

#### **Review Article**

#### ROLE OF BIOTECHNOLOGY IN ABIOTIC STRESS TOLERANCE IN PLANTS

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# (Received, 30<sup>th</sup> May 2024, Revised 18<sup>th</sup> September 2024, Published 29<sup>th</sup> September 2024)

Abstract As population grows, abiotic stresses reduce plant growth and agricultural output, affecting global food security. Heat, cold, drought, salinity, and heavy metals affect crops. Heat stress from climate change disrupts cellular metabolism and lowers productivity. Tropical and subtropical crops suffer physiologically from cold stress. Climate variability affects drought, reducing crop output and germination. Salinity stunts growth and causes nutritional imbalances in big agricultural areas. Heavy metal buildup in soils threatens plant health. To overcome these problems, plants use osmotic adjustment, antioxidant defenses, and stress signaling pathways. CRISPR/Cas9 and other genetic engineering and molecular breeding methods may improve crop stress tolerance. Using omics technologies (genomics, transcriptomic, proteomics, and metabolomics) in breeding programs helps us understand stress tolerance processes and generate resilient crop types. This research is crucial for sustainable agriculture and global food security.

Keywords: abiotic stresses; drought stress; genomics; transcriptomic; CRISPR/Cas9

#### Introduction

Abiotic stress is any environmental condition that reduces plant growth and productivity. Rapid worldwide growing populations challenged food security; thus, plant scientists and agronomists have concentrated on abiotic stress for 50 years (Hasanuzzaman et al., 2019). Rising food demand necessitate agricultural production reductions, previously overlooked. Boyer believed environmental issues might limit crop productivity by 70% in the early 1980s 1. Since then, many papers have highlighted abiotic stress-induced crop losses 23. Some of these pressures permanently damage farmed areas, affecting crop quality and yield (Fischer and Connor, 2018). Although measuring the effects of abiotic pressures on agricultural land is difficult, estimated that 96.5% of global rural land is affected. Heat, cold, drought, and salinity constantly threaten agricultural land and crop productivity (El Sabagh et al., 2020).

#### **Heat Stress**

Global warming is an serious concern for plant scientists due to greenhouse gas emissions and human activities (Xi-Liu and Qing-Xian, 2018). Greenhouse gases including CO2, methane, nitrous oxide, and chlorofluorocarbons are expected to gradually raise global ambient temperature in global circulation models. The IPCC predicts a 0.3°C rise in global average temperature every decade 1. A 1°C global temperature rise by 2025 presents enormous hurdles, especially given the requirement to feed nine billion people by 2050 (Loboguerrero et al., Plants experience heat stress when 2019). temperatures exceed normal ranges, which affects cellular metabolism. According to Wahid et al., heat stress occurs when temperatures reach a threshold, damaging plant health permanently. While brief temperature spikes (10-15°C) are called 'heat shock,' heat stress involves complicated mechanisms relating to intensity, duration, and temperature increase above acceptable ranges (Abbas et al., 2024; ALI, 2022; Ul Hassan et al., 2021). Heat stress relies on daytime and nighttime heat exposure in certain climates. Heat disrupts cellular balance, slowing development and killing plants. This stress affects world crop production greatly. Rising temperatures may indirectly influence agriculture and livestock by changing rice, wheat, and corn growing seasons and geographical distribution (Malhi et al., 2021). Porter suggested that warmer temperatures could extend pasture grasses' growing season. Most cereals have a small temperature tolerance range, and exceeding it during floral transition might impair seed production and crop yields.

#### **Cold Stress**

Many plant species' growing seasons are limited by cold stress 1. Since tropical or subtropical crops



including maize, rice, tomatoes, bananas, mangoes, and papayas are vulnerable to chilling, understanding its effects on crops is critical. Depending on degree and duration, cold stress can cause freezing or chilling. Seedlings are especially susceptible to cold stress (Bhattacharya, 2022). Surface lesions, dehydration, browning processes induced senescence, shelf life reduction, and ethylene generation are common. Chilling stress increases cellular Ca+ levels, accumulates reactive oxygen species (ROS) 2, affects membrane fluidity due to fatty acid unsaturation, and modifies cell membrane lipid-to-protein ratios (Cheong et al., 2022). Dehydration can also result from low temperatures impairing water intake. However, freezing damages membranes by dehydrating and icing intracellular spaces. Ice production damages cells and tissues and denaturates proteins and precipitates solutes. Both freezing and chilling stress produce ROS, upsetting cellular redox equilibrium. As with other abiotic stresses, knowing these pathways is crucial to producing plant resistance methods (Souza et al., 2017).

### **Drought Stress**

Drought is a major crop productivity inhibitor. Climate change may increase water deficits due to rainfall variability, increased water demand, and diminishing supply (AHMAD et al., 2023; Arshad et al., 2024; Guo and Shen, 2016; Rasheed and Malik, 2022). This will harm all organisms, but it will hurt plants without mobile structures that may migrate when food and water are low, hurting agricultural production. Due to rainfall patterns, soil water holding capacity, and evapotranspiration, drought stress damage is hard to assess(Tramblay et al., 2020). Well-studied drought stress impacts on crop physiology and development. A global 1980-2015 study found drought reduced wheat and maize production by 21% and 40%. Poor germination, seedling growth, food availability, photosynthesis, leaf number and size, fresh and dry weight, etc. are caused by drought. Dry weather can also make pearl millet (Pennisetum glaucum L.) sterile by disrupting nutrition transfer to the growing ear. Coffee production may decline 80% due to dehydration. Abrahão and Costa (2018) showed that a 45-day Parana, Brazil, drought reduced soybean harvest by 80% in 2008/2009. Drought causes stomatal closure, decreased transpiration, etc. Plants also alter gene expression to resist drought (BASHIR et al., 2023; Cao et al., 2017; Haider et al., 2023).

#### Salt Stress

Water and soil salinity can impair crop productivity in dry or semi-arid regions. Salinity affects 800 million hectares worldwide, according to 2011 research (Junaid and Gokce, 2024; Kumar and Sharma, 2020). The same study anticipated 17 million hectares of salinity-affected farmland by

2050. Salinity damages plants due to soil low osmotic potential, nutritional imbalance, ion effect, or a combination. These variables adversely affect plant growth and development. Growth slows, leaves decrease, and roots shorten (Lambers et al., 2019; Mushtaq et al., 2024; Rasheed et al., 2024; REHMAN et al., 2020). Osmotic effects of salinity affect leaf color, root/shoot ratio, and maturity. Salinity, like other abiotic stressors, impacts plants differently depending on duration and severity. Salinity increases cytotoxic ion levels, slows metabolic activity, and causes early senescence and cell death (Isayenkov and Maathuis, 2019). It also closes stomata and inhibits shoot-specific cell expansion. Plants withstand salinity by physiomolecular mechanisms, osmotic and ionic tolerance, and tissue tolerance (Khan et al., 2019). Osmotic tolerance reduces stomatal conductance during fast root-shoot transmission to preserve water. Activating several signaling cascades restricts net Na+ inflow and translocation for ionic tolerance (Fatima et al., 2023; Ketehouli et al., 2019). Translocating toxic ions to the vacuole prevents cytoplasmic metabolic effects and develops tissue-specific tolerance. The salt overload sensitive (SOS) pathway drives detrimental ions to the vacuole via many SOS genes (Yuan et al., 2019).

### **Heavy Metal Stress**

Heavy metals (HMs) like Mn, Cu, Ni, Co, Cd, Fe, Zn, and Hg have accumulated in soils due to fertilizer application, incorrect industrial waste disposal, unregulated sewage discharge, and casual automotive effluent disposal. Metals leak into groundwater or collect on soil. HM stress is nonbiodegradable and cannot naturally disappear from the environment (Chowdhury and Rahman, 2024). Enzymes are inactivated or denaturated by HM stress. Reports show that HMs impair biomolecule substitution processes with critical metallic ions, impacting membrane integrity, photosynthetic capabilities, and respiration. Furthermore, HMs cause oxidative stress by increasing H2O2, O-2, and OH radical generation (Al Mahmud et al., 2019). HM accumulation, especially in roots (owing to Casparian strips or root cell walls), inhibits plant physio-morphological functions and reduces agricultural output (Wahab et al., 2022).

#### Mechanisms of Abiotic Stress Tolerance Abiotic Stress Resilience in Plants

Drought, salinity, extreme temperatures, and heavy metals reduce plant growth and productivity. Plants have developed complex mechanisms to survive and thrive in harsh conditions. These mechanisms must be understood to develop biotechnological plant resilience strategies.

#### Adjusting Osmotically

• Drought and salinity cause plants to adjust osmotically.

- To maintain cellular turgor and structure, they store proline, glycine betaine, and sugars.
- Osmolytes remove ROS and stabilize membranes and proteins (Khan et al., 2023).
- Plants improve water uptake and retention by changing root architecture and expressing aquaporins.

### Antioxidant Defences:

- Plants use antioxidant defenses to combat ROSinduced oxidative stress.
- Antioxidants include ascorbate, glutathione, catalase, peroxidases, and superoxide dismutase.
- ROS are eliminated by these antioxidants, protecting cells.
- ABA and other stress-induced signaling pathways regulate antioxidant enzyme expression.

### **Stress Signaling**

- Abiotic stress signals trigger complex plant responses.
- Ca2+, ROS, and phytohormones like ABA, ethylene, and jasmonic acid are crucial.
- These molecules activate stress-responsive genes via protein kinases and transcription factors.
- In drought, ABA accumulates and activates genes involved in stomatal closure (Ha et al., 2018), osmoprotectant synthesis, and stress-related protein production.

# Molecular Chaperones and HSPs

- These proteins prevent stress-induced protein misfolding.
- For cellular protein balance, HSPs fold, stabilize, and degrade proteins.
- To protect vital cellular machinery, HSPs are upregulated in response to heat, cold, or other stresses.

# Traditional Breeding vs. Biotechnological Approaches

Plant breeding programs improve crop productivity and nutrition by improving resilience to biotic or abiotic stress and quality traits. Agrochemical use is restricted, and insects, fungi, bacteria, viruses, and nematodes are becoming more virulent (Deka et al., 2021). Due to climate change, plant breeders must select water- and thermal-resistant genotypes to adapt to changing rainfall patterns and rising temperatures.

Raina et al. (2020) developed genetically improved crop plants with higher yield, quality, and stress resilience through mutagenesis and crossings. Since germplasm introgression was impossible in the 1920s, radiation or chemicals have been used to mutate. Genetic engineering and biotechnologies have given geneticists and breeders new tools to create GMOs in the last century (Ricroch et al., 2022). This approach has improved disease resistance, abiotic stress resistance, nutritionally improved GMOs, and GMO limitations and concerns.

Plant breeders can now isolate and transfer genes from sexually incompatible plant species and other organisms to crops (Muñoz-Sanz et al., 2020). While GM crops covered 191.7 million hectares in 2018 with great benefits, public health and environmental concerns remain. Many citizens oppose transgene insertion in crop genomes, which contain selectable markers like antibiotic resistance. Many methods have been developed to overcome GM crop limitations, including genome editing (Kamthan et al., 2016).

NGS technologies have advanced crop genomics and published many reference crop genomes in the last years. High-throughput re-sequencing of 15 hundreds of genotypes revealed domesticated and wild plant allele diversity (Razzaq et al., 2021). Genome structure data helped understand plant history. identify domestication genes for agrochemical traits and gene functions, and develop NPBTs to overcome major GMO regulatory evaluation processes and public concerns. NPBTs transfer one gene, like cisgenesis and genome editing.

Potato, apple, grapevine, melon, wheat, barley, poplar, rice, and strawberry were GMOs. Many studies focused on pathogen resistance, but some on quality trait improvement. Haverkort et al. (2016) created four cisgenic late blight (Phytophthora infestans)-resistant potato varieties without markers by transferring one to three resistance genes. Inserting the apple scab (Venturia inaequalis) resistance gene Rvi6 into the susceptible cultivar 'Gala' created cisgenic apple varieties (Švara et al., 2024). Recombinase R was induced with dexamethasone to remove the selectable marker gene. Three years after field testing, cisgenic plants were resistant .Kleidon et al. (2020) found that the same recombinase system can remove the green fluorescent protein reporter gene in bananas.

In plant immune responses, pathogen virulence, and plant-microbe interactions, sRNAs and RNAi regulate gene expression. After RNAi machinery was discovered, plant protection efforts increased (Liu et al., 2021). RNAi down-regulates target RNA post-transcriptionally for endogenous transcript regulation and viral defense, a conserved plant regulatory strategy. RNase III DICER-LIKE endonucleases cleave dsRNA into 21-24 nt siRNAs after cell production (Fukudome and Fukuhara, 2017). One siRNA strand forms RISCs with ARGONAUTE (AGOs) proteins after cleavage (Willkomm et al., 2017). RISCs cleave or repress mRNA in post-transcriptional gene silencing (PTGS) using sequenced-based complementarity (Figure 4). Repressive chromatin marks in target genomic DNA

<sup>[</sup>Citation: Abbas, A., Riaz, S., Waleed, R., Ali, Q. (2024). Role of biotechnology in abiotic stress tolerance in plants. *Biol. Clin. Sci. Res. J.*, **2024**: *1142*. doi: https://doi.org/10.54112/bcsrj.v2024i1.1142]

sequences can also cause siRNA-induced TGS. Plant host-pathogen interactions involve siRNAs. Viral cells directly produce siRNAs by processing viral genome dsRNA molecules (Santos et al., 2019). Evidence suggests that siRNAs produced in one cell can spread via plasmodesmata to surrounding cells and distal plant parts via the vascular system, causing systemic silencing. Studies are ongoing on SiRNA short- and long-distance transport to the plant (Kehr et al., 2022).

**Genetic Engineering for Abiotic Stress Tolerance** Molecular breeding and genetic engineering allow quick genome-edited crop output. Genetic engineering may help plants overcome reproductive difficulties (Parmar et al., 2017). Transgenic breeding has increased crop productivity due to its shortened breeding period. Despite their large and complicated genomes, Arabidopsis, cucumber, tomato, and rice have genome sequences. The development of new molecular breeding markers is made easier by cost-effective sequencing of novel crops and agricultural kinds using 'next-generation sequencing' technology (Khan et al., 2017).

Plant physiology advances our understanding of drought-tolerant systems and characteristics. Molecular and genomic selection methods identify QTLs and genes linked with these features (Yadav and Sharma, 2016). To develop drought-resistant transgenic crops, scientists must identify candidate genes for plant tolerance to various abiotic stresses. QTL mapping identifies drought tolerance genes that can be introduced into suitable crops via genetic engineering and marker-assisted selection (Kiriga et al., 2016).

Plant engineering methods for abiotic stress tolerance have been studied for 20 years. Research on gene expression in plant defense signaling networks found abiotic stress-tolerant proteins (Aftab and Roychoudhury, 2021). Recent genetic transformation has improved plant stress tolerance.

### **Transgenic Plants and Stress Resistance**

Abiotic stressors have been mitigated by plant defenses. Breeding or genetically engineering stresstolerant plants requires understanding stress response systems and key regulators (Marwein et al., 2019). Recent advances in plant genomes and omics have illuminated stress signaling pathways and abiotic stress response components.

# The general signaling route for any abiotic stress has several main steps:

- i.Signal Perception: Plants receive stress signals directly or indirectly from other organs.
- ii.Signal Transduction: Stress signals activate stresstype-specific genes.
- iii. These genes produce proteins that help plants withstand stress.
- iv.Complex gene networks control plant metabolism, cell development, division, and differentiation.
- v.Many stress transduction pathways rely on protein kinases and phosphatases for signal relay. Terminal transducers (TFs) interact with gene promoter ciselements to directly influence downstream gene expression. Researchers have identified and characterized TFs implicated in plant abiotic stress responses, including AP2/EREBP, MYB, WRKY, NAC, and bZIP.

### CRISPR/Cas9 and Genome Editing

The resilient CRISPR-Cas9 genome engineering technique has been used in bacteria, mammals, and plants. Effective gene editing makes it useful for crop development and offers many research opportunities (Arora and Narula, 2017). CRISPR-Cas9 works on Arabidopsis, Oryza, Nicotiana, wheat, maize, tomato, soybean, and sorghum.

Several components are needed to improve plant abiotic stress tolerance utilizing CRISPR/Cas9 gene editing:

- i. Selecting an appropriate Cas9 promoter.
- ii. Designing gene-targeted guide RNAs.
- iii. Generate novel alleles for abiotic stressresponsive genes.
- iv. Effectively delivering altered genes to plant cells.

CRISPR/Cas9 has been used to improve Arabidopsis drought stress tolerance by leveraging the ABRE1 genetic characteristic (Table 1). Rice mutants (OsPQT3) with increased paraquat tolerance were created using CRISPR/Cas9 (Alfatih et al., 2020). The CRISPR/Cas system has quickly replaced ZFN and TALEN as a standard genome editing approach due to its simplicity, adaptability, stability, and ease of transformation (Li and Xia, 2020).

Table 1. Stress responsive enzymes and techniques for modifications						
Osmotical	Antioxidant	Stress	Molecular	Genetic	Transgenic	CRISPR/Cas9
Adjustment	Defences	Signaling	Chaperones	Engineering	Plants and	
			and HSPs	for Abiotic	Stress	
				Stress	Resistance	
				Tolerance		
Proline	Enzymatic	Calcium	Heat Shock	Overexpression	Drought-	Gene
Accumulation	Antioxidants	Signaling	Proteins	of Stress-	Resistant	Knockouts
			(HSPs)	Responsive	Transgenic	
				Genes	Plants	

 Table 1. Stress responsive enzymes and techniques for modifications

Glycine Betaine	Non-	ABA	Small Heat	Gene Silencing	Salt-	Gene Insertions
Offenne Detaille	Enzymatic	Signaling	Shock Proteins	(RNAi)	Tolerant	Gene miseruons
	Antioxidants	Signaning	(sHSPs)		Transgenic	
	7 miloxidants		(31151 3)		Plants	
Compatible	Reactive	MAP Kinase	Chaperonins	Transcriptional	Cold-	Targeted
Solutes	Oxygen	Pathways	1	Regulation	Resistant	Mutations
	Species	2		U	Transgenic	
	(ROS)				Plants	
	Scavenging					
Osmoprotectants	Ascorbate-	Reactive	Co-chaperones	Stress-Induced	Heat-	Promoter
	Glutathione	Oxygen		Promoters	Tolerant	Editing
	Cycle	Species			Transgenic	
		(ROS)			Plants	
Osmolytes	Catalase	Mitogen-	Chaperone-	Gene Stacking	Multi-Stress	Site-Specific
	Activity	Activated	Assisted		Resistant	Mutagenesis
		Protein	Protein		Transgenic	
		Kinases	Folding		Plants	
	a . 1	(MAPKs)	HODZO		D	
Cellular Water	Superoxide	Hormonal	HSP70,	Use of Model	Pest-	Base Editing
Balance	Dismutase	Cross-Talk	HSP90	Plants for Stress	Resistant	
	(SOD)			Studies	Transgenic	
	D 11	G 1	<b>D</b> 11		Plants	
Salt Exclusion	Peroxidases	Secondary	Folding	Marker-Assisted	Nutrient-Use	Genome-Wide
Mechanisms		Messengers	Enzymes	Selection	Efficiency in	Association
					Transgenic	Studies
					Plants	(GWAS)

This RNA-guided defense system evolves to protect bacteria and archaea from mobile genetic elements and bacteriophages (Rocha and Bikard, 2022). Recent initiatives include SpCas9-VRQR, xCas9, and Cas9-NG, which target non-canonical PAM sites in plants, to enhance its genome-targeting capabilities (Table 2).

#### Omics Technologies in Abiotic Stress Research Genomics

Molecular marker and genomic technologies can improve breeding efficiency, especially abiotic stress tolerance. Genomic integration into breeding programs is exciting with next-generation sequencing and high-throughput phenotyping (D'Agostino and Tripodi, 2017). Genome-assisted breeding uses our knowledge of genetic characteristics and sequence variants to modify genomes precisely. The richness of 'omics' data is quickly improving our understanding of plant abiotic stress tolerance genes and variations (Yang et al., 2021). Given the urgency of global food security, crop development is becoming more viable with this information.

#### Transcriptomic

Transcriptomic studies all RNAs transcribed by cells or tissues, including coding and non-coding. It illuminates gene transcription, structure, function, and control. Technology advances in transcript sequencing and analysis have advanced this field (Hedlund and Deng, 2018). Northern blotting and RT-PCR only examined single transcripts or small groups. However, microarrays enabled simultaneous investigation of thousands of genes, revolutionizing transcript profiling. RNA-Seq and real-time RT-PCR have improved our understanding of gene regulation networks and epigenetics (Steyaert et al., 2016). ESTs, SAGE, and digital gene expression analysis were also used in early transcriptomics research. These methods led to present transcriptomics technologies, which help explain gene expression regulation and biological activities (Casamassimi et al., 2017).

#### Proteomics

Targeted techniques require proteomic investigations to investigate complex cellular relationships and comprehend protein function during abiotic stress (Kosová et al., 2018). Recent advances in methods, sample regimens, and bioinformatics enable highthroughput mass-spectrometry protein research. Proteomics processes use gel-free and 2-DE methods. For instance, nonreducing circumstances and MS-based proteomics revealed 60 novel peanut proteins. Subcellular proteomics helps study localized cellular responses and subcellular interactions during plant growth under abiotic stressors (Kosová et al., 2018).

#### Metabolomics

Metabolomics analyzes all biological metabolites, including volatile primary metabolism components. Due to their complexity, metabolites cannot be detected simultaneously. NMR is a common analytical instrument but more sensitive than others (Marshall and Powers, 2017). GC-MS is a standard method for detecting metabolites, however it heats

samples to gas. LC-MS is more adaptable and no chemical derivatization of nonvolatile metabolites is needed (Sun et al., 2021). Metabolomics research often uses ultra-performance liquid chromatography (UPLC) for its speed and sensitivity (Perez de Souza et al., 2021). Metabolomics studies use LC/GC-MS,

NMR, chemometrics, and multivariate statistical analysis to study plant metabolites, which are quite varied. The goal of guided metabolomics is to identify and quantify as many metabolites as possible within a chemical group.

Genomics	Transcriptomics	Proteomics	Metabolomics	Role of	Genome	Metal Stress
				<b>Epigenetics in</b>	Sequencing	Tolerance
				Stress		
				Tolerance		
Identification	Differential Gene	Stress-Induced	Metabolite	DNA	Whole-	Heavy Metal
of Stress-	Expression	Protein	Profiling	Methylation	Genome	Accumulation
Responsive		Changes		Patterns	Sequencing	
Genes						
Gene	RNA Sequencing	Protein	Secondary	Histone	Next-	Metal
Mapping and	(RNA-Seq)	Quantification	Metabolite	Modifications	Generation	Transporters
Functional			Analysis		Sequencing	
Genomics					(NGS)	
Comparative	Expression of	Post-	Primary	Chromatin	Whole-	Chelation
Genomics	Stress-Related	Translational	Metabolite	Remodeling	Genome	Mechanisms
	Transcripts	Modifications	Analysis		Resequencing	
Gene	Transcript	Proteome	Metabolite	Small RNA	Comparative	Metal
Functional	Profiling	Changes in	Pathway	Involvement	Genomics and	Detoxification
Annotation		Response to	Analysis		Sequencing	
		Stress				
Structural	Gene Expression	Protein-Protein	Metabolite	Epigenetic	High-	Genetic
Genomics	Dynamics	Interactions	Quantification	Regulation of	Throughput	Variants
			and	Stress Genes	Sequencing	Associated with
			Identification			Metal
						Tolerance

# Table 2. Stress types and genetic response

#### Biotechnological Innovations in Stress Signal Transduction Pathways

Biotechnological advances in stress signal transduction pathways have helped researchers understand how plants perceive and respond to stress. MicroRNAs (miRNAs) govern plant processes indirectly by regulating target mRNA abundance and spatial-temporal accumulation (Song et al., 2019). MiRNA fine-tuning is a strong biotechnology technique to improve abiotic or biotic stress tolerance in economically important crops.

# **Role of Epigenetics in Stress Tolerance**

Plant stress tolerance, acclimatization, adaptation, and evolution depend on epigenetics. Progenies inherit stress-induced chromatin alterations that change gene expression but not DNA sequence (Kumar, 2018). These alterations affect DNA bases, histones, and tiny non-coding RNAs. Methylation of a promoter can suppress gene transcription, while methylation of a coding area can silence a gene posttranscriptionally (Zhong et al., 2023). Epialleles also contribute to agricultural enhancement. DNA methylation is mostly found in transposable elements (TEs) and genes in eukaryotic genomes. Genome

sequencing has revealed the structure and function of protein-coding genes in any species' chromosomes, as well as non-coding elements like transposons and promoters (Polychronopoulos et al., 2017). This is essential for understanding evolution and diversification.

# Genome Sequencing and Pangenomics in Improving Drought Tolerance

Genome sequencing can identify upstream regulatory elements of each gene and directly access the structural and functional aspects of proteincoding genes organized in any species' chromosomes to improve drought tolerance. Pangenome, which includes all genetic information in species accessions, has garnered attention (Zhang et al., 2021). Plant pangenomics depends on the reference assembly's size, completeness, annotation, selection, and extensive phenotyping of relevant genotypes (Hurgobin and Edwards, 2017).

**Role of NGS in Abiotic Stresses** 

Next-generation sequencing, high-throughput genotyping, precision editing, space and technologies have boosted crop improvement efforts, creating more resilient cultivars (Singh et al., 2020). Studies on plant responses to single and combined stress have revealed molecular processes governing tolerance to different stress levels. Many researchers examine salt damage and salt-tolerance pathways, and transgenic technology is a fast and effective way to create salt-tolerant types (Muchate et al., 2016). To combat osmotic and ionic stress, plants evolve biochemical and molecular mechanisms like selective accumulation or exclusion of ions, ion uptake control, and compartmentalization, synthesis of compatible solutes, photosynthetic pathway changes, antioxidant enzyme induction, and plant hormones.

Rapid industrialization and urbanization limit plant heavy metal stress tolerance. Modern biotechnology may design legumes and other plants to overexpress stress-induced genes, transcription factors, proteins, and metabolites for heavy metal stress resistance (Rai et al., 2021). Researchers are still trying to understand how plants may endure severe metal stress and flourish without harm.

#### **Biotechnology and Heavy Metal Stress Tolerance** in Plants

Plant responses and tolerance to heavy metal stress are being studied fast, with omics techniques identifying genes activated by metal stress (Jamla et al., 2021). Arabidopsis, Brassica, and Lycopersicum transcriptome research revealed the role of TFs like bHLH, bZIP, AP2/ERF, and DREB. In Oryza sativa, Zea mays, Arabidopsis, and Populus sp., heavy metal detoxifying proteins are implicated (Benyó et al., 2016). Heavy metals increase calcium-dependent, mitogen-activated protein kinase, ROS, and hormone signaling in plants. Plants have several Ca2+ sensors that detect, decipher, and report cytosolic Ca2+ changes for stress response. In Medicago sativa, high Cu and Cd levels activate MAPKs, whereas ROS disrupt phytohormone signaling (Mondal, 2023). Changes in phytohormone levels affect metal stress response. In the next century, high temperatures, submergence, soil salinization, and drought, nutritional imbalances are expected to worsen. Agronomic technologies like abiotic stressmitigating microorganisms are needed to maintain crop output in suboptimal growing conditions.

# Role of Microbiomes and Endophytes in Abiotic Stress Tolerance

Microbial endophytes, plant tissue symbionts, are essential to plant health and growth under abiotic stress (Lata et al., 2018). Secondary active substances that protect plants from infections and extracellular enzymes for colonization boost plant growth. Endophytes produce phytohormones and help plants flourish in polluted soils. Plants' complicated abiotic stress response encompasses physiological, biochemical, and gene expression alterations. Understanding and promoting stress tolerance in commercially significant plant species has been done in many ways. various endophytes use various signaling pathways for different abiotic stresses (Phurailatpam and Mishra, 2020).

# Future Perspectives and Challenges in Biotechnology for Stress Tolerance

Biotechnology for stress tolerance may generate genetically modified (GM) plants with pest-resistant or herbicide-tolerant agronomic features. Science progress in research and development and regulatory restrictions in nations with awaiting marketing approvals determine market release timing. Biotechnology helps address global issues including food security and climate change. Many plants are tested for high production despite biotic and abiotic stressors. Enhancing carbon absorption, growth, biomass yield, and stress-dependent and tissuespecific promoters are future research priorities.

# Conclusion

In conclusion, abiotic stresses reduce plant growth and agricultural output, threatening global food security. Climate change, heat, cold, drought, salt, and heavy metal contamination are endangering crop harvests worldwide. Plants have developed methods to cope with these challenges, and researchers are using genetic engineering, molecular breeding, and omics to improve crop resilience. Sustainable agriculture and food security for a growing population depend on understanding and improving plant stress tolerance. Research and innovation in this subject are needed to meet food demands and maintain ecological balance in an uncertain environment.

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<sup>[</sup>Citation: Abbas, A., Riaz, S., Waleed, R., Ali, Q. (2024). Role of biotechnology in abiotic stress tolerance in plants. *Biol. Clin. Sci. Res. J.*, **2024**: *1142*. doi: <u>https://doi.org/10.54112/bcsrj.v2024i1.1142</u>]

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<sup>[</sup>Citation: Abbas, A., Riaz, S., Waleed, R., Ali, Q. (2024). Role of biotechnology in abiotic stress tolerance in plants. *Biol. Clin. Sci. Res. J.*, **2024**: *1142*. doi: https://doi.org/10.54112/bcsrj.v2024i1.1142]

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#### Declaration

Ethics Approval and Consent to Participate Not applicable. Consent for Publication The study was approved by authors. Funding Statement Not applicable Authors' Contribution All authors contributed equally. Conflict of interest There is no conflict of interest among the authors of the manuscript.



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