



# Omics strategies for crop improvement in response to climate change-imposed abiotic stress

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SUBMITTED 13 February 2024 REVISED 30 October 2024 ACCEPTED 4 November 2024

**ABSTRACT** Given the current status of climate change and its impact on global food security, it is imperative to improve the abiotic stress tolerance of crop plants to enhance productivity. Traditional plant breeding methods have been widely employed to develop climate-resilient crops; however, their success has been limited due to the lack of understanding of the complex relationships between genes and stress-related phenotypes. The advent of modern genomics has enabled the expression analysis of stress genes in plants, as genome-wide information is readily accessible and can be utilized to assign and validate the gene functions. This article highlights the potential applications and limitations of present-day genomic technologies based on genome mapping, gain or loss-of-function analysis for identification of the role of a particular gene in abiotic stress response in plants. Such technologies are highly efficient in candidate gene identification; gene-trait relationships establishment; functional elucidation of genes; and stress genes modification in crop plants. Modern high throughput genomic technologies offer wide scope for deciphering the complexities of genetic regulation of stress in plants; modulating stress responses; and developing stress tolerance in crop plants against drought, temperature, salinity, osmotic imbalance, herbicides and heavy metal toxicity.

**KEYWORDS** CRISPR; Gene function identification; Gene-trait relationships; Genome editing; GWAS; High throughput genomic technologies

## 1. Introduction

The world's rapidly growing population has raised concerns regarding sustainability, particularly in terms of food security, adequacy, and nutritional deficiency, which are among the most critical global challenges. The Food and Agriculture Organization (FAO) has envisaged a roadmap to achieve food security and fulfill the SDG2 ensuring zero hunger through C-neutral agri-food systems, complying with the limits of 1.5 °C of Paris Agreement (FAO 2023). This necessitates an increase in agricultural productivity for which there could be several possibilities such as increasing arable land area, increasing food imports to meet the food demand, and increasing the use of fertilizers and pesticides to enhance crop yield. However, each of these options has an unsustainability factor associated with it that jeopardize the sustainable development goals in one or the other way. The population growth of a country and its subsequent industrial and economic development together exert pressure on land resource and force to put the agricultural land into other uses, contributing to reduction in agricultural productivity. Higher food imports result in a larger carbon footprint, and increased use of chemical fer-

tilizers and pesticides poses significant hazards to human and environmental health. Further, about 50% reduction in global crop production has been attributed to environmental stresses which not only account for economic loss of nearly 20 million USD, but also pose a serious threat to food security (Kaur et al. 2022).

Climate change imposed environmental perturbations cause various stresses to crop plants, such as salinity stress, osmotic stress, drought stress, high temperature stress, and cold or chilling stress. Aggravating the situation, the anthropogenic pollution harming soil and water leads to pollutant stress in plants such as heavy metal toxicity. These stress factors not only impact the growth, development, phenology and vigor of crops, but also reduce the yield and productivity. Therefore, achieving the food security goal in a sustainable manner would require a substantial shift to climate resilient agriculture. This is only possible if the crop productivity is enhanced by cultivating climate resilient and abiotic stress tolerant crops. Traditional methods of producing abiotic stress tolerant crops were largely dependent upon the plant breeding approaches of desired trait selection following various crosses between

suitable parents. However, the success used to be very limited due to many reasons such as the epigenetic variations, polygenic inheritance of characters, complexity of gene/loci architecture and lack of precise information about the gene-trait relationship.

The exposure of plants to one or multiple stressors elicits many responses simultaneously at physiological and genetic/epigenetic level, as a strategy to combat and tolerate stress. These responses are often manifested as altered phenotypes that would enable the plants to survive under stress. Stress defense in plants is widely studied based on morphology and appearance, antioxidant mechanisms, signaling pathways, growth regulation and secondary metabolism (Vijayalakshmi and Shourie 2019). The clues for underlying molecular basis of the stress defense mechanism in plants lie in stress-guided gene programming, therefore it is important to identify the genes involved in generating the stress trait and understand the gene functions governing the stress responses (He et al. 2018). Several decades ago, the researchers ascertained the function of the genes implicated directly or indirectly in the abiotic-stress response often through gene-by-gene method. However, with the advent of modern genomics, the expression analysis of stress related genes in plants has become very convenient as genome-wide information is readily accessible to be utilized to assign and validate the gene functions. Such information is highly valuable for genetic engineering of target genes at precise loci and modification of crop plants to improve stress tolerance and produce a desired trait (Bhat et al. 2021).

Exploration of different genes functions in a high-throughput mode is possible through functional genomics that combines many parallel techniques, such as global transcript profiling and the application of mutants and transgenics. Present day genomics technologies like genome-wide association studies (GWAS) provide a significant tool to uncover genetic intricacies of abiotic stress signal transduction and subsequent plant responses. The techniques also offer the foundation to device novel strategies leading to increased stress-tolerance in crop plants, such as gene editing through clustered regulatory interspaced short palindromic repeats (CRISPR) or by using customized nucleases, to prevent the chronic loss in crop yield (Bhat et al. 2021), as depicted in Figure 1.

This review explores the suitability and potential of

genomics to impart abiotic-stress tolerance targeting crop improvement. The critical commentary on various genomics tools like GWAS, QTL mapping, ESTs, CRISPR, engineered nucleases and gene silencing strategy is presented to give a bird's eye view of their advantages, applications, and drawbacks. The focus of this article is placed on summarizing the existing research to present a broad account of usage of genomics techniques in past two decades for developing tolerance of plants against abiotic stresses like drought, salinity, temperature, osmotic imbalance, herbicides, and heavy metal toxicity, which would be useful for further exploration of the opportunities offered by the advances in high-throughput genomics.

## 2. Discussion

### 2.1. Identification of abiotic stress related genes

#### 2.1.1 Quantitative trait loci (QTL) mapping

Mapping of quantitative trait loci has become a popular tool to elucidate the complexities of trait gene relationship, unravel gene architecture related to the trait and decipher the candidate genes. It has emerged as a modern approach to crop plant breeding due to its potential to reveal the genes consistently expressing desired agronomic traits under various abiotic stresses, which could act as valuable molecular markers (Raj and Nadarajah 2023).

QTL associated with drought related traits in maize were mapped through single nucleotide polymorphism (SNP) genotyping which could be useful in plant breeding through marker-assisted selection. Similarly, novel SNPs responsible for imparting drought tolerance in *Phaseolus vulgaris* were also identified (Villordo-Pineda et al. 2015). The study revealed major QTLs related to growth, physiology, stress signaling and yield under drought conditions in maize (Sarkar et al. 2023). Genes contributing to drought tolerance were analyzed and QTLs associated with drought stress index were identified in germinating seeds of *Brassica napus* and in cotton (Gad et al. 2021; Abdelraheem et al. 2021).

Plant genomes are large and highly repetitive, making it difficult to correlate stable and reliable QTLs with specific traits. The QTLs are typically mapped in large confidence intervals (CI) and are restricted only to the genetic diversity of the segregating population parents. They can

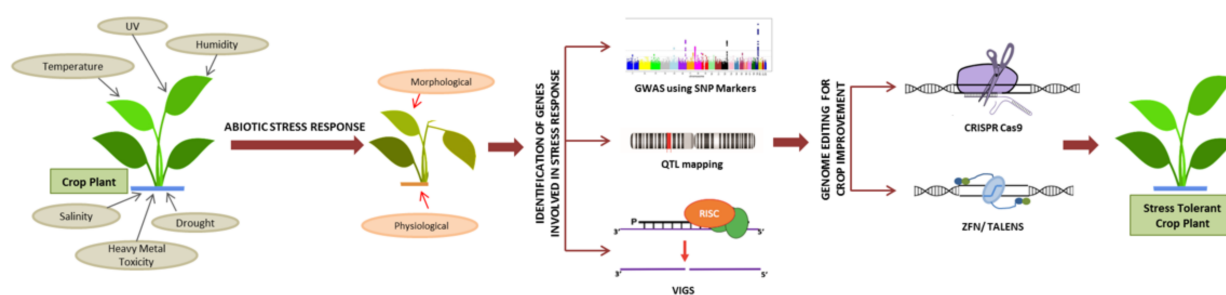


FIGURE 1 Genomics approach for developing abiotic stress tolerance in crop plants.

leave some loci undetected as they map multiple loci that influence the same trait (Ma et al. 2022). Low mapping resolution limits the identification of complete functional diversity within the natural population (Xu et al. 2017).

### 2.1.2 Genome-wide association studies (GWAS)

GWAS provide an opportunity to scan the genome for the identification of the associations between molecular markers and desired traits. However, this requires genotyping a sufficient number of markers throughout the genome that exhibit strong linkage disequilibrium (LD) with the functional variant(s) particularly in cases where multiple traits are correlated or interactions like epistasis exist (Devate et al. 2022). Thus, irrespective of the kind of mapping population, GWAS use a large number of molecular markers for mapping of QTLs to gauge the genetic diversity among various cultivars. The application of GWAS in unraveling tolerance mechanisms in plants is studied against several stress factors like drought, salinity, temperature and osmotic imbalance. It has been utilized effectively to identify cold tolerance genes in the 1033 rice diversity panel and cold tolerance-associated genetic loci namely LOC\_Os10g34840 was attributed to the cold-tolerance in the seedlings (Xiao et al. 2018). A genome wide study analyzing 33,009 SNPs found 31 SNPs on chromosome 3, one SNP on chromosome 2 and 7 as novel QTLs to be associated significantly with salt tolerance, and recommended several markers on chromosomes 2, 3, 14, 16, and 20 for marker-assisted selection and breeding of salt-tolerant soybean varieties (Zeng et al. 2017).

GWAS mapping is highly recommended for traits that are controlled by a minor number of loci having large effects; however, its application is limited for complex genetic architecture which presents a number of common variations of small phenotypic effects. Thus, due to the highly quantitative nature of many abiotic stress related phenotypes in plants, GWAS may not successfully identify the causative loci (Saini et al. 2022).

### 2.1.3 Expressed sequence tags (EST)

ESTs are small fragments of mRNA sequences derived from cloned cDNA, sequenced in a single shot. High throughput ESTs are very useful in delineating the genomic regions of crops and uncovering the expression patterns of genes, thus are used as efficient and rapid strategy to identify target genes. The EST databases are the most abundant source of coding sequences and despite their low accuracy they are highly useful for similarity searches. Mulberry EST encoding remorin (MiREM) was identified and used in the molecular and functional characterization involved in abiotic stresses mainly mediating osmotic stress (Checker and Khurana 2013). The major disadvantage associated with the usage of ESTs as genetic markers is that they are derived from expressed genes which are mostly conserved, thus exhibit less polymorphism than genomic simple sequence repeats (SSRs). Since high de-

gree of redundancy exists in EST libraries, it is difficult to ascertain the relative modifications in transcript levels (Guzinski et al. 2016).

### 2.1.4 Virus-induced gene silencing (VIGS)

VIGS is a versatile and powerful tool used in both forward and reverse genetics through silencing of transcriptional genes, off-target genes, post-transcriptional genes (PTGS), non-integration-based transmissible PTGS, and heterologous genes (Ramegowda et al. 2014). VIGS could help in identifying numerous abiotic stress-related genes implicated in drought (Ogata et al. 2017), deficiency of nutrients (Atwood et al. 2014), salinity stress and oxidative stress (Cai et al. 2017).

There are various VIGS vectors like the Tobacco Rattle Virus (TRV) which are used for overcoming abiotic stresses like heat (Singh et al. 2017), cold (Jia et al. 2016), drought (Liang et al. 2016a), oxidative (Singh et al. 2019), drought and salinity (Liang et al. 2016b). In cotton, by using CLCrV as VIGS vector and target gene as *GhNAC79* reduced drought stress (Guo et al. 2017). *TaBTF3* was used as the target gene and BSMV as the VIGS vector to reduce drought stress in wheat (Kang et al. 2013).

VIGS is a robust genomics tool for high throughput forward genetic screening as well as gene validation; however, it has certain limitations that should be addressed. Silencing a gene that imparts tolerance to plant against certain abiotic stress might enhance its susceptibility many times and elicit other defense response genes similar in function. Virus infection can itself cause concurrent abiotic and biotic stresses. In both the cases, the overlapping responses might make it difficult to ascertain the gene function. The factors influencing rate of viral infection and multiplication can also affect the silencing, resulting into delayed gene expression or appearance of a different phenotype. In several instances it was found that VIGS did not completely suppress the target gene and even after the down-regulation of its expression by 75–90%, the reduced transcript levels could still result in functional proteins and phenotypes (Rössner et al. 2022).

## 2.2. Genome editing for abiotic stress tolerance

### 2.2.1 Zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs)

ZFNs and TALENs are site-specific nuclease systems that have transformed genome editing and have allowed modification of precisely targeted specific gene of interest. ZFNs identify a broad range of triplet nucleotides and allow zinc fingers to join at the target of interest (Figure 2). TALENs can target any sequence because they have non-specific DNA-cleaving nuclease at the DNA-binding domain. TALENs are often preferred over ZFNs as they are easy to design and use, exhibit higher specificity towards target and show less off-target cleavages. ZFN-mediated targeted transgene integration was achieved in maize genome leading to stacking of herbicide resistance traits. Genome editing targeting SWEET genes encoding

sugar transporters have been performed for disease resistance in cassava, cotton and rice (Zhou et al. 2015; Cox et al. 2017). Another customized *dTALE* gene activated the recessive resistance allele *xa13* of susceptibility gene *Os8N3* (a member of SWEET gene family) and conferred resistant to *Xoo* susceptible rice cultivar IRBB13 (Li et al. 2013b).

ZFNs and TALENs consist of a specific DNA-binding sequence and a FokI nuclease domain that is functional only as a dimer. The DNA binding modules in each pair of ZFN and TALEN are required to be designed to provide the target specificity so that nuclease is able to dimerize and introduce double-stranded DNA breaks at the required site. The target-specific designing is difficult and expensive (Wada et al. 2020). The use of homo-dimeric FokI domains in ZFNs could lead to appearance of undesirable dimeric species. This has been overcome by designing hetero-dimeric FokI domains which prevents formation of homodimers and enhances the specificity of cleavage (Kaur et al. 2022).

### 2.2.2 Clustered regularly interspaced short palindromic repeat (CRISPR)-Cas9

CRISPR-Cas9 is a popular genome-editing tool involving an RNA-guided endonuclease Cas9 (Figure 3). The 366 bp deletion in *dst* mutants down-regulated the genes involved in development of stomata, thus the mutants exhibited improved tolerance against drought and salt stress via reduced stomatal density (Santosh Kumar et al. 2020). The native maize *GOS2* promoter was used to replace the native *ARGOS8* promoter using CRISPR-Cas, which resulted in the increased levels of *ARGOS8* transcripts conferring drought tolerance (Shi et al. 2017a). In tomato, *SIMAPK3* gene which contains TEY motifs (refers to a conserved Thr-Glu-Thy motif) that regulate abiotic stress responses, was edited through this technology to develop drought tolerance (Wang et al. 2017b). In cotton, the genes *GhRDL1* and *GhPIN1-3* were edited by the CRISPR-Cas technique to overcome drought stress (Dass et al. 2017).

*APL* and *APS* genes encoding the large and small subunits of ADP-glucose pyrophosphorylase (AGPase) enzyme involved in starch biosynthesis, show differential responses to abiotic and biotic stresses in banana (Miao et al. 2017). Single and double mutants of *APL* and *APS* both genes were created in rice using CRISPR-Cas9 system and the two genes were implicated in alteration of phosphorus (P) homeostasis and Pi starvation signaling (Meng et al. 2020).

The application of CRISPR in plants has several inherent limitations, such as the need for detailed information and accessibility of the plant genome sequence. Without this information, it is difficult to find potential editing targets, create the complementary guide RNA (gRNA) sequences required to guide Cas nucleases to the target site, or evaluate gRNAs' off-target activity (Venezia and Creasey Krainer 2021). CRISPR/Cas is likely to cause the off-target mutations, which in certain cases could raise concerns. Further there could be possibilities of on-target unintentional mutations like insertions, deletions or inversions, which have not been well researched in plants as of now (Hahn and Nekrasov 2019).

### 2.2.3 Homing endonucleases or mega-nucleases

Mega-nucleases are classified based on their sequence and motifs into five families which are LAGLIDADG (most widely used), GIY-YIG, His-Cyc box, HNH and PD-(D/E)XK (Silva et al. 2011). One of the successful applications of mega-nuclease techniques has been seen in gene stacking in cotton where precision-targeted transgene insertion was achieved at a cotton elite locus. Targeted nucleic acid cleavage by nucleases combined with tailored specificities and homologous recombination mediated double-strand break (DSB) repair by naturally occurring DNA repair pathways makes this conceivable. In cotton, two transgenes for herbicide tolerance i.e. *hppd* of *Pseudomonas fluorescens* and *epsps* gene of maize, were introduced near a locus consisting of insect resistance transgene, to generate a gene-stack (D'Halluin et al. 2013). Meganucleases are also employed to generate tar-

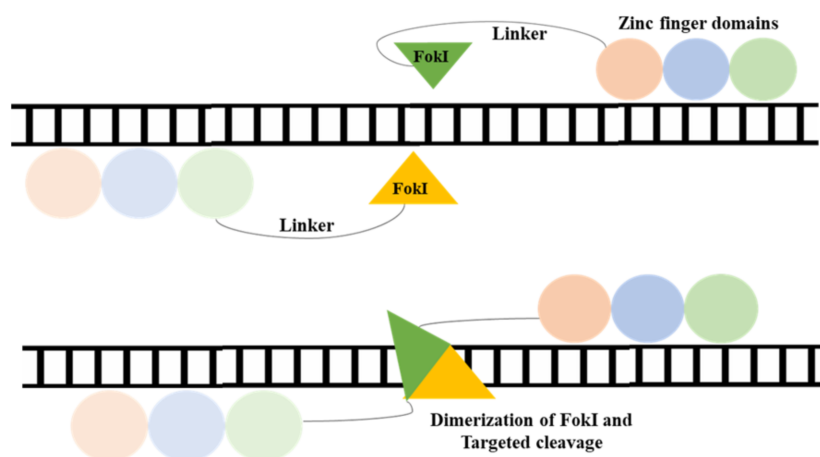


FIGURE 2 Genome editing using zinc-finger nucleases (ZFN).



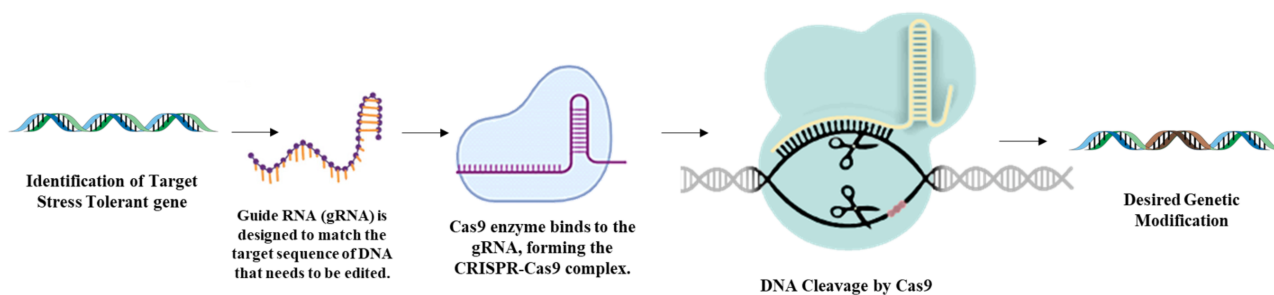


FIGURE 3 Genome editing using CRISPR-Cas9.

geted knockout-mutations. For example, two maize loci, *ms 26* and *ligules-1* were mutagenized using CreI-based meganuclease from *C. reinhardtii* to induce male sterility (Djukanovic et al. 2013). Although mega-nucleases are useful tools for deletion and insertion at the targeted loci with good frequency, because of certain limitation their use in genome engineering is not as prevalent as ZFNs or TALENs. Some of the drawbacks associated with them are first the overlap of DNA binding and cleavage domains cause decrease in rate of catalysis of the enzyme; second, unlike ZFNs and TALENs, mega-nucleases lack the modular DNA-binding domain architecture found in ZFNs and TALENs; and third, sequence degeneracy in mega-nucleases can occasionally occur, which increases

the possibility of off-target binding (Stoddard 2011).

### 2.3. Improvement of crops against abiotic-stress through genomics approaches

The crop responses against different abiotic stresses are highly complex and multidimensional and several regulatory networks involving genes, transcription factors and proteins interact together to impart tolerance to plant against the stress (Shourie et al. 2014). Often the gene-trait relationships are not direct and very difficult to predict, therefore genomics offer several advantages for high throughput and accurate screening that could be utilized in crop improvement (Figure 4).

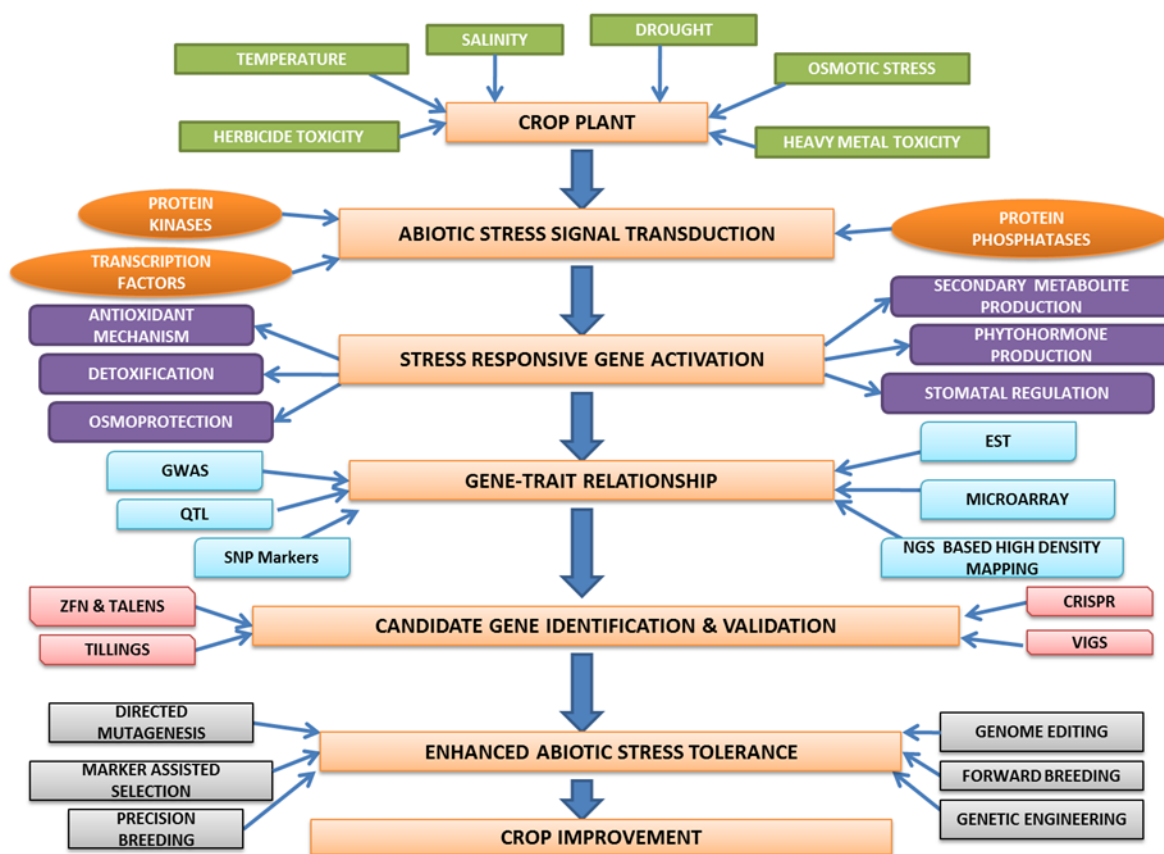


FIGURE 4 Schematic for improvement of abiotic stress tolerance in crop plants through genomics.

Applications of genomics in enhancing tolerance of crops against drought, temperature, salinity, osmotic stress and heavy metal toxicity through identification of candidate gene and its subsequent silencing, editing or engineering are summarized in Table 1.

### 2.3.1 Drought stress

One of the main factors reducing agricultural yields globally is drought. There are several other environmental stress factors that accompany drought such as high temperature, low soil and air moisture, poor soil health and lack of nutrient availability to plants. Thus, plants exposed to drought experience a multitude of stresses which causes a series of overlapping molecular and physiological events to occur. Identification of genes involved in

drought stress signaling is imperative for crop improvement. GWAS was performed on *Aegilops tauschii*, a close relative of wheat *Triticum aestivum* L., using around 7185 SNP markers to understand and identify QTLs functional against drought stress (Qin et al. 2016). In a study, genes *OMTN3*, *OMTN4*, *OMTN2*, and *OMTN6* were targeted in rice as their overexpression resulted into a negative effect on drought resistance (Zhang et al. 2017a).

Role of *SINPR1* I was studied in regulating stress response through generation of *sinpr1* mutants using the CRISPR/Cas9 system in tomato and the reduced tolerance of mutants towards drought indicated that *SINPR1* was involved in the expression of drought related genes like *SIGST*, *SIDHN*, and *SIDREB* (Li et al. 2019). CRISPR/Cas9 editing was applied in wheat on drought responsive genes such as TaDREB2 and TaERF3, which

**TABLE 1** Applications of genomics for crop improvement against abiotic stress.

No	Abiotic Stress	Plant	Identification of Target Gene/Loci	Genomics Tool/Technology implemented	References
1	Drought stress	<i>Arabidopsis thaliana</i>	<i>miR169a</i> as negative factor for drought tolerance	CRISPR	(Zhao et al. 2016)
2		<i>Solanum piminellifolium</i>	SpMPK2, SpMPK1 & SpMPK3	VIGS	(Li et al. 2013a)
		<i>Brassica napus</i>	<i>BnaC09.RPS6</i> , <i>BnaC09.MATE</i> , <i>BnaA10.PPD5</i> and <i>BnaC09.Histone</i>	GWAS	(Shahzad et al. 2021)
3		<i>Vicia faba</i> L.	29 SNPs identified	GWAS	(Gutiérrez et al. 2023)
4		<i>Triticum aestivum</i> L.	94 SNPs identified	GWAS	(Reddy et al. 2023)
5		<i>Solanum lycopersicum</i> L. cv. Ailsa Craig	Silencing of <i>WRKY81</i>	VIGS	(Ahammed et al. 2020)
6	Temperature Stress	<i>Oryza sativa</i>	<i>SPKST</i> for spikelet sterility	GWAS	(Lafarge et al. 2017)
7			<i>OsAnn3</i> for cold tolerance	CRISPR/Cas9	(Shen et al. 2017)
8		<i>Sorghum bicolor</i>	<i>Sb06g025040</i> for temperature-resistant	GWAS	(Chopra et al. 2017)
9		<i>Solanum lycopersicum</i>	<i>BZR1</i> for heat stress response	CRISPR/Cas9	(Yin et al. 2018)
10	Salinity Stress	<i>Triticum aestivum</i> L.	Five genes encoding kinase family protein, transmembrane protein ligase-like protein, E3 ubiquitin-protein, and ligase-like protein	GWAS	(Quan et al. 2021)
11		<i>Zea mays</i>	<i>ZmHKT1</i> - a major salt-tolerant QTL	CRISPR/Cas9	(Zhang et al. 2017b)
12		<i>Oryza sativa</i>	<i>OTS1</i> - a salt stress regulator	CRISPR/Cas9	(Zhang et al. 2019)
13			<i>OsNAC041</i>	CRISPR/Cas9	(Bo et al. 2019)
14			<i>SIT1</i>	CRISPR/Cas9	(Li et al. 2014)
15	Heavy metal Stress	<i>Oryza sativa</i>	<i>qSdw12</i> , <i>qSdw3b</i> , <i>qSdw3a</i> and <i>qSFe5</i> / <i>qSZn5</i> -QTLs linked to Zn and Fe toxicity	GWAS	(Zhang et al. 2017c)
16		<i>Brassica napus</i>	<i>BCATs</i> , <i>TBR</i> , <i>GSTUs</i> <i>UBP13</i> , and <i>HIPP01</i> - QTLs linked to Pb tolerance	GWAS	(Zhang et al. 2020)
17	Drought and Salinity Stress	<i>Brassica juncea</i>	Four SRAP and two EST-SSRs	EST	(Saini et al. 2019)
18	Drought and Heat Stress	<i>Oryza sativa</i>	<i>OsNAC006</i>	CRISPR/ Cas9	(Wang et al. 2020)
19	Salinity and Heavy metal Stress	Sugarcane	<i>ScGluD2</i>	CRISPR/Cas9	(Su et al. 2016)

improved drought tolerance (Kim et al. 2018). With the help of CRISPR-Cas9 editing of salt and drought tolerance gene *OsDST*, indica mega rice cultivar MTU1010 *dst* mutant was generated having broader leaves with less stomatal density that could enhance the leaf water retention to tolerate drought condition (Ganie et al. 2021; Santosh Kumar et al. 2020).

### 2.3.2 Temperature stress

Climate change caused by rising temperatures is a global concern as it has resulted into poorer crop yields. Impacts of temperature stress can be observed in different ways such as loss of chlorophyll, low rate of photosynthesis, slower pace and low rate of germination. Several genome editing techniques have been applied to plants to achieve temperature tolerance and improve plant performance under non-ambient temperature. Identification of temperature-tolerant genes using GWAS was applied in Sorghum seedlings and 30 SNPs were found to be associated with cold stress, while 12 SNPs with thermal stress (Chopra et al. 2017). Another GWAS was employed on 167 rice varieties to evaluate the influence of temperature on plants during anthesis by studying the secondary traits like panicle micro-climate, fertilization process and spikelet sterility (SPKST), and it was concluded that genes at SPKST locus and its nearby loci were associated with many temperature stress related traits (Lafarge et al. 2017). The role of the tomato C-repeat binding factor 1 (*CBF1*) gene in conferring cold stress tolerance in tomato was discovered by mutagenesis using CRISPR/Cas9, where the *cbf1* mutant tomato plants were shown to be sensitive to stress, accumulating more indole acetic acid and experiencing increased electrolyte leakage (Li et al. 2018). VIGS technology was applied to potatoes for transformation of five genes - *StWTF*, *StSSH2*, *StFLTP*, *StBHP*, *StUGT* and *StFLTP*, that were involved in heat stress and the transformed plants were successfully made resistant to heat stress (Tomar et al. 2021).

### 2.3.3 Salinity stress

Salinity significantly affects the agricultural productivity, particularly in semi-arid and arid regions. Exposure of crop plants to excessive salinity leads to an increased influx of  $\text{Na}^+$  and  $\text{Cl}^-$  ions in tissues of plants, which further induces ROS generation and significant ion disruption in cells. Several genes have been validated to improve salt-stress resistance using CRISPR/Cas editing. In rice, the multifunctional gene *OsBBS1* was found to be involved in salt stress sensitivity and early leaf senescence and the *OsMIR528* gene was reported to be a positive regulator of salt stress (Ganie et al. 2021). Through CRISPR/Cas9 technology, a rice gene called *Osmir535* that encodes microRNA was modified. This gene controls the expression of abiotic stress-responsive genes at the post-transcriptional level. In comparison to the control plants, the knockout *osmir535* rice mutant plants performed better when ex-

posed to salt and drought stress (Yue et al. 2020). Salt-tolerant genes across the crop diversity were identified through GWAS in many crops. In a study, 6,361,920 SNPs from 478 various rice varieties were investigated for seven seed germination-related parameters under control and salt-stress situations and the connection between traits and SNPs was identified using a mixed linear model (Shi et al. 2017b).

Comparative analysis of ESTs related to various stresses like salinity and drought was conducted in seedlings of *Lablab purpureus*. ESTs were characterized for their putative functions and relative fold expression was estimated. It was proposed that different gene sets, differentially express under the two stresses which could lead to prediction of functions of specific ESTs (Kokila and Devaraj 2021). In cotton, miRNA ghr-miR414c and iron superoxide dismutase gene *GhFSD1* collaborate in response to salt-stress, resulting in enhancement of *GhFSD1* expression. Silencing *GhFSD1* in cotton resulted in an abnormally hypersensitive phenotype to salt stress, whereas overexpression of miR414c reduced *GhFSD1* expression while increasing tolerance to salinity stress, provided a phenotype that was very similar to *GhFSD1*-silenced cotton (Wang et al. 2019).

### 2.3.4 Osmotic stress

Plants are put under osmotic stress due to several environmental conditions such as cold or chilling, excessive salinity, and drought. The soil's excess ions cause osmotic stress, which reduces the osmotic potential and prevents the uptake of water and nutrients. Functional redundancy between *HSA6a* and *HSA6b* and their role in abiotic stress tolerance in *Arabidopsis thaliana* was investigated. The two genes, edited using CRISPR/Cas9, were suggested to offer osmotic stress tolerance by regulating the reactive oxygen species (ROS) homeostasis in plants (Wenjing et al. 2020). The involvement of the nuclear protein coilin in the stress resistance mechanisms of *Solanum tuberosum* cultivar Chicago was investigated using CRISPR-Cas9 technology. Editing of one allele of the gene not only increased tolerance of potato against salinity and osmotic stress, but also enhanced the resistance against potato virus Y (Makhotenko et al. 2019).

CRISPR/Cas9 technology was used as an effective tool for functional reverse genetics to improve salinity and osmotic tolerance in tomato by antisense downregulation and loss of function of Auxin Response Factors ARF4 (Bouzroud et al. 2020). A group of 56 phenotypic features were subjected to genome-wide association mapping in salt and osmotic stress tests on winter barley using 4885 gene-based SNP markers. The researchers found 28 quantitative trait loci (QTLs), of which 10 dealt with salinity stress and 20 with osmotic stress (Xue et al. 2019). Additionally, GWAS has been used to analyze 150,325 SNPs across 175 upland rice accessions (*Oryza sativa*) under investigation with and without drought exposure. The study discovered 50 genes, 30 of which were annotated, and 10

of which had previously been related to drought and/or abiotic stress tolerance (Pantalião et al. 2016).

### 2.3.5 Herbicide stress

Herbicides are widely used in agriculture worldwide, but many herbicides decrease germination and growth, affect plant reproduction and impact crop production. Herbicide tolerance has been successfully induced in crop plants using functional genomics. Genes encoding enzymes of amino acid pathways have been widely engineered using ZFNs, TALENs, and CRISPR-Cas to confer herbicide tolerance to plants. Many herbicides such as imidazolinones, pyrimidinylthio (or oxy)-benzoates, triazolopyrimidines, and sulfonylureas, impede the activity of the ALS (acetolactate synthase) gene. Thus, this gene was edited using the ZFN genome editing tool resulting in tolerance in tobacco for sulfonyl urea herbicides (Townsend et al. 2009). In corn, *ALS1*, *ALS2*, and *MoPAT* genes were successfully edited using CRISPR/Cas9 editing tool to achieve herbicide tolerance. The *ALS2* gene was edited utilizing single-stranded (ss) oligonucleotides or ds DNA vectors as repair templates, resulting in chlorsulfuron-resistant plants (Svitashev et al. 2015). Using the CRISPR/Cas9 editing tool, the precise editing of EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) genes, lead to the advancement of herbicide-resistant trait in flax (Sauer et al. 2016). Herbicides such as phenylpyrazoline (PPZ), aryloxyphenoxypropionate (APP), and cyclohexanedione (CHD) act on ACCase, a key enzyme in fatty acid production. In rice and wheat, this gene has been edited using CRISPR/Cas to achieve herbicide resistance (Zhang et al. 2019). In a study, genome-wide association mapping analysis was undertaken to utilize field-collected sorghum biomass panel (SBP) data to investigate the underlying tolerance mechanism, and a greenhouse test was constructed to confirm the field phenotypes. A total of 26 SNPs on chromosome 3 were identified as targets of protoporphyrinogen oxidase (PPO)-inhibiting herbicides (Adhikari et al. 2020).

### 2.3.6 Heavy metal stress

Heavy metals like Pb, Ni, Fe, Co, Hg, Cu, and As are highly toxic and are found in high concentrations in industrial waste and sewage. The metabolism, physiology and development, of plants can all be negatively impacted by excessive amounts of these metals, even though many of them are essential micronutrients that are involved in a variety of plant activities. Many genome-editing techniques have been applied to overcome heavy metal stress in crop plants and improve crop production. Using the CRISPR/Cas9 technique in rice, it was analyzed that transcription factors OsARM1, an R2R3 MYB are involved in the regulation of the response to As- stress and controlled the root-to-shoot translocation of As (Wang et al. 2017a). The molecular mechanisms underlying selenium (Se) resistance and Se hyperaccumulation in the Se hyperaccumulator *Stanleya pinnata* were investigated by con-

trasting it to the Se-resistant species *S. albescens* using a combination of structural, physiological, biochemical, and genomic techniques (Freeman et al. 2010). Cas 9 assisted knockout of target gene *OsNramp5* in *Oryza sativa* led to reduced accumulation of cadmium in plants (Tang et al. 2017).

### 2.4. Transgenics for abiotic stress tolerance in crops

Transgenic technology has immense potential for developing stress tolerant plants and there are many studies in which genetic transformation have been accomplished for crop improvement. Transgenic rice was developed expressing *AtDREB1A* transcription factor using *Agrobacterium*-mediated transformation which successfully imparted resistance against drought stress without compromising other important agronomic traits (Ravikumar et al. 2014). Transgenic rice was made salt tolerant by overexpressing the stress-inducible *SNAC1* gene (Nakashima et al. 2007) and *OsWRKY45-2* gene for higher ABA sensitivity (Ye et al. 2009). Transgenic *Arabidopsis* plants expressing *AtCBF3* demonstrated freezing tolerance, while *AtCBF4* overexpression provided freezing and dehydration tolerance. Similarly, transgenic *Arabidopsis* that expressed *GmDREB2* under both constitutive and stress inducible promoters demonstrated drought and salt stress tolerance (Chen et al. 2007). Transgenic tobacco was developed that expressed *LeNCED1* gene from tomato that encodes a 9-cis-epoxycarotenoid dioxygenase implicated in ABA biosynthesis; the transformants showed enhanced ABA biosynthesis, increased guttation reduced stomatal conductance and increased seed dormancy. Transgenic potato cv. IPB CP1 was made through genetic transformation incorporating MmCuZn SOD transgene isolated from *Melastoma malabathricum* L., encoding superoxide dismutase, which conferred resistance against multiple abiotic stresses such as salinity, aluminium and drought stress (Musawira et al. 2022). Proline biosynthesis genes have been transferred to economically important crop plants, resulting in increased proline accumulation and tolerance to environmental stresses. Inducing constitutive expression of these genes has shown pleiotropic effects (Kavi Kishor and Sreenivasulu 2014).

## 3. Conclusions

Genomics have indeed contributed immensely not only to identify but also to modify abiotic stress-related genes in crop plants, thereby alleviating the plants from stress. Traditional methods of crop improvement against environmental stresses such as variant selection, induced mutation selection and hybridization had very limited success rate, whereas genomics technologies are far better and efficient in many ways (Table 2). The integrated nature of abiotic stress signaling pathways results into high degree of variability in response traits and enhances the complexity of gene functions. Despite remarkable advancements in genomics, major information gaps regarding the molecular regulation of key biological processes still ex-



**TABLE 2** A comparison of traditional and modern technologies used for crop improvement against abiotic stress

Aspect	Traditional Approaches	Modern Technologies	References
Technologies Used	Plant Domestication Pure line and Mass selection Pedigree Breeding Ideotype Breeding Hybrid Breeding	Molecular Breeding Genomic Selection CRISPR-Cas9 & Editing High-Throughput Phenotyping Transcriptomics and Proteomics	(Gupta and Shaw 2020; Banerjee et al. 2024)
Data Accuracy	Subjective and liable to human error	Objective, precise, and reproducible data from automated systems	(Chauhan et al. 2022; Jangra et al. 2021)
Time Requirement	Time-consuming, often requires weeks or months to assess stress impacts	Rapid, high-throughput assessments in real time	(Muhammad Aslam et al. 2021; Zhang et al. 2024)
Cost	Relatively low, but with high labor costs due to manual processes	High initial cost due to equipment, but reduced long-term labor costs	(Akinyi et al. 2022; Li et al. 2021)
Sensitivity	Low sensitivity, cannot detect subtle molecular changes	High sensitivity to detect early and minor molecular changes related to stress	(Murmu et al. 2024; Chaturvedi et al. 2024)
Scalability	Limited, difficult to scale due to labor-intensive nature	Highly scalable using automated systems and robotics	(Lowry et al. 2024; Poorter et al. 2023)
Complexity of stress tolerance traits	Limited by the complexity of stress tolerance traits, low genetic variance of yield components under stress conditions	High complexity of stress tolerance traits, high genetic variance of yield components under stress conditions	(Raza et al. 2020)
Non-invasive Techniques	Generally invasive (e.g., sampling plant tissue)	Non-invasive techniques (e.g., imaging technologies, spectrometry, and fluorescence)	(Ye et al. 2023; Rico-Cambren et al. 2023)
Skill Requirement	Requires experienced personnel for proper visual assessment	Requires trained personnel for operating sophisticated equipment	(Singh et al. 2020; Zhang et al. 2024)
Environmental Impact	Can disturb the plant or environment due to manual intervention	Minimal environmental disturbance through remote sensing and imaging	(El-Ramady et al. 2019; Al-Tamimi et al. 2022)

ist which impede the development of abiotic stress tolerant plants. There is a huge need to comprehend the composite connections between genes related to different plant traits under distinct environmental situations. Precise gene functions often cannot be determined using a single approach; to fully utilize genomics, a multidisciplinary approach and comprehensive understanding of the molecular and other biological processes underlying various phenotypes are required. Currently, abiotic stress-responsive genes are identified using a sequence similarity approach to identify abiotic stress-responsive genes in model crops. Advances in systems biology have resulted in a high-speed and cost-effective method for creating a massive number of sequences, which facilitates the identification of genes that regulate stress tolerance. Various high-throughput techniques like next generation sequencing generate massive amount of data that is useful to decipher the molecular mechanisms underlying stress tolerance in plants. Novel bioinformatics tools and software with higher resolving power and other technological advancements are instrumental in rapid estimation and comparison of gene functions. A multidisciplinary integrated strategy permits the functional characterization of plant genes to fully exploit the available genetic infor-

mation. Thus, the progress in developing stress-resilient crop plants is dependent upon the development of high-throughput technologies and the integration of multidisciplinary methodologies such as genomics, transcriptomics, proteomics, epigenomics, and bioinformatics.

## Acknowledgments

The authors acknowledge the support provided by the Department of Biotechnology, School of Engineering and Technology, Manav Rachna International Institute of Research and Studies, Faridabad in accomplishment of this manuscript.

## Authors' contributions

All the authors substantially contributed to the study: the conception and design were done by AS; literature survey, analysis and preparation of first draft of the manuscript were done by PS and NM; revision and redrafting were done by PS, editing, finalization and submission of manuscript were done by AS.

## Competing interests

The authors have no relevant financial or non-financial interests to disclose.

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