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Breeding for Climate Resilience: Genetic Strategies for Developing Drought and Heat Tolerant Crops

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ABSTRACT

Climate change constitutes the greatest threat to food security ever seen in the history of humankind, associated mainly with the increasing number and severity of abiotic stresses (e.g., drought and heat). These climatic impacts are huge hampering factors in terms of the growth, development, and yield of crops resulting in a far-reaching economic loss that intensifies the problem of malnutrition among the debt populations. The most relevant aspect of ensuring sustainable agriculture and supplying food to an increasingly larger world population against a changing climate revolves around developing climate-resilient crops. This is a review that gives an overview of existing knowledge regarding the physiological, morphological and biochemical adaptation of crops to drought and heat stress as well as the complex genetic basis of the mechanisms behind the tolerance to stress. It also critically looks at traditional breeding methods, their achievements and their shortcomings and also the advanced strategies of genetics which are dealt with in great length. These new techniques are molecular-based breeding strategies like Marker-Assisted Selection (MAS) and Genomic Selection (GS) which utilizes the use of genetic markers to increase the rate of selection. Besides, the review explains the revolutionary nature of genetic engineering and genome editing as a technology (e.g., CRISPR/Cas9) capability that can be undertaken to specifically target and leverage genes to strengthen stress tolerance. High-throughput phenotyping has been mentioned as an integrative technology with the next generation omics technologies including: genomics, transcriptomics, proteomics, and metabolomics to address the synergistic need to identify new genes, the complex signaling pathways involved in stress response, and to conduct more accurate breeding. They have been used to produce stress-tolerant varieties as shown by case studies on major crops such as rice, wheat, maize, and legumes with the practical application and effect. Challenges do exist even though much improvement has been achieved, such as the polygenic nature of traits associated with tolerance, the interaction between a given genotype and environmental factors, and an effective phenotyping platform. Future outlooks base the need to develop multidisciplinary, integrated solutions through a combination of conventional and molecular technologies with systems biology and digital agriculture to provide resilient and sturdy, widely adapted, and enduring climate-resilient crops. The report highlights the importance of a coordinated international effort towards research and development so that agricultural systems can be reinforced to withstand the unavoidable effects of climate change.

Keywords: *Climate resilience, Drought tolerance, Heat tolerance, Genetic strategies, Crop breeding, Molecular breeding, Genetic engineering, Genome editing, Omics technologies, Food security.*

1. Introduction

Global climate change represents one of the most pressing challenges of the 21st century, with profound implications for all facets of human life, particularly agriculture. The escalating atmospheric concentrations of greenhouse gases have led to a discernible increase in global temperatures, altered precipitation patterns, and a rise in the frequency and severity of extreme weather events [1]. Among these climatic shifts, drought and heat stress emerge as the most devastating abiotic factors, singularly and synergistically threatening crop productivity and, consequently, global food security [2]. Approximately 80% of the world's agricultural land is rain-fed, making it highly susceptible to drought, while rising average temperatures and heat waves threaten crop growth and development even in irrigated areas [3, 4].

Drought that involves inadequate supply of water, interferes with practically all the biological activities that plants engage in, including cellular metabolism and photosynthesis, uptake and absorption of nutrients and the development of reproductive structures, and eventually results in drastic loss of yield or crop failure [5]. Just like DHS, heat stress also compromises the growth and development of plants, which is characterized as a situation where plants are exposed to non-optimal temperatures (high or low) for an extended duration of time by affecting proteins and membranes, and inefficiency of photosynthesis, particularly during sensitive reproductive phases [6, 7]. It is even more problematic that drought is often accompanied by heat stress; a phenomenon that is on the rise in most agricultural areas and where the result is usually a more significant effect than when either of the two stresses occurs alone [8].

In this context, the ability to create crops that can endure these harsh conditions with stable yields is more than an academic exercise because it is an emergency measure in the sustainable practice of agriculture and food security, as the world has to feed almost 10 billion people in 2050 [9]. Conventional crop breeding has been very effective in increasing yield potential under ideal conditions; whereas, increasing stress tolerance to abiotic stresses has been relatively sluggish because these characters are normally complex, polygenic in nature, and highly influenced by genotype-environment and environmental management practices [10].

The review will offer an integrative review of the genetic measures adopted in the crop breeding concerning drought and heat tolerance. It will closely examine the physiological, morphological and biochemical aspects through which plants respond to these stresses thereby providing a basis on which genetic enhancement can be targeted. This will then be followed by a critical evaluation of the way breeding has progressed, in terms of traditional selection methods into the latest advances in molecular breeding, with such techniques as Marker-Assisted Selection (MAS), and Genomic Selection (GS). In addition to that, the innovative potential of genetic engineering as well as precision genome editing applications, including CRISPR/Cas9, to precisely edit the plant genome to improve resilience will be examined. The combinations of high throughput phenotyping with other 'omics or high-throughput technologies - genomics, transcriptomics, proteomics and metabolomics will be outlined as a synergetic route in facilitating breeding speed and gene identification. Lastly, the key cases of advancements made in main crop species followed by the remaining issues and prospects on how to build resilient, widely adapted and sustainable climate-resilient crops in a fast-changing world, will be provided in this review.

2. Impact of Drought and Heat Stress on Crop Plants

Drought and heat stress, either individually or in combination, severely disrupt various physiological, morphological, and biochemical processes in crop plants, leading to significant reductions in growth, development, and ultimately, yield. Understanding these impacts is fundamental for identifying target traits and designing effective breeding strategies.

2.1. Physiological Responses

Plants respond to drought and heat stress through a cascade of physiological adjustments aimed at minimizing damage and conserving resources.

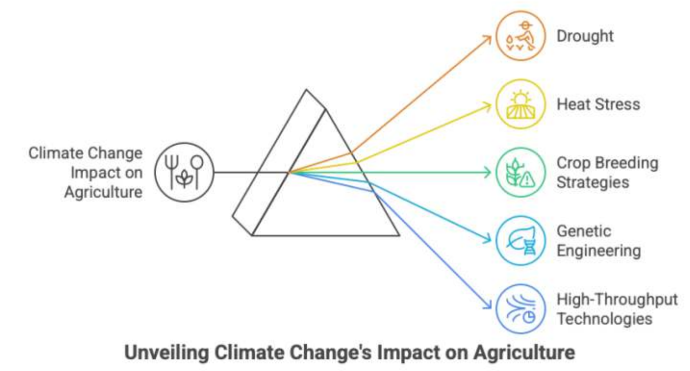


Fig. 1

Water Potential and Turgor Loss: Drought stress primarily manifests as a reduction in water potential within plant tissues, leading to turgor loss. This affects cell expansion, stomatal opening, and overall plant water relations. Turgor maintenance is crucial for cell elongation and, consequently, growth [11]. Under severe drought, cells lose turgor, leading to wilting and compromised physiological functions.

Photosynthesis Inhibition: Both drought and heat stress adversely affect photosynthesis, the cornerstone of plant productivity.

Stomatal Closure: Under drought, plants close their stomata to reduce transpirational water loss. While this conserves water, it simultaneously limits CO$_2$ uptake, leading to reduced photosynthetic rates [12].

Non-Stomatal Limitations: Beyond stomatal closure, heat and severe drought can cause non-stomatal limitations to photosynthesis. High temperatures directly damage photosynthetic machinery, including photosystem II (PSII) and Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase), leading to reduced light capture and CO$_2$ fixation efficiency [13]. Drought also impairs PSII efficiency and electron transport.

Chlorophyll Degradation: Prolonged stress can lead to the degradation of chlorophyll pigments, reducing the plant's ability to capture light energy and contributing to reduced photosynthetic capacity [14].

Respiration and Energy Balance: While photosynthesis declines, respiration rates can initially increase under moderate stress, consuming energy reserves. Under severe stress, however, respiration can also be inhibited due to cellular damage [15]. Maintaining an optimal balance between energy production and consumption is critical for survival under stress.

Oxidative Stress: A common consequence of both drought and heat stress is the generation of reactive oxygen species (ROS), such as superoxide radicals (O2.−), hydrogen peroxide (H_2O_2), and hydroxyl radicals (.OH) [16]. ROS are highly reactive molecules that can cause oxidative damage to lipids (membrane peroxidation), proteins (enzyme inactivation), and nucleic acids (DNA damage), leading to cellular dysfunction and programmed cell death. Plants possess an intricate antioxidant defense system to scavenge ROS, including enzymatic antioxidants (e.g., superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase) and non-enzymatic antioxidants (e.g., ascorbate, glutathione, tocopherols, carotenoids) [17]. The efficiency of this system is critical for stress tolerance.

2.2. Morphological Adaptations

Plants exhibit various morphological adjustments to cope with drought and heat stress, often representing adaptive strategies to minimize water loss or maximize water uptake.

Root Architecture: A well-developed root system is crucial for drought avoidance.

Deeper Roots: Plants with deeper root systems can access water from deeper soil layers, especially during prolonged dry spells [18].

Increased Root Density and Fine Roots: A higher density of fine roots and increased root length density can enhance water and nutrient uptake efficiency from the soil [19].

Root Angle: Steeper root angles can contribute to deeper rooting, as observed in some maize genotypes.

Leaf Traits:

Leaf Rolling and Folding: Many cereal crops, like maize and rice, respond to water deficit by rolling or folding their leaves. This reduces the exposed leaf surface area, thereby minimizing water loss through transpiration and decreasing light interception, which can lower leaf temperature [20].

Reduced Leaf Area: Plants may reduce their total leaf area through smaller leaves or premature senescence of older leaves to decrease transpirational demand [14].

Leaf Angle: Altering leaf angle to become more erect can reduce the amount of absorbed radiation and thus leaf temperature, particularly under heat stress [21].

Thicker Cuticle and Wax Production: A thicker cuticular layer and increased wax deposition on the leaf surface act as physical barriers, reducing non-stomatal water loss [22].

Trichomes: Increased trichome density can create a boundary layer of still air, reducing air flow over the leaf surface and thus reducing transpiration and reflecting incoming radiation [23].

Early Senescence/Stay-Green: Under severe or prolonged stress, plants may accelerate senescence to remobilize nutrients from vegetative parts to developing grains, a survival strategy. However, the "stay-green" phenotype, where leaves remain photosynthetically active longer under stress, is desirable for maintaining carbon assimilation during grain filling and is associated with higher yields under drought [24].

2.3. Biochemical Changes

Plants initiate a myriad of biochemical adjustments to mitigate the harmful effects of drought and heat stress.

Osmoprotectants Accumulation: To maintain cell turgor and protect cellular components, plants synthesize and accumulate compatible osmolytes (osmoprotectants) such as proline, glycine betaine, sugars (e.g., trehalose, sucrose), and sugar alcohols (e.g., mannitol, sorbitol) [25]. These compounds are non-toxic at high concentrations and help in osmotic adjustment, stabilizing proteins and membranes, and scavenging ROS.

Antioxidant Defense System Enhancement: As mentioned, plants ramp up their enzymatic (e.g., SOD, CAT, APX, GR) and non-enzymatic (e.g., Ascorbate, GSH, tocopherols, carotenoids) antioxidant systems to counteract the increased production of ROS under stress [26].

Heat Shock Proteins (HSPs): Under heat stress, plants rapidly synthesize Heat Shock Proteins (HSPs). These molecular chaperones prevent the denaturation and aggregation of other proteins, facilitate the refolding of misfolded proteins, and aid in their transport and degradation [27]. HSPs are crucial for maintaining protein homeostasis and cellular integrity at high temperatures.

Stress Hormones: Plant hormones play critical roles in mediating stress responses. Abscisic acid (ABA) is a key hormone in drought signaling, promoting stomatal closure, root growth, and regulating the expression of many stress-responsive genes [28]. Other hormones like salicylic acid, jasmonates, auxins, cytokinins, and gibberellins also participate in complex crosstalk networks to modulate stress responses [29]

Changes in Photosynthetic Pigments and Metabolites: Stress can lead to changes in chlorophyll and carotenoid content. Furthermore, primary and secondary metabolism are reconfigured, affecting pathways involved in amino acid synthesis, carbohydrate metabolism, and the production of secondary metabolites like flavonoids and phenolics, which often have antioxidant properties [30].

2.4. Yield Losses and Food Security Implications

The combined impact of these physiological, morphological, and biochemical alterations is a significant reduction in crop yield. Drought and heat stress affect different growth stages differently, but reproductive stages (flowering and grain filling) are particularly sensitive. Pollen viability, fertilization success, and grain development are highly susceptible to stress, leading to fewer and smaller grains [7, 31].The cumulative effect of these stresses can result in:

Reduced Biomass and Plant Growth: Overall plant size, root and shoot biomass are significantly reduced.

Lower Harvest Index: The ratio of grain yield to total biomass can decrease due to poor reproductive development.

Compromised Quality: Beyond yield, nutritional quality (e.g., protein content in cereals, oil content in oilseeds) can also be negatively impacted [32].

Increased Volatility in Food Production: The unpredictable nature of extreme weather events leads to greater volatility in regional and global food supplies, driving price spikes and increasing food insecurity, especially in developing countries where a large portion of the population relies directly on agriculture for livelihood [33]

Developing crops with enhanced tolerance to these stresses is therefore not just about increasing production but about stabilizing agricultural output, reducing vulnerability, and building resilient food systems in the face of climate change.

3. Genetic Basis of Drought Tolerance

Understanding the genetic basis of drought tolerance is crucial for developing effective breeding strategies. Drought tolerance is a complex quantitative trait governed by multiple genes with small effects, highly influenced by environmental interactions (G x E). The genetic architecture involves a network of genes controlling various physiological, morphological, and biochemical responses.

3.1. Quantitative Trait Loci (QTLs) for Drought Tolerance

QTL mapping has been a cornerstone in dissecting the genetic architecture of complex traits like drought tolerance. By analyzing the co-segregation of phenotypic traits and molecular markers in mapping populations (e.g., recombinant inbred lines, backcross populations, doubled haploids), researchers can identify genomic regions (QTLs) associated with drought-responsive traits. Numerous QTLs have been identified across various crop species for traits associated with drought tolerance.

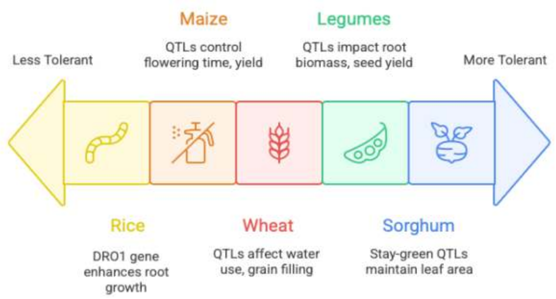


Fig. 2

Rice (*Oryza sativa*): One of the most significant achievements in rice breeding for drought tolerance has been the identification and deployment of the DRO1 (Deeper Rooting 1) gene. This QTL, located on chromosome 9, enhances root growth angle, allowing roots to penetrate deeper into the soil and access sub-surface water [34].Other important QTLs in rice include those associated with spikelet fertility under drought (e.g., qDTY1.1, qDTY2.1, qDTY3.1, qDTY12.1) and yield under drought (YLD-QTLs) [35, 36].These QTLs often explain a small to moderate proportion of the phenotypic variation but collectively contribute to tolerance.

Maize (*Zea mays*): Extensive QTL mapping efforts in maize have identified genomic regions controlling various drought-adaptive traits, including root traits (root depth, root biomass), flowering time (silking delay), leaf rolling, stay-green phenotype, and grain yield under stress [37, 38]. For example, QTLs for stay-green in maize have been found on multiple chromosomes, suggesting a complex genetic control [39].

Wheat (*Triticum aestivum*): Drought tolerance in wheat is particularly important given its widespread cultivation in rain-fed areas. QTLs have been identified for traits such as water use efficiency, canopy temperature, carbon isotope discrimination, tiller number, grain filling rate, and yield under terminal drought stress [40, 41]. The 1B and 2B chromosomes have been frequently implicated in drought tolerance in wheat.

Sorghum (*Sorghum bicolor*): Sorghum is inherently more drought-tolerant than maize, making it an excellent model for studying drought adaptation. Key QTLs in sorghum include those associated with stay-green (maintaining green leaf area during grain filling) [42]. Osmotic adjustment, and yield under drought [43]. The stay-green QTLs (Stg1, Stg2, Stg3, Stg4) have been fine-mapped and are extensively utilized in breeding programs [44].

Legumes (e.g., Chickpea, Common Bean): In legumes, drought tolerance QTLs have been identified for traits such as canopy temperature, root biomass, flowering time, podding efficiency, and seed yield under water deficit [45, 46]. For example, in chickpea, QTLs for root traits and drought tolerance indices have been mapped to various linkage groups.

3.2. Candidate Genes Involved in Water Use Efficiency, Root Development, and Stress Signaling

Beyond broad QTL regions, fine-mapping and association studies have led to the identification of specific candidate genes underlying drought tolerance. These genes often belong to several functional categories:

Genes involved in Abscisic Acid (ABA) Signaling Pathway: ABA is a crucial plant hormone mediating drought stress responses. Genes encoding components of the ABA signaling pathway, such as receptors (PYR/PYL/RCARs), protein phosphatases (PP2Cs), and protein kinases (SnRK2s), play vital roles in regulating stomatal closure, root development, and stress gene expression [47, 48]. Overexpression of certain ABA-responsive transcription factors (e.g., AREB/ABF) has been shown to improve drought tolerance in various crops.

Transcription Factors (TFs): TFs regulate the expression of large sets of genes in response to stress. Key families of TFs implicated in drought tolerance include:

DREB/CBF (Dehydration-responsive Element Binding/C-repeat Binding Factor): These TFs regulate genes containing DRE/CRT elements in their promoters, which are involved in cold and dehydration responses [49]. Overexpression of DREB TFs from *Arabidopsis* and other plants has significantly enhanced drought tolerance in rice, wheat, and maize.

NAC (NAM, ATAF1/2, CUC2): A large family of plant-specific TFs involved in development and stress responses. Many NAC TFs (e.g., *OsNAC6*, *OsNAC10*) are induced by drought and contribute to tolerance through various mechanisms, including regulating root development and senescence [50, 51].

MYB (Myeloblastosis): MYB TFs regulate diverse processes, including stress responses. Specific MYB TFs (e.g., *OsMYB4*, *ZmMYB31*) are known to enhance drought tolerance by modulating osmolyte accumulation, stomatal density, or ROS scavenging [52].

bZIP (Basic Leucine Zipper): bZIP TFs are involved in ABA signaling and other stress pathways. Examples like *OsbZIP23* and *TRAB1* have been shown to improve drought tolerance [53].

Genes encoding Osmoprotectant Synthesis Enzymes: Genes involved in the biosynthesis of compatible solutes like proline (e.g., *P5CS* - pyrroline-5-carboxylate synthetase), glycine betaine (e.g., *BADH* - betaine aldehyde dehydrogenase), and sugars (e.g., *TPS* - trehalose-6-phosphate synthase) are crucial. Enhancing their expression can lead to increased osmolyte accumulation and improved osmotic adjustment [54].

Genes involved in Root Architecture: Beyond DRO1 in rice, genes like those coding for auxin transporters, aquaporins, or cell wall-modifying enzymes can influence root development and water uptake efficiency [55].For example, aquaporins (AQPs) facilitate water transport across membranes, and their regulation is critical under drought [56].

Genes related to Water Use Efficiency (WUE): While WUE is complex, genes influencing stomatal density, stomatal conductance, or photosynthetic carbon fixation pathways indirectly contribute to WUE. For example, genes involved in C4 photosynthesis are inherently more water-efficient than C3 plants.

3.3. Omics Approaches for Gene Discovery

The advent of high-throughput 'omics' technologies has revolutionized the ability to dissect the genetic and molecular mechanisms of drought tolerance, moving beyond single-gene analyses to systems-level understanding.

Genomics: Next-Generation Sequencing (NGS) has enabled rapid and cost-effective sequencing of entire crop genomes and re-sequencing of diverse germplasm collections. This provides a comprehensive catalog of genetic variation (SNPs, indels, SVs) which can be linked to drought tolerance through Genome-Wide Association Studies (GWAS) or used for Genomic Selection [57].Comparative genomics can also identify conserved drought-responsive genes across species.

Transcriptomics: RNA sequencing (RNA-Seq) allows for the global profiling of gene expression patterns under drought stress. This can identify differentially expressed genes (DEGs) and gene networks that are activated or repressed in response to water deficit. Studies have revealed thousands of DEGs involved in signaling, metabolism, transport, and defense [58].For example, transcriptomic analyses in drought-stressed maize have revealed coordinated changes in genes involved in ABA biosynthesis, osmoprotectant synthesis, and cell wall modification.

Proteomics: Proteomic approaches (e.g., 2D-PAGE, LC-MS/MS) aim to identify and quantify proteins whose abundance changes under drought stress. This provides insights into the post-transcriptional regulation and functional machinery involved in stress responses. Proteins related to photosynthesis, carbohydrate metabolism, antioxidant defense, and chaperones are often found to be altered [59].

Metabolomics: Metabolomics, the study of small molecule metabolites, provides a functional readout of the plant's physiological state. By analyzing changes in metabolite profiles under drought, researchers can identify key metabolic pathways that are altered and identify novel biomarkers for stress tolerance. For instance, drought stress often leads to the accumulation of specific sugars, amino acids, and secondary metabolites [60].

Integrative Omics: The most powerful approach involves integrating data from multiple omics platforms (genomics, transcriptomics, proteomics, metabolomics) with phenomic data. This systems biology approach allows for a holistic understanding of the complex interplay between genes, transcripts, proteins, and metabolites in conferring drought tolerance, enabling the identification of key regulatory nodes and novel targets for genetic manipulation [61].

4. Genetic Basis of Heat Tolerance

Similar to drought tolerance, heat tolerance is a complex quantitative trait governed by multiple genes and pathways, often exhibiting significant G x E interactions. High temperatures affect a myriad of plant processes, from photosynthesis and respiration to membrane stability and reproductive development.

4.1. QTLs for Heat Tolerance

QTL mapping has been instrumental in dissecting the genetic architecture of heat tolerance in various crop species. Traits such as membrane stability index (MSI), chlorophyll content, pollen viability, spikelet fertility, and grain yield under heat stress are frequently targeted for QTL analysis.

Wheat (*Triticum aestivum*): Heat stress, especially during the grain-filling period, significantly reduces wheat yield. Numerous QTLs for heat tolerance have been identified, particularly for traits like grain weight, grain number, and yield per plant under heat stress conditions [62, 63]. QTLs affecting physiological traits such as canopy temperature depression (CTD), stay-green, and membrane stability index have also been reported. Chromosomes 3A, 4A, 5A, 7A, 2D, 3D, and 5D have frequently been associated with heat tolerance.

Rice (*Oryza sativa*): Rice is highly sensitive to heat stress, especially during anthesis (flowering), where temperatures above 35∘°C can cause spikelet sterility. QTLs for heat tolerance in rice have been identified for traits like spikelet fertility, pollen viability, grain yield, and seed setting rate under high temperatures [7, 64].For example, a major QTL for high-temperature tolerance at the reproductive stage was mapped on chromosome 4, and another QTL on chromosome 1 for pollen viability.

Maize (*Zea mays*): Heat stress, particularly during tasseling and silking, can lead to asynchronous flowering and reduced kernel set in maize. QTLs for heat tolerance have been mapped for traits such as tassel-silking interval, pollen viability, ear length, kernel number, and grain yield under heat stress [65, 66].The physiological trait of membrane stability index (MSI) is often used as a proxy for heat tolerance and has associated QTLs.

Sorghum (*Sorghum bicolor*): Sorghum, being a C4 plant, is generally more heat-tolerant than C3 crops. However, extreme heat still impacts its productivity. QTLs have been identified for various heat-responsive traits, including flowering time, biomass, and grain yield under heat stress [67].

Legumes (e.g., Common Bean, Soybean): Legumes are also vulnerable to heat stress, especially during flowering and pod development. QTLs for heat tolerance in common bean have been mapped for traits like pod set percentage, seed weight, and chlorophyll content under high temperatures [68].In soybean, QTLs associated with flower abortion and yield components under heat stress have been reported [69].

4.2. Candidate Genes Related to Heat Shock Response, Cellular Protection, and Membrane Stability

Through fine-mapping, GWAS, and functional genomics, several candidate genes influencing heat tolerance have been identified. These genes are often involved in heat shock response, antioxidant defense, osmoprotection, and maintaining cellular integrity.

Heat Shock Proteins (HSPs) and Heat Shock Factors (HSFs): HSPs are chaperones critical for protein homeostasis under heat stress, preventing denaturation and aiding refolding [27]. HSFs are transcription factors that regulate the expression of HSPs and other heat-responsive genes [70].Overexpression of specific HSFs or HSPs has been shown to enhance heat tolerance in model plants and crops (e.g., *OsHSP17.7* in rice, *AtHSP101* in *Arabidopsis*).

Genes encoding Antioxidant Enzymes: Heat stress, like drought, induces oxidative stress. Genes encoding key antioxidant enzymes (e.g., *SOD*, *CAT*, *APX*, *GR*) and enzymes involved in the synthesis of non-enzymatic antioxidants are crucial for scavenging ROS and mitigating cellular damage [71].Enhancing the capacity of the antioxidant system is a common strategy for improving heat tolerance.

Genes involved in Osmolyte Synthesis: Accumulation of compatible osmolytes (e.g., proline, glycine betaine, trehalose) is also a response to heat stress, helping to protect cellular structures and enzymes [71].Genes like *P5CS* for proline synthesis or *TPS/TPPC* for trehalose synthesis are important candidates.

Genes maintaining Membrane Stability: High temperatures can increase membrane fluidity and permeability, leading to cellular leakage and dysfunction. Genes involved in lipid metabolism, fatty acid desaturation (e.g., *SAD* - stearoyl-ACP desaturase), and those encoding proteins that protect membranes (e.g., LEA proteins, specific aquaporins) are relevant for maintaining membrane integrity under heat stress [73].For example, genetic manipulation of fatty acid desaturases can alter membrane lipid composition and improve heat tolerance.

Genes involved in Photosynthesis Protection and Repair: Genes protecting photosystem II (PSII) from heat damage, such as those encoding components of the D1 protein repair cycle or chaperones specific to chloroplasts, are vital. Genes related to Rubisco activase, which is heat-labile and critical for Rubisco activity, also play a role [74].

Transcription Factors (TFs): Besides HSFs, other TF families are involved in heat stress response:

HSFA4a: Involved in ROS signaling and tolerance.

MYB/NAC/WRKY: Specific members of these families have been found to regulate genes involved in heat tolerance [75].For instance, *OsNAC066* in rice has been linked to improved heat tolerance.

4.3. Omics Approaches for Heat Tolerance

Omics technologies are indispensable for unraveling the complex molecular networks underlying heat tolerance, providing a holistic view of gene regulation, protein dynamics, and metabolic adjustments.

Genomics: Genome sequencing and re-sequencing of heat-tolerant and sensitive genotypes enable the identification of genetic variations (SNPs, indels, CNVs) associated with heat tolerance through GWAS [76].This facilitates the discovery of novel heat-tolerant alleles and their use in breeding.

Transcriptomics: RNA-Seq studies under heat stress reveal massive reprogramming of the transcriptome. These studies identify thousands of differentially expressed genes (DEGs) related to heat shock response, antioxidant defense, secondary metabolism, and hormone signaling [77].For example, transcriptomic analysis in heat-stressed maize silk identified genes involved in pollen-stigma interaction and ovule development.

Proteomics: Proteomic analyses provide insights into changes in protein abundance and post-translational modifications in response to heat stress. Common findings include up-regulation of HSPs, antioxidant enzymes, and proteins involved in energy metabolism and protein synthesis [78].This helps pinpoint key functional proteins directly involved in tolerance.

Metabolomics: Metabolomics helps elucidate the metabolic adjustments plants make under heat stress. Accumulation of osmolytes, changes in carbohydrate metabolism, and shifts in secondary metabolite production (e.g., flavonoids, phenolics) are common observations [79].These metabolic signatures can serve as biomarkers for heat tolerance and reveal critical pathways.

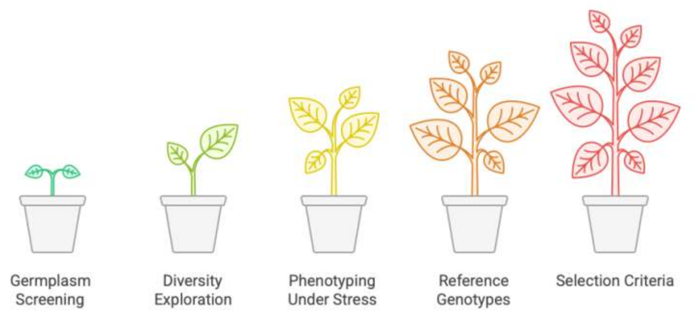
Integrative Omics and Systems Biology: Combining data from genomics, transcriptomics, proteomics, and metabolomics, along with detailed phenotyping, allows for a systems-level understanding of heat tolerance. This integrative approach helps to construct gene regulatory networks, identify core heat stress response pathways, and prioritize candidate genes for functional validation and breeding applications [75].This provides a more complete picture of how a plant tolerates heat, moving beyond individual components to the entire stress response system.

Table 1: Key Physiological and Morphological Traits for Drought and Heat Tolerance

Trait Category	Specific Trait	Relevance to Drought Tolerance	Relevance to Heat Tolerance
Water Use & Conservation	Stomatal Conductance	Lower conductance reduces water loss.	Can indirectly reduce leaf temperature.
	Water Use Efficiency (WUE)	Maximize biomass/yield per unit water.	Maintains photosynthetic efficiency.
	Canopy Temperature Depression (CTD)	Indicator of effective transpiration/cooling.	Direct measure of evaporative cooling (key for heat).
	Leaf Rolling/Folding	Reduces transpirational surface area.	Reduces direct solar radiation absorption.
	Cuticular Wax Content	Reduces non-stomatal water loss.	Helps in reducing water loss under high temperature.
Root System	Deep Rooting	Access deeper soil moisture.	Can access moisture to support transpirational cooling.
	Root Biomass/Density	Greater water/nutrient absorption.	Supports overall plant vigor and water uptake.
Cellular & Biochemical	Membrane Stability Index (MSI)	Indicates cellular integrity under dehydration.	Indicates cellular integrity under high temperature.
	Osmolyte Accumulation (Proline, GB)	Osmotic adjustment, cell protection.	Protects proteins and membranes from heat damage.
	Antioxidant Enzyme Activity	Scavenges ROS, reduces oxidative damage.	Scavenges ROS, reduces oxidative damage.
	Heat Shock Protein (HSP) levels	General stress protein, sometimes induced by drought.	Crucial for protein folding and preventing denaturation.
Growth & Phenology	Stay-Green Phenotype	Prolongs photosynthesis, improves grain filling.	Maintains photosynthetic activity, reduces premature senescence.
	Early Flowering/Maturity	Drought/heat escape strategy (escape terminal stress).	Avoids most severe late-season heat.
	Leaf Area Index (LAI)	Reduced LAI can decrease water demand.	Reduced LAI can decrease heat load.
Reproductive Traits	Spikelet Fertility	Direct impact on yield under stress.	Direct impact on yield under stress (pollen viability).
	Pollen Viability	Crucial for successful fertilization.	Highly sensitive to high temperatures.
	Grain Filling Rate	Sustains yield under terminal stress.	Sustains yield under high temperatures.

5. Conventional Breeding Strategies for Drought and Heat Tolerance

Conventional plant breeding has been the backbone of crop improvement for centuries, relying on phenotypic selection and recombination to develop superior varieties. While the complex and polygenic nature of drought and heat tolerance makes direct selection challenging, significant progress has been made using traditional approaches.



Steps to Identify Stress-Tolerant Plants

5.1. Germplasm Screening and Selection

The initial and crucial step in any conventional breeding program is the identification of promising genetic variation within existing germplasm.

Diversity Exploration: Screening diverse germplasm collections, including landraces, wild relatives, and exotic accessions, is essential. These traditional varieties and wild species often harbor valuable stress tolerance genes that have been lost in modern, high-yielding cultivars bred under optimal conditions [80].For instance, wild progenitors of many crops demonstrate superior stress adaptation.

Phenotyping Under Stress Conditions: Rigorous and systematic phenotyping is critical to identify genuinely tolerant genotypes. Plants are grown under controlled or field conditions designed to impose relevant levels of drought or heat stress at critical growth stages (e.g., flowering, grain filling). This often involves:

Rainout shelters: Used to control water availability for drought screening.

Heat chambers/Greenhouses with temperature control: For controlled heat stress experiments.

Managed Stress Environments (MSEs): Field sites where water and temperature can be manipulated or where natural stress conditions are reliably present (e.g., arid or semi-arid regions for drought, tropical lowlands for heat) [81].

Reference Genotypes: Including known tolerant and sensitive checks in every screening trial is vital for comparison and validation.

Selection Criteria: Selection is based on easily measurable traits (yield, biomass, flowering time) and specific physiological or morphological indicators of stress tolerance (e.g., stay-green, canopy temperature depression, leaf rolling, spikelet fertility, membrane stability index) [10]. Repeated screening across multiple years and locations with varying stress intensities helps to identify consistently performing genotypes and mitigate the impact of G x E interactions.

5.2. Phenotyping Techniques (High-Throughput Phenotyping - HTP)

Phenotyping, especially under stress, is often a bottleneck in breeding programs due to its labor-intensive and time-consuming nature. The emergence of High-Throughput Phenotyping (HTP) technologies is transforming this process.

Traditional Phenotyping: Involves manual measurements of traits like plant height, biomass, yield components, root length, chlorophyll content, etc. While precise, it's slow, expensive, and destructive for many traits.

High-Throughput Phenotyping (HTP): HTP integrates sensor technologies, automation, robotics, and image analysis to rapidly and non-destructively quantify complex plant traits on a large scale [82].

Imaging Techniques:
RGB Imaging: Captures visible light images to assess plant growth, biomass, leaf area, color, and senescence (e.g., stay-green).

Near-Infrared (NIR) Imaging: Used for estimating plant water content, biomass, and nutrient status.

Thermal Imaging: Measures canopy temperature, which correlates with stomatal conductance and transpirational cooling (lower canopy temperature indicates higher transpiration and potentially better cooling under heat, or better water uptake under drought) [83].This is a highly valuable tool for heat and drought screening.

Hyperspectral Imaging: Captures reflectance across a wide range of the electromagnetic spectrum, providing detailed information on plant health, pigment content, water status, and stress indicators [84].

Fluorescence Imaging: Measures chlorophyll fluorescence, an indicator of photosynthetic efficiency and PSII health, which is sensitive to both heat and drought stress [85].

Automated Platforms:
Greenhouse/Growth Chamber Systems: Robotic systems move plants through imaging stations, collecting data automatically.

Field-based Platforms: Unmanned Aerial Vehicles (UAVs or drones), ground-based phenotyping robots, and sensor-equipped tractors can collect high-resolution data from large field plots [4]. This allows for rapid assessment of large breeding populations across diverse environments.

Data Analysis: Sophisticated image processing and machine learning algorithms are used to extract meaningful phenotypic data from the raw images and sensor readings.
HTP greatly enhances the capacity for accurate and rapid phenotyping, enabling breeders to screen vast populations and identify superior genotypes more efficiently.

5.3. Conventional Breeding Methods
Once tolerant germplasm is identified, conventional breeding methods are employed to incorporate these traits into elite cultivars.

Selection: The simplest method, involving direct selection of superior individuals from diverse populations or crosses under stress. This can be mass selection, progeny selection, or pure-line selection, depending on the crop's reproductive biology.

Hybridization and Pedigree Method: This is a widely used method. Tolerant donors are crossed with high-yielding but stress-sensitive elite lines. The progeny (F1) are self-pollinated, and subsequent generations (F2 to F6/F7) are advanced by selfing, with selection occurring in each generation based on desired stress tolerance traits and agronomic performance. Pedigree records are maintained for each plant. This method is effective for traits with relatively high heritability.

Backcross Method: This method is ideal for transferring one or a few major genes (or QTLs with large effects) for tolerance from a donor parent into a recurrent elite parent, while largely maintaining the genetic background of the elite parent. Repeated backcrossing to the recurrent parent is followed by selection for the desired trait (e.g., a specific drought tolerance gene) and recovery of the recurrent parent's genome [86].This is less effective for complex, polygenic traits unless combined with MAS.

Recurrent Selection: This method aims to gradually increase the frequency of desirable alleles for polygenic traits within a population. It involves repeated cycles of selection, intermating, and re-selection of individuals from a broad-based population. This is particularly useful for improving complex traits like stress tolerance that are controlled by many genes with small effects [87].

Population Breeding Methods: Other methods include the bulk method, single seed descent, which are often used in combination with selection strategies depending on the breeding objective and crop type.

5.4. Challenges and Limitations of Conventional Breeding
Despite its successes, conventional breeding for drought and heat tolerance faces several inherent challenges:
Complexity of Traits: Drought and heat tolerance are highly complex quantitative traits controlled by numerous genes, each with a small effect, making precise phenotypic selection difficult [10].

Genotype x Environment (G x E) Interaction: The expression of stress tolerance genes is highly influenced by environmental conditions (e.g., stress severity, timing, duration, soil type, nutrient availability). A genotype performing well in one stress environment may not perform similarly in another, necessitating multi-location and multi-year trials [88].

Phenotyping Bottleneck: As discussed, accurately and efficiently phenotyping large populations under realistic stress conditions is labor-intensive, time-consuming, and expensive, limiting the population size that can be evaluated.

Long Breeding Cycles: Developing a new variety using conventional methods can take 10-15 years or more, which is slow given the rapid pace of climate change.

Linkage Drag: In backcrossing or even pedigree methods, transferring desirable stress tolerance genes from a donor can inadvertently bring along undesirable genes (linkage drag) from the donor, requiring extensive backcrossing and careful selection to break these linkages.

Genetic Variation: The availability of sufficient genetic variation for stress tolerance within the accessible germplasm can be a limiting factor.
These limitations highlight the need for integrating conventional breeding with more advanced molecular and biotechnological tools to accelerate the development of climate-resilient crops.

6. Molecular Breeding Approaches

Molecular breeding integrates molecular biology tools, particularly DNA markers, into traditional breeding programs. It aims to increase the efficiency and precision of selection by directly or indirectly tracking desirable genes, thereby overcoming some limitations of conventional breeding.

6.1. Marker-Assisted Selection (MAS)

Marker-Assisted Selection (MAS) involves using molecular markers (DNA sequences linked to genes or QTLs of interest) to select individuals with desired traits, often at early growth stages or even in tissue culture. This avoids the need for extensive field phenotyping, especially for traits that are difficult, expensive, or time-consuming to phenotype conventionally.

Principles of MAS:

Identification of Markers: First, molecular markers (e.g., SNPs, SSRs, RFLPs) tightly linked to a target gene or QTL for drought/heat tolerance must be identified. This is typically achieved through QTL mapping, GWAS, or fine mapping studies [89].

Validation of Markers: The identified markers need to be validated across different genetic backgrounds and environments to ensure their consistent association with the trait.

Marker-Assisted Backcrossing (MABC): This is the most common application of MAS. Instead of relying solely on phenotypic selection, breeders use markers to:

Foreground Selection: Select for the presence of the desired stress tolerance allele from the donor parent at each backcross generation.

Background Selection: Simultaneously select for the recovery of the recurrent parent's genetic background across the rest of the genome, thereby reducing linkage drag and accelerating the development of improved varieties [89].

Marker-Assisted Pedigree Selection: Markers can be used in early generations (e.g., F2 or F3) to select superior individuals carrying desired alleles, enriching the breeding population for desired traits before extensive field evaluation.

Marker-Assisted Pyramiding: MAS facilitates the stacking or pyramiding of multiple desirable stress tolerance genes or QTLs into a single genotype. This is particularly valuable for complex traits like drought and heat tolerance, which are polygenic [91].

Types of Markers:

Restriction Fragment Length Polymorphisms (RFLPs): Early markers, less used now due to their laborious nature.

Simple Sequence Repeats (SSRs or Microsatellites): Highly polymorphic, abundant, and easy to use, making them widely adopted in MAS [92].

Single Nucleotide Polymorphisms (SNPs): The most abundant type of genetic variation, highly stable, and amenable to high-throughput genotyping platforms. SNPs are increasingly preferred for MAS and GS [93].

Insertion-Deletion Polymorphisms (InDels): Also common and useful markers.

Applications in Drought/Heat Tolerance:

Rice: MAS has successfully been applied to introgress drought tolerance QTLs (e.g., DRO1, qDTYs) into popular susceptible rice varieties, leading to the development of drought-tolerant lines that maintain yield under water deficit [35, 34].

Wheat: MAS has been used to select for traits like canopy temperature depression, stay-green, and yield-related QTLs under drought and heat stress [62, 63].

Maize: MAS is employed to improve drought tolerance traits such as anthesis-silking interval (ASI), stay-green, and grain yield under stress [94].

Sorghum: The stay-green QTLs in sorghum (Stg1, Stg2, Stg3, Stg4) have been successfully introgressed into elite lines using MAS, contributing to enhanced drought tolerance [44].

6.2. Genomic Selection (GS)

Genomic Selection (GS) is a more recent and powerful molecular breeding approach that aims to predict the breeding values (or genetic merit) of individuals based on genome-wide marker data, rather than relying on a few markers linked to specific QTLs [95].This is particularly advantageous for complex traits like drought and heat tolerance, which are controlled by hundreds or thousands of genes with small effects.

Principles of GS:

Training Population: A reference or training population, consisting of historically phenotyped and genotyped individuals (e.g., elite breeding lines, diverse germplasm), is established.

Genomic Estimated Breeding Values (GEBVs): Statistical models (e.g., GBLUP, Bayesian methods) are used to estimate the effects of all genome-wide markers simultaneously from the training population. These marker effects are then used to predict the Genomic Estimated Breeding Values (GEBVs) for unphenotyped individuals in the breeding (selection) population [96].

Selection: Individuals with high GEBVs are selected for advancement or crossing, without the need for individual phenotypic evaluation in every cycle.

Prediction Accuracy: The success of GS depends on the prediction accuracy of GEBVs, which is influenced by factors such as the size and genetic relatedness of the training population, marker density, and the heritability of the trait.

Advantages over MAS:

Handles Polygenic Traits: GS effectively captures the effects of all genes, including those with small effects that are difficult to detect by traditional QTL mapping or MAS. This is crucial for complex quantitative traits like stress tolerance [97].

Faster Breeding Cycles: By eliminating the need for extensive field phenotyping in every generation, GS can significantly shorten breeding cycles, accelerating genetic gain per unit time.

Early Selection: Selection can be performed at the seedling stage, saving resources.

Reduced G x E Interaction Impact: By incorporating environmental data and considering genetic correlations across environments, GS can potentially lead to the selection of genotypes that are more robust across varying stress conditions.

Efficient Resource Allocation: Resources can be concentrated on phenotyping the training population accurately, and then applied more broadly through genotyping.

Application in Complex Traits: GS has shown great promise for improving drought and heat tolerance in crops.

Maize: GS has been successfully applied to improve grain yield under drought stress in maize, demonstrating higher prediction accuracies than MAS [98].

Wheat and Rice: Researchers are increasingly exploring GS for improving yield and stability under drought and heat stress in wheat and rice, demonstrating its potential for more efficient selection [99, 100].

Other Crops: GS models are being developed and tested in various other crops for stress tolerance, including sorghum, chickpea, and common bean.

6.3. Gene Pyramiding

Gene pyramiding, also known as gene stacking, involves combining multiple desirable genes or QTLs into a single genotype. This is particularly important for complex traits like drought and heat tolerance, which are influenced by several genes contributing to different tolerance mechanisms.

Mechanism: Pyramiding is typically achieved through sequential backcrossing or complex crossing schemes combined with MAS (Ye et al., 2012). For example, a breeder might first introgress a QTL for deeper rooting into an elite line, then cross that improved line with another line carrying a QTL for stay-green, using markers to track both QTLs.

Benefits: Pyramiding multiple genes, each contributing to a different aspect of tolerance (e.g., root architecture, water use efficiency, oxidative stress protection), can lead to more robust and broad-spectrum tolerance compared to improving a single trait [101].

Challenges: The major challenge lies in tracking multiple genes simultaneously and ensuring their effective combination without significant linkage drag. High-throughput genotyping platforms and efficient marker assays are crucial for successful gene pyramiding.

6.4. Speed Breeding

Speed breeding is a technological innovation that accelerates plant generation cycles, dramatically reducing the time required for breeding programs. It is not a genetic strategy itself but a method that enhances the efficiency of all breeding approaches, including those targeting stress tolerance.

Mechanism: Speed breeding involves manipulating environmental conditions (e.g., extended photoperiod, controlled temperature, humidity, light intensity, and often early harvesting of seeds) in controlled environments (e.g., growth chambers, greenhouses) to shorten the time to flowering and seed set [102]. For example, wheat can go from seed to seed in 60-70 days instead of 120-150 days.

Benefits:
Accelerated Genetic Gain: More breeding cycles per year translate to faster accumulation of desirable alleles and quicker release of new varieties [103].

Faster Gene Pyramiding: Multiple genes can be stacked more rapidly.

Quicker Trait Introgression: Desirable stress tolerance traits can be transferred into elite lines much faster.

Improved Screening: Allows for more rapid screening of germplasm under controlled stress conditions.

Applications: Speed breeding is increasingly being applied to accelerate breeding for drought and heat tolerance in major crops like wheat, rice, barley, and canola, enabling breeders to respond more quickly to evolving climate challenges [103].
Molecular breeding approaches, particularly MAS and GS, offer powerful tools to overcome the complexities of breeding for drought and heat tolerance. When combined with speed breeding, they provide a robust framework for accelerating the development and deployment of climate-resilient crop varieties.

7. Genetic Engineering and Genome Editing for Enhanced Tolerance

Beyond traditional and molecular breeding, biotechnological approaches, particularly genetic engineering and more recently, genome editing, offer precise and powerful tools to introduce or modify specific genes for enhanced stress tolerance. These methods allow for the direct manipulation of the plant genome in ways that are often difficult or impossible to achieve .

7.1. Transgenic Approaches (Genetic Engineering)

Genetic engineering, or transgenesis, involves the introduction of foreign DNA (transgenes) from any species (plant, microbe, animal) into a plant's genome to confer a desired trait. This approach has been explored extensively for enhancing abiotic stress tolerance.

Principles:
Gene Identification: Identify candidate genes (e.g., from stress-tolerant wild relatives, model plants, or microbes) that are known to play a role in stress response pathways.

Vector Construction: Clone the gene of interest into a suitable plant expression vector, typically containing a promoter (e.g., constitutive, stress-inducible), the gene sequence, and a terminator. Often, a selectable marker gene (e.g., antibiotic resistance) is included for distinguishing transformed cells.

Transformation: Introduce the vector into plant cells. The most common methods are *Agrobacterium tumefaciens*-mediated transformation (utilizing its natural ability to transfer DNA) or direct gene transfer methods like particle bombardment (gene gun).

Regeneration and Selection: Transformed cells are regenerated into whole plants using tissue culture techniques. Selection markers help in identifying and growing only the transformed cells.

Validation: Transgenic plants are then evaluated for transgene integration, expression, and most importantly, for the enhanced stress tolerance phenotype in controlled environments and field trials.

Genes Targeted and Examples of Successful Transformations: Transgenic strategies for drought and heat tolerance often target genes involved in:

Osmolyte Biosynthesis: Overexpression of genes encoding enzymes for proline (e.g., *P5CS*), glycine betaine (e.g., *BADH*, *CMO*), or trehalose (*TPS*, *TPPC*) synthesis has shown promise. For instance, overexpression of *P5CS* has enhanced drought tolerance in rice, wheat, and tobacco by increasing proline accumulation [105].

Transcription Factors (TFs): TFs are master regulators of stress responses.

DREB/CBF: Constitutive overexpression of *DREB1A* from *Arabidopsis* enhanced drought and heat tolerance in rice, wheat, and canola by activating downstream stress-responsive genes [106, 107].However, this can sometimes lead to a growth penalty under non-stress conditions due to the constitutive expression.

NAC/MYB/bZIP: Overexpression of specific NAC TFs (e.g., *OsNAC6*, *OsNAC10*) has improved drought tolerance in rice by regulating root development and senescence (Hu et al., [108].Similarly, some MYB and bZIP TFs have shown potential.

Antioxidant Enzymes: Enhancing the expression of antioxidant enzymes (e.g., *SOD*, *CAT*, *APX*, *GR*) can improve the plant's capacity to scavenge ROS. For example, overexpression of *APX* in tobacco and rice improved tolerance to oxidative stress and drought [109].

Water Channel Proteins (Aquaporins): Overexpression of certain aquaporins (AQPs) has been shown to improve water uptake and transport efficiency under drought conditions in some studies [110].

Heat Shock Proteins (HSPs): Overexpression of small HSPs (sHSPs) or other HSPs has been effective in enhancing heat tolerance by stabilizing proteins and maintaining cellular integrity [111].

Challenges of the Transgenic Approach:

Public Perception and Regulatory Hurdles: Concerns about "genetically modified organisms" (GMOs) and stringent regulatory frameworks have hindered the widespread adoption of transgenic crops for abiotic stress tolerance, particularly in many parts of the world.

Off-target effects: Unintended insertion of transgenes and pleiotropic effects leading to growth penalties.

Cost and Time: The process of creating and deregulating a transgenic crop is often costly and time-consuming.

7.2. CRISPR/Cas9 and Other Genome Editing Tools

Genome editing technologies, particularly the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas system, represent a revolutionary advancement that allows for precise, targeted modifications to the plant genome. Unlike transgenesis, which introduces foreign DNA, genome editing primarily modifies existing genes, resulting in changes that are often indistinguishable from naturally occurring mutations. This has significant implications for regulatory acceptance.

Principles of Genome Editing (CRISPR/Cas9):

Targeting: A synthetic guide RNA (gRNA) is designed to be complementary to a specific 20-nucleotide target sequence in the plant's DNA.

Binding: The gRNA forms a complex with the Cas9 nuclease. This complex then binds to the target DNA sequence.

Cleavage: Cas9, guided by the gRNA, creates a double-strand break (DSB) at the target site.

DNA Repair: The plant's own DNA repair mechanisms are activated:

Non-Homologous End Joining (NHEJ): The most common repair pathway, which is error-prone and often leads to small insertions or deletions (indels) at the break site, effectively creating gene knockouts (frameshift mutations).

Homology-Directed Repair (HDR): If a repair template (donor DNA with homology to the target site) is provided, HDR can be used for precise gene editing, such as introducing specific point mutations, gene knock-ins, or replacing gene sequences.

Delivery: CRISPR/Cas components can be delivered into plant cells via *Agrobacterium*-mediated transformation, particle bombardment, or direct delivery of pre-assembled Cas9-gRNA ribonucleoproteins (RNPs).

Applications in Enhancing Stress Tolerance: Genome editing offers unparalleled precision for enhancing drought and heat tolerance by:

Knock-out of Negative Regulators: Disabling genes that negatively regulate stress response pathways can enhance tolerance. For instance, knocking out genes that promote sensitivity or inhibit adaptive responses can improve performance under stress.

Enhancing Promoter Activity: Modifying promoter regions of stress-responsive genes to increase their expression levels under stress.

Introducing Targeted Mutations: Creating specific point mutations in key enzyme active sites or regulatory regions to improve their function under stress conditions (e.g., making a protein more stable at high temperatures).

Gene Knock-in/Precise Replacement: Introducing specific alleles from tolerant germplasm or modifying existing alleles to improve their function (e.g., swapping a sensitive allele with a tolerant one).

Optimizing Gene Regulation: Altering microRNA (miRNA) binding sites or other regulatory elements to fine-tune gene expression.

Examples of CRISPR/Cas9 in Stress Tolerance:
Rice: CRISPR has been used to knock out genes like *OsERF71*, which improved drought resistance by enhancing root growth [112]. Editing other genes involved in ABA signaling or stomatal development has also shown promise.

Wheat: Genome editing has been used to target genes related to grain size, yield, and potentially stress tolerance by modifying genes like *TaGW2* and *TaGASR7* [113]. Efforts are underway to edit genes involved in heat shock response and water use efficiency.

Maize: CRISPR/Cas9 has been successfully applied to modify genes related to plant architecture and potentially stress response. For example, editing genes like *ZmMPK6* and *ZmABA8ox1* could alter stress signaling (Du et al., 2020).

Tomato: Knockout of *SlGRF4* in tomato improved heat tolerance and yield by enhancing chloroplast development and photosynthesis [114].

Other Genome Editing Tools:
TALENs (Transcription Activator-Like Effector Nucleases): An Earlier generation of site-specific nucleases, also used for gene knockout and targeted modifications.

ZFNs (Zinc Finger Nucleases): Even earlier, less precise, and more complex to design than TALENs or CRISPR.

Base Editors and Prime Editors: Newer, more advanced tools that allow for precise single-nucleotide changes (base editors) or insertions/deletions of short sequences (prime editors) without creating double-strand breaks, offering even higher precision and fewer off-target effects [116]. These are highly promising for fine-tuning stress tolerance genes.

7.3. Ethical and Regulatory Considerations
Ethical Concerns: Public acceptance of genetically modified crops remains a significant hurdle for transgenic crops. Genome-edited crops, especially those with small, targeted changes that could occur naturally, are generally perceived as less controversial and may face fewer regulatory burdens, but ethical discussions continue regarding unintended effects or altering natural genetic diversity.

Regulatory Frameworks: Regulations for GMOs are stringent and vary widely across countries, often requiring extensive testing and approval processes. For genome-edited crops, regulatory landscapes are evolving. Some countries (e.g., Argentina, Brazil, Japan) have adopted a product-based approach, classifying non-transgenic genome-edited plants (without foreign DNA) similarly to conventionally bred varieties, while others (e.g., the EU) continue to regulate them as GMOs [116]. This regulatory diversity impacts the adoption and commercialization of stress-tolerant biotech crops.
Genetic engineering and genome editing offer powerful avenues for developing climate-resilient crops with enhanced drought and heat tolerance, especially by targeting specific genes that confer robust and stable phenotypes. As genome editing technologies become more precise and accessible, they are poised to play an increasingly central role in crop improvement.

8. Physiological and Morphological Traits as Selection Criteria
Effective breeding for drought and heat tolerance requires accurate and reliable selection criteria. While yield under stress is the ultimate target, it is a highly complex and integrative trait influenced by many underlying physiological and morphological processes. Therefore, focusing on these component traits, often called secondary traits, can increase selection efficiency, especially when yield is unstable due to high G x E interactions.

8.1. Root Traits
Root systems are the primary interface for water and nutrient uptake, making their architecture and efficiency critical for drought tolerance.

Root Depth and Angle: Deeper roots can access water from deeper soil profiles during prolonged dry periods, enhancing drought avoidance [18].A steeper root growth angle allows for more rapid penetration into deeper soil layers.

Measurement: Root depth can be assessed directly by excavation or indirectly by observing wilting patterns in specific soil types. Root angle can be measured in hydroponic or soil-based phenotyping systems.

Significance: Genotypes with inherently deeper or more steeply angled roots are often more drought-tolerant, as exemplified by the DRO1 gene in rice [34].

Root Biomass and Density: A larger total root biomass and a higher density of fine roots within the soil profile enhance the surface area for water and nutrient absorption, contributing to more efficient water uptake [19].

Measurement: Destructive sampling (digging up roots, washing, drying, weighing) is common but labor-intensive. Non-destructive methods include root imaging systems (rhizotrons), minirhizotrons, and ground-penetrating radar (GPR).

Significance: Higher root biomass and density under water-limited conditions are strong indicators of drought adaptation.

8.2. Leaf Traits
Leaves are central to photosynthesis and transpiration, making their characteristics critical for water conservation and temperature regulation.

Stomatal Conductance: Stomata regulate uptake for photosynthesis and water release through transpiration. Lower stomatal conductance under drought stress signifies better water conservation, but if too low, it impairs photosynthesis [12].

Measurement: Porometers or infrared gas analyzers (IRGAs) are used to measure stomatal conductance. HTP techniques using thermal imaging can indirectly estimate stomatal conductance through canopy temperature.

Significance: A balance is needed: the ability to close stomata rapidly under stress, but also to open them and recover quickly when water is available.

Canopy Temperature (CT) / Canopy Temperature Depression (CTD): CT is the temperature of the plant canopy. CTD (difference between air temperature and canopy temperature) is a robust indicator of water status and transpirational cooling (Blum, 2011). A lower CTD (cooler canopy) indicates greater transpirational cooling, suggesting better water uptake and/or more efficient water use, beneficial under both drought and heat stress.

Measurement: Handheld infrared thermometers or, more efficiently, thermal cameras mounted on ground vehicles or UAVs for HTP [83].

Significance: Cooler canopies are often associated with higher yields under drought and heat stress.

Leaf Rolling and Folding: A common drought avoidance mechanism in cereals where leaves roll or fold to reduce exposed surface area, thereby reducing light interception and transpirational water loss.

Measurement: Visual scoring based on a scale (e.g., 1-5 or 1-9).

Significance: While reducing water loss, excessive rolling can limit photosynthesis. A moderate and timely response is desirable.

Stay-Green Phenotype: The ability of a plant to maintain green leaf area (photosynthetic activity) during stress, particularly during grain filling [24].

Measurement: Visual scoring, chlorophyll content meters (SPAD readings), or HTP using RGB/NIR imaging to quantify greenness over time.

Significance: Directly correlates with sustained photosynthesis and improved grain filling under terminal drought and heat stress, leading to higher yields. Highly valuable in sorghum, maize, and wheat.

8.3. Water Use Efficiency (WUE) Components

WUE refers to the ratio of biomass or grain yield produced per unit of water consumed. It can be measured at different levels: instantaneous (physiological), whole-plant (gravimetric), or canopy/field (yield per unit of rainfall/irrigation).

Carbon Isotope Discrimination (Δ13C): This physiological trait is inversely related to WUE. Plants with higher WUE discriminate less against the heavier isotope of carbon (13C) during photosynthesis.

Measurement: Mass spectrometry of plant tissue (leaves, grains).

Significance: Useful for identifying genotypes with inherently higher WUE, but requires specialized equipment [118].

8.4. Membrane Stability Index (MSI)

Cell membranes are highly sensitive to oxidative stress induced by drought and heat. MSI is a measure of cellular membrane integrity, often assessed by electrolyte leakage (leakage of ions from damaged cells) from leaf tissues subjected to stress.

Measurement: Conductivity meter to measure ion leakage after heating or drought treatment, compared to untreated samples [119].

Significance: Higher MSI indicates greater membrane integrity and thus better tolerance to oxidative damage caused by stress. It is a widely used and relatively simple physiological indicator for both drought and heat tolerance.

8.5. Chlorophyll Fluorescence

Chlorophyll fluorescence measures the efficiency of photosystem II (PSII), which is highly susceptible to both drought and heat stress. Changes in fluorescence parameters (e.g., Fv/Fm, ΦPSII) indicate damage to the photosynthetic machinery.

- **Measurement:** Pulse-amplitude modulation (PAM) fluorometers, or HTP fluorescence imaging systems [85].
- **Significance:** A decrease in chlorophyll fluorescence efficiency indicates stress-induced damage to the photosynthetic apparatus, making it a valuable tool for early stress detection and tolerance assessment.

These physiological and morphological traits serve as valuable proxy traits for yield under stress. Their precise measurement, especially with the aid of HTP technologies, enables breeders to screen large populations more effectively and select for genotypes with desirable adaptive mechanisms, contributing to the overall drought and heat tolerance of new crop varieties.

9. Integration of Omics Technologies in Breeding Programs

The integration of various 'omics' technologies—genomics, transcriptomics, proteomics, metabolomics, and phenomics—represents a paradigm shift in plant breeding. This multi-pronged approach allows for a comprehensive, systems-level understanding of stress responses, accelerating gene discovery, functional validation, and ultimately, the development of climate-resilient crops.

9.1. Transcriptomics for Identifying Differentially Expressed Genes

Transcriptomics, primarily using RNA sequencing (RNA-Seq), provides a global view of gene expression changes under stress.

Application: By comparing gene expression profiles of tolerant vs. sensitive genotypes, or plants under stress vs. optimal conditions, researchers can identify:

Differentially Expressed Genes (DEGs): Genes whose expression levels significantly change in response to drought or heat. These DEGs often include genes involved in signal transduction, transcription factors, enzymes for osmolyte synthesis, antioxidant defense, and heat shock proteins [58].

Stress-Responsive Pathways: Identification of entire biological pathways that are activated or repressed, providing insights into the plant's adaptive mechanisms.

Integration with Breeding: DEGs and their associated pathways can serve as a rich source of candidate genes for functional validation (e.g., through genetic transformation or genome editing) or for developing molecular markers for MAS. Transcriptomic data can also inform the design of gene expression assays for high-throughput screening of breeding lines.

9.2. Proteomics for Understanding Protein Networks

Proteomics focuses on the identification and quantification of proteins, providing insights into the actual functional machinery of the cell under stress.

Application:

Protein Abundance Changes: Identifying proteins whose levels change in response to drought or heat. These often include HSPs, antioxidant enzymes, proteins involved in energy metabolism, and proteins related to protein folding and degradation [59].

Post-Translational Modifications (PTMs): Identifying PTMs (e.g., phosphorylation, ubiquitination) that regulate protein activity and stability, which are crucial for rapid stress responses.

Integration with Breeding: Proteomic data can validate findings from transcriptomics (confirming whether changes in mRNA levels translate to protein changes) and identify key regulatory proteins. Proteins identified as crucial for tolerance can become targets for genetic engineering or be used to develop biochemical markers for selection.

9.3. Metabolomics for Stress-Induced Metabolic Changes

Metabolomics, the study of small molecule metabolites, offers a functional snapshot of the plant's physiological state and metabolic adjustments to stress.

Application:

Biomarker Identification: Identifying specific metabolites that accumulate or deplete under drought/heat stress, which can serve as biomarkers for tolerance. Examples include osmolytes (proline, sugars, polyols), amino acids, and secondary metabolites (flavonoids, phenolics) with antioxidant properties [25].

Pathway Flux Analysis: Understanding how metabolic pathways are reconfigured to cope with stress, for instance, shifts in primary metabolism to produce stress-protective compounds.

Integration with Breeding: Metabolite profiles can be used as a high-throughput phenotyping trait for screening germplasm. Identifying key metabolites can lead to the discovery of novel metabolic pathways or enzymes that can be targeted for genetic improvement through breeding or engineering.

9.4. Phenomics for High-Throughput Phenotyping

As discussed earlier, phenomics involves the high-throughput measurement of plant phenotypes using various imaging and sensor technologies.

Application: Enables rapid, non-destructive, and precise quantification of complex traits (e.g., growth rate, biomass, water use, canopy temperature, chlorophyll content, senescence) across large populations under controlled stress conditions or in the field [82].

Integration with Breeding: Phenomics generates massive datasets that are critical for:

QTL Mapping and GWAS: Providing precise phenotypic data to link with genomic data for identifying stress-tolerance QTLs and genes.

Genomic Selection: Supplying accurate phenotypic records for the training population, which is essential for building robust predictive models.

Validation of Gene Function: Rapidly evaluating the phenotypic effects of genes identified through other omics approaches or genetically engineered for tolerance.

9.5. Systems Biology Approach

The most powerful strategy involves the synergistic integration of all these omics datasets within a systems biology framework.

Concept: This approach aims to build comprehensive models that describe the complex interplay between genes, transcripts, proteins, and metabolites, and how these molecular components interact to produce the observed phenotypic responses under stress [61].

Data Integration and Network Analysis: Advanced bioinformatics and computational tools are used to integrate disparate omics datasets. Network analysis (e.g., gene regulatory networks, protein-protein interaction networks, metabolic networks) helps to identify central 'hubs' or key regulatory nodes that are critical for orchestrating stress responses.

Benefits for Breeding:

Holistic Understanding: Provides a deeper and more holistic understanding of stress tolerance mechanisms than individual omics approaches.

Identification of Novel Targets: Uncovers novel genes, pathways, and regulatory elements that might not be apparent from single-layer analyses, providing new targets for breeding and genetic engineering.

Prioritization of Candidate Genes: Helps prioritize the most promising candidate genes for functional validation, gene editing, or marker development based on their central role in stress response networks.

Predictive Models: Contributes to developing more accurate predictive models for stress tolerance, enabling more efficient selection in breeding programs.

By integrating the wealth of data generated by omics technologies, breeders can move from a 'trial-and-error' approach to a more 'knowledge-based' and predictive breeding strategy, significantly accelerating the development of climate-resilient crop varieties.

10. Case Studies: Progress in Major Crop Species

Significant progress has been made in breeding for drought and heat tolerance across major crop species, demonstrating the effectiveness of the genetic strategies discussed. These advancements are critical for safeguarding global food production.

10.1. Cereals

Cereals, including rice, wheat, maize, and sorghum, form the staple food for a large portion of the world's population, making their resilience to climate change paramount.

Rice (*Oryza sativa*):
Drought Tolerance: The International Rice Research Institute (IRRI) has led extensive efforts. The identification and deployment of the DRO1 (Deeper Rooting 1) gene has been a landmark achievement [34].This gene allows roots to grow deeper, accessing sub-surface water. Varieties like *Sahbhagi Dhan* (India) and *Tardiva* (Philippines) were developed through conventional breeding and MAS, exhibiting improved yield stability under drought. MAS for QTLs related to yield under drought (qDTYs) on chromosomes 1, 3, and 12 has also been instrumental in developing improved drought-tolerant rice varieties such as *DRR Dhan 44* and *DRR Dhan 46* [35, 120].

Heat Tolerance: Rice is highly sensitive to heat stress at flowering. IRRI's work has focused on identifying heat-tolerant donors and QTLs for spikelet fertility and pollen viability under high temperatures. Markers linked to these QTLs are being used in breeding programs to develop heat-tolerant varieties suitable for hotter climates [7].

Wheat (*Triticum aestivum*):
Drought Tolerance: CIMMYT (International Maize and Wheat Improvement Center) has spearheaded global efforts. Breeding programs emphasize traits like early vigor, deeper rooting, water use efficiency (e.g., higher canopy temperature depression), and the "stay-green" phenotype. Varieties like *CIMMYT's Dryland Wheat* lines (e.g., *Borlaug 100*) are examples of successful breeding for rain-fed conditions. MAS has been applied to introgress QTLs for grain yield under drought and canopy temperature [62].Genomic selection is increasingly being used to improve drought-adaptive traits across diverse wheat breeding populations [63].

Heat Tolerance: Breeding for heat tolerance in wheat focuses on maintaining grain filling under high temperatures. Traits like membrane stability index, grain weight, and grain number are key. Heat-tolerant varieties have been developed in India and other regions through conventional breeding. New heat-tolerant lines are often characterized by better flag leaf photosynthesis and efficient assimilate partitioning under high temperatures [63].

Maize (*Zea mays*):
Drought Tolerance: CIMMYT and various national programs have made significant progress. Drought-tolerant maize hybrids exhibit reduced anthesis-silking interval (ASI), improved pollen viability, and sustained kernel set under water deficit [121]. Molecular breeding, particularly MAS and GS, has been critical. For example, MAS has been used to introgress drought-adaptive QTLs into elite maize hybrids.
Several drought-tolerant maize hybrids are now commercially available, demonstrating improved yield stability in drought-prone areas (e.g., *DroughtTEGO* series from Syngenta, *DRM* series from CIMMYT).

Heat Tolerance: Maize is also sensitive to heat during flowering. Breeders are selecting for genotypes with better pollen viability and reduced ASI under high temperatures. Some drought-tolerant maize lines also exhibit improved heat tolerance due to overlapping physiological mechanisms.

Sorghum (*Sorghum bicolor*):
Drought Tolerance: Sorghum is naturally more drought-tolerant due to its efficient C4 photosynthesis and robust root system. The "stay-green" phenotype is a cornerstone of drought tolerance in sorghum, where the leaves remain green and photosynthetically active during the grain-filling stage, allowing for continued assimilation even under terminal drought [42]. The underlying QTLs (Stg1, Stg2, Stg3, Stg4) have been identified and successfully introgressed into elite lines using MAS, leading to superior drought-tolerant varieties [44].

Heat Tolerance: Sorghum's C4 pathway contributes to its high-temperature tolerance. Breeding efforts focus on maintaining yield under extreme heat, with traits like flowering time and grain filling being key targets.

10.2. Legumes

Legumes are vital for food security and soil fertility, but they are also highly susceptible to drought and heat stress.

Chickpea (*Cicer arietinum*):
Drought Tolerance: Chickpea is often grown in rain-fed environments and is highly vulnerable to terminal drought. ICARDA (International Center for Agricultural Research in the Dry Areas) and ICRISAT (International Crops Research Institute for the Semi-Arid Tropics) have focused on breeding for traits like deeper rooting, early maturity (drought escape), and improved water use efficiency. A major achievement has been the identification of robust QTLs for drought tolerance (e.g., for root traits, canopy temperature, and yield under drought) through extensive phenotyping and mapping [45].MAS is being used to accelerate the development of drought-tolerant chickpea varieties.

Heat Tolerance: Heat stress significantly impacts chickpea yield, especially during flowering. Breeding efforts are directed towards developing heat-tolerant genotypes that can set pods and fill grains under high temperatures.

Common Bean (*Phaseolus vulgaris*):
Drought Tolerance: CIAT (International Center for Tropical Agriculture) has led efforts to breed drought-tolerant common beans. Traits like deeper rooting, efficient water extraction, and the ability to recover after stress are targeted. Molecular breeding has identified QTLs associated with drought tolerance, and MAS is being used to introgress these into elite varieties [46].

Heat Tolerance: Common bean is highly sensitive to heat stress during flowering, leading to flower abortion and yield loss. Heat-tolerant common bean lines have been identified and used in breeding programs, with selection based on pod set percentage and seed weight under high temperatures [68].

Soybean (*Glycine max*):
Drought and Heat Tolerance: Soybean is sensitive to both stresses, particularly during reproductive stages. Breeding strategies focus on traits like efficient water use, maintenance of photosynthetic activity, and sustained pod development. Genomics and transcriptomics have identified genes and QTLs related to these traits. Some drought-tolerant soybean varieties have been developed that exhibit improved yield stability in water-limited environments. Genetic engineering has also explored enhancing tolerance through the overexpression of stress-responsive genes in soybean [122].

10.3. Other Important Crops

Cotton (*Gossypium hirsutum*): Drought tolerance in cotton is critical for rain-fed cultivation. Breeding efforts target improved water use efficiency, deeper root systems, and maintenance of boll retention under stress. QTLs for root traits, biomass, and lint yield under drought have been identified.

Potato (*Solanum tuberosum*): Potato is a highly water-demanding crop. Breeding for drought tolerance focuses on efficient water use, the ability to recover from stress, and the maintenance of tuber yield. Physiological traits like stomatal conductance and canopy temperature are often used as selection criteria.

Groundnut (*Arachis hypogaea*): Groundnut is grown in many semi-arid regions. Breeding for drought tolerance emphasizes traits such as "stay-green," biomass production under stress, and pod filling efficiency. MAS for QTLs associated with terminal drought tolerance has been applied to develop improved varieties [123].These case studies underscore the diverse approaches employed and the tangible progress achieved in breeding for climate resilience across a spectrum of vital food crops. The combination of conventional methods with advanced molecular and biotechnological tools is proving to be a powerful strategy in developing varieties capable of withstanding the intensifying challenges of drought and heat stress.

Table 2: Major QTLs/Genes Identified for Drought and Heat Tolerance in Key Crop Species

Crop Species	Stress Type	Trait (Phenotype)	Key QTLs/Genes (Example)	Mechanism/Function
Rice (*Oryza sativa*)	Drought	Deeper Rooting	DRO1 (Deeper Rooting 1)	Modulates root growth angle, enabling deeper water access.
	Drought	Yield under Drought	qDTY1.1, qDTY12.1, qDTY3.1	Contribute to yield stability under water deficit.
	Drought	Water Use Efficiency	OsDREB1A, OsNAC6, OsNAC10	Transcription factors regulating stress response genes, root development.
	Heat	Spikelet Fertility, Pollen Viability	QTLs on Chr 1, 4, OsHSP17.7	Involved in reproductive stage heat tolerance, protein protection.
Wheat (*Triticum aestivum*)	Drought	Grain Yield, Stay-Green	QTLs on 1B, 2B, 3A, 4A, 5A, 7A, 2D, 3D, 5D	Improve yield stability, prolong photosynthesis under terminal drought.
	Drought	Canopy Temperature Depression	QTLs linked to CTD	Indirectly related to water use efficiency and cooling.
	Heat	Grain Weight, Membrane Stability	QTLs on 3A, 5A, 7A, 5D	Maintain grain filling and cellular integrity under high temperatures.
Maize (*Zea mays*)	Drought	Grain Yield, ASI	QTLs for ASI, Stay-Green	Reduce flowering asynchronous, maintain green leaf area.
	Drought	Root Traits	QTLs on various chromosomes	Influence root depth and biomass for water uptake.
	Heat	Pollen Viability, Kernel Number	QTLs linked to pollen viability, yield under heat	Sustain reproductive success under heat stress.
Sorghum (*Sorghum bicolor*)	Drought	Stay-Green	Stg1, Stg2, Stg3, Stg4	Maintain green leaf area during grain filling, crucial for terminal drought.
	Drought	Osmotic Adjustment	QTLs for osmotic potential	Contribute to cellular turgor maintenance.
Chickpea (*Cicer arietinum*)	Drought	Root Mass/Depth, Yield	QTLs for root architecture, yield indices	Enhance water extraction and yield stability under water deficit.
	Heat	Pod Set, Seed Weight	QTLs for podding efficiency under heat	Maintain reproductive success and yield under high temperatures.
Common Bean (*Phaseolus vulgaris*)	Drought	Yield, Water Uptake	QTLs on various chromosomes	Improve water extraction efficiency and yield stability.
	Heat	Pod Set, Seed Weight	QTLs for reproductive heat tolerance	Maintain pod development and seed filling under high temperatures.
Soybean (*Glycine max*)	Drought/Heat	Yield, Photosynthesis	QTLs/Genes like GmNAC, GmDREB	Transcription factors regulating stress response.

Note: This table provides examples and is not exhaustive. The precise genomic locations and functional validation of many QTLs/genes are ongoing research areas.

11. Challenges and Future Perspectives

Despite the remarkable progress in understanding and breeding for climate resilience, significant challenges remain. Drought and heat tolerance are complex traits, influenced by multiple genes and strong interactions with the environment. Addressing these challenges and integrating cutting-edge technologies will be crucial for securing future food production.

11.1. Complexity of Stress Tolerance (Multi-genic, G x E Interaction)

Polygenic Nature: Both drought and heat tolerance are controlled by numerous genes, each contributing a small effect. This polygenic architecture makes it challenging to identify and manipulate all contributing genes through traditional breeding or even simple MAS. The combined effect of many small-effect genes often underlies robust tolerance.

Genotype-by-Environment (G x E) Interaction: The expression of stress tolerance genes and the phenotypic performance of a genotype are highly dependent on the specific environmental conditions, including the severity, duration, and timing of stress, soil type, and nutrient availability [88].A genotype tolerant in one environment might be sensitive in another, making the identification of broadly adapted, stable tolerant varieties difficult. This necessitates extensive multi-location and multi-year trials.

Combined Stresses: The increasing co-occurrence of drought and heat stress, along with other abiotic (e.g., salinity, nutrient deficiency) and biotic stresses, adds another layer of complexity. Responses to combined stresses are often unique and not simply additive to individual stress responses [8].Breeding for multiple stresses simultaneously is a formidable challenge.

11.2. Phenotyping Bottlenecks

As previously discussed, accurate, precise, and high-throughput phenotyping under realistic stress conditions remains a significant bottleneck.

Cost and Labor: Manual phenotyping is laborious and expensive, limiting the size of populations that can be evaluated.

Scalability: While HTP technologies (drones, robotics, sensors) are advancing, their widespread adoption and affordability for all breeding programs remain challenges, particularly in developing countries.

Environmental Control: Mimicking real-world, dynamic stress conditions in controlled environments (greenhouses, growth chambers) is difficult, and field phenotyping introduces uncontrollable environmental variability.

Root Phenotyping: Characterizing root architecture and function non-destructively in the field is still a major challenge, despite its critical importance for drought tolerance [55].

11.3. Integrating Different Strategies

The optimal path forward involves the judicious integration of conventional breeding, molecular breeding, and biotechnological approaches.

Seamless Integration: Developing a pipeline that seamlessly integrates phenotypic data from HTP, genomic data from high-throughput genotyping, transcriptomic/proteomic/metabolomic insights, and functional validation from genetic engineering/editing is essential. This requires interdisciplinary teams and robust data management and analytical platforms.

"Omics-to-Field" Translation: Translating discoveries from lab-based omics studies (genes, pathways) into tangible improvements in field-grown crops remains a major challenge. Validating the function of candidate genes in diverse genetic backgrounds and environments is critical.

Pre-breeding and Germplasm Enhancement: Strengthening pre-breeding efforts to introduce novel stress tolerance alleles from wild relatives and landraces into elite breeding lines is crucial. This widens the genetic base available for breeding.

11.4. Climate-Smart Agriculture and Sustainable Practices

Breeding efforts must be complemented by the adoption of climate-smart agricultural (CSA) practices.

CSA Practices: These include water-saving irrigation techniques (e.g., drip irrigation, deficit irrigation), conservation agriculture (e.g., no-till, cover cropping to improve soil moisture retention), improved nutrient management, and diversified cropping systems.

Systems Approach: Resilient agricultural systems require not just tolerant varieties but also improved management practices, policies that support adaptation, and robust knowledge-sharing mechanisms.

Agro-ecology: Integrating principles of agro-ecology to enhance ecosystem resilience and reduce reliance on external inputs can complement genetic improvements.

11.5. Ethical and Societal Acceptance of New Technologies

Public Dialogue: Continued open and transparent dialogue with the public about the benefits and risks of genetic engineering and genome editing is essential for gaining societal acceptance.

Regulatory Harmonization: Harmonizing regulatory frameworks globally for genome-edited crops that do not contain foreign DNA could accelerate their deployment and commercialization, especially in regions facing severe food security challenges.

11.6. Future Research Directions

Future research should focus on several key areas to accelerate the development of climate-resilient crops:

Functional Genomics and Gene Stacking: Deeper understanding of gene function and regulatory networks underlying stress tolerance. Prioritizing the identification of pleiotropic genes (genes affecting multiple traits) or 'hub' genes controlling multiple tolerance mechanisms. Efficiently stacking multiple stress tolerance genes using advanced molecular tools and speed breeding.

Exploiting Genetic Diversity: Systematically exploring and phenotyping underexploited genetic resources, including wild relatives and neglected and underutilized species (NUS), which may harbor novel adaptive genes.

Multi-Stress Tolerance: Shifting from single-stress tolerance to breeding for combined or multi-stress tolerance, reflecting real-world agricultural conditions.

CRISPR/Cas and Beyond: Further refining genome editing tools (e.g., base editing, prime editing) for more precise and efficient targeted modifications, reducing off-target effects, and expanding their applicability to a wider range of crops. Developing transgene-free genome-edited plants for easier regulatory approval.

Artificial Intelligence (AI) and Machine Learning (ML) in Breeding: Leveraging AI and ML for:

Predictive Breeding: Improving the accuracy of genomic prediction models by integrating diverse omics and environmental data.

Phenotype Prediction: Developing robust models to predict complex phenotypes from genomic and environmental data.

Trait Discovery: Identifying novel stress-tolerant traits from large-scale phenomic datasets.

Optimal Crossing Strategies: Using algorithms to design optimal crossing schemes to combine desired alleles.

High-Throughput Root Phenotyping: Developing non-destructive, high-throughput technologies for detailed root phenotyping in field conditions.

Microbiome Engineering: Exploring the role of plant microbiomes (beneficial microbes associated with roots and shoots) in enhancing stress tolerance and integrating microbiome-based solutions into breeding strategies [124].

Farmer Participation and Co-creation: Involving farmers in the breeding process to ensure that developed varieties meet local needs, preferences, and environmental conditions (participatory plant breeding).

12. Conclusion

Agricultural challenges on the global scale have now reached a tipping point with unprecedented changes of increasing drought and heat stress, which have occurred as a result of climate change. The way to achieve this response level of food security in the face of a growing population is dependent on the capability of coming up with and implementing climate-resistance crop varieties. Through this extensive review, it can be emphasized that there have been considerable developments towards the realization of complex physiological, morphological, and biochemical means that are utilized by plants in responding to these abiotic stress conditions. This complements the genetic approaches at the conventional side of breeding and advanced molecular breeding methods such as Marker-Assisted Selection (MAS) or Genomic Selection (GS) but also the fine-grained manipulations of genetic engineering on the one hand and the genome editing methods revolutionizing bio-engineering (e.g., CRISPR/Cas9) on the other.

An example is the identification and deployment of key Quantitative Trait Loci (QTLs) and candidate genes e.g.-, in rice, deeper rooting via DRO1; in sorghum, stay-green QTLs. Furthermore, phenotyping with high-throughput systems, coupled with the panel of the so-called omics technologies, i.e., genomics, transcriptomics, proteomics, and metabolomics, is revolutionizing our capacity to delink the stress response networks, and identify new genetic targets, as well as fast-tracking the breeding pipeline

Nevertheless, these breakthroughs come with big challenges that persistently exist and these include the multifactorial polygenic character of stress tolerance, the widespread effect of genotype-environment interactions, and difficulties in assessing high throughput phenotypes, particularly that of root traits. The breeding of climate resilience in the future requires a strongly multidisciplinary approach. This includes a synergistic use of conventional and molecular breeding as part of the equation, the specificity of post-genomic biotechnologies, the force of systems biology and artificial intelligence to resolve and control the complex genetic framework of stress tolerance. More so, the need to adopt climate-smart farming methodologies and the promotion of candid open forums among the populace about the new breeding technologies are also important in the scope of sustaining sustainable production and mass adoption of the resistant crops. Finally, all the countries of the world should unite their efforts in research, development, and deployment to build resistant agricultural structures to face the involuntary consequences of climate change, preserve lives and guarantee tomorrow food security of the world.

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