlled environment has opened new avenues for the multifaced integration of technologies. Thus, the amalgamation of omics and genome editing in conjunction with speed breeding can achieve significant results for sustainable agricultural production to feed the billions by achieving the sustainable development goal of "zero hunger."

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Author contributions

AR, RKV, and WZ conceived the idea. AR, JT, AZF, RS, and RKS contributed to the writing and literature search. AR prepared the figures. AR, JT, and AZF designed the tables. LJ, VF, KS, RKS, RKV, and WZ reviewed and edited the manuscript. All authors have read and approved the final version of the manuscript.

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