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Review

Confronting Heat Stress in Crops Amid Global Warming: Impacts, Defense Mechanisms, and Strategies for Enhancing Thermotolerance

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ABSTRACT

Crops constantly face a range of biotic and abiotic stresses that negatively affect their growth and productivity. Among these, temperature-related stress, exacerbated by global climate change, poses a significant threat to crop production worldwide. Heat stress, in particular, leads to a cascade of detrimental effects, including impaired photosynthesis, increased respiration rates, membrane damage, protein denaturation, inhibited cell division and elongation, and reduced reproductive success, ultimately resulting in yield loss. In response, plants activate several defense mechanisms such as the biosynthesis of heat shock proteins, antioxidants, and reactive oxygen species scavengers, alongside the activation of heat stress-responsive transcription factors. Enhancing thermotolerance in crop plants is thus crucial for sustaining global food security. Key approaches include breeding, transgenic technologies, and the exogenous application of thermos-protectants. This review explores the different impacts of heat stress, plant responses, and the latest strategies to strengthen heat stress tolerance in major crop species.

KEYWORDS: heat stress; heat stress responses; thermotolerance

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ABBREVIATIONS

HSP, heat shock protein; HSF, heat stress transcription factors; ROS, reactive oxygen species; QTLs, quantitative trait loci; RT-PCR, real-time polymerase chain reaction; IPCC, Intergovernmental Panel on Climate Change; OEC, Oxygen Evolving Complex; PSI, Photosystem I; PSII, Photosystem II; PEP, phosphoenolpyruvate; SA, salicylic acid; IAA, indole acetic acid; JA, Jasmonic acid; ABA, Abscisic acid; PUFA, polyunsaturated fatty acids; APX, ascorbate peroxidase; SOD, superoxide dismutase; POX, peroxidase; GR, glutathione reductase; CAT, catalase; ASC, ascorbate; GSH, glutathione; GPX, glutathione peroxidase; PAL, phenylalanine ammonia-lyase; CDPK, calcium-dependent protein kinases; SSA, sulphosalicylic acid;

MeSA, methyl salicylate; MAGIC, Multi-parents advanced generation intercross; GWAS, genome-wide association studies; GBS, genotyping by sequencing; SNP, single nucleotide polymorphism

INTRODUCTION

External environmental factors often have detrimental effects on the growth, development, and productivity of crops, especially since they are sessile. These factors can be divided into biotic and abiotic factors, which can cause various stresses in plants. Biotic stresses involve diseases caused by various pathogens including bacteria, fungi, nematodes, viruses and damage by insects, herbivores, and even weeds. While abiotic stresses primarily pertain to non-living environmental factors (either physical or chemical conditions) and their extremes, including drought, heat, salinity, waterlogging, radiation and contaminants such as heavy metals (an emerging concern associated with agrochemical usage and industrialization) [1]. Major abiotic stresses encountered by plants include heat stress, cold stress (especially in temperate regions), salinity stress, drought stress, and toxin stress [1]. Some of these abiotic stress conditions are interconnected. For example, salinity stress is correlated with osmotic stress and iron toxicity, while heat stress is connected to osmotic stress and oxidative stress [2]. Heat and drought stresses are interrelated abiotic stress conditions in plants, as heat stress leads to a reduction in water levels by increasing the transpiration rate [3]. Additionally, the phenological and anatomical changes induced by elevated temperatures are generally similar to the responses observed in drought stress.

Among all the abiotic stresses, heat stress is an important aspect of abiotic stress attributed to global warming. Global warming, or the gradual increase in the Earth's overall atmospheric temperature, is mainly caused by the emission of greenhouse gases like carbon dioxide, methane, chlorofluorocarbons, and nitrous oxide. According to the report of the Intergovernmental Panel on Climate Change (IPCC), a rise of 1.5 ℃ in global warming from 2021 to 2040 has been estimated [4]. This will profoundly affect agriculture, especially the geographical distribution and growth of food crops. According to Kan et al., a 1 ℃ rise in global temperature leads to an average yield reduction in three main grain crops and it is approximately 6.0% in wheat (*Triticum aestivum*), 3.2% in rice (*Oryza sativa*) and 7.4% in maize (*Zea mays*) [5]. Furthermore, Huang et al. have stated that the increase in global temperature has affected not only the harvest of these major grain crops but also legume crops [6].

As each plant exhibits normal metabolism and activities within an optimum temperature range. Beyond this range, extremes hinder the plant's growth and development, triggering unfavorable physiological, biochemical and molecular responses including reduced photosynthesis and reproduction, altered nutrient uptake and metabolism, impaired water and osmotic potentials, and damaged cell structures. The temperature at which a detectable reduction in growth begins is referred to as the temperature stress threshold for that specific plant [7]. According to the definition, there should be a lower and an upper developmental threshold which is defined as maximum temperature and minimum temperature that minimize the growth and cause heat stress and cold stress, respectively.

Plants can actively respond to the stresses at phenotypic, physiological, and molecular levels through various defense mechanisms [1,8]. Most of the responses initiate through a signal transduction pathway, culminating in cellular responses that aid the plant in surviving, flourishing, or developing tolerance to stress. This encompasses changes in gene expression, metabolism, growth, and development [8]. These include defense mechanisms such as the accumulation of heat shock proteins (HSPs), activation of heat stress transcription factors (HSF), and acquired thermotolerance by the accumulation of antioxidants and other reactive oxygen species (ROS) scavengers. It is important to characterize the molecular basis of natural heat stress tolerance by identifying major quantitative trait loci (QTLs) and candidate genes. This effort will aid in accelerating crop breeding programs, using marker-assisted selection to develop varieties with enhanced heat stress tolerance. Researchers identified major genes and QTLs related to heat stress tolerance through methods such as QTL mapping, microarray analysis, transcriptome analysis, or real-time polymerase chain reaction (RT-PCR). Various studies have attempted to enhance thermotolerance and mitigate the adverse effects of heat stress by employing strategies and mechanisms such as the exogenous application of heat damage protectants, conventional and marker-assisted breeding, transgenic and other biotechnological approaches such as heat-responsive genes knocking out or overexpression.

This review is essential as rising global temperatures are an imminent threat to agriculture, directly impacting crop yields and food security. In this review, we summarize relevant literature on different studies related to heat stress responses and thermotolerant mechanisms of plants in a broader scope. We focus on integrating physiological, molecular, and biotechnological perspectives to address the modern demands of crop improvement. Rather than merely cataloging heat stress responses, this review provides an assessment of multi-dimensional defense mechanisms and explores practical, actionable strategies to enhance thermotolerance. By combining traditional breeding approaches with cutting-edge biotechnological tools such as CRISPR and other transgenic methods it offers insights into the latest advancements for improving crop resilience to heat stress. The review also aims to provide a comprehensive overview of plant responses to heat stress by exploring key mechanisms across a wide range of species. The species discussed were selected based on their agricultural significance, importance as model plants, availability of experimental data, and their ability to represent diverse plant systems, including monocots, dicots, C3, and C4 plants. By highlighting examples from various species, such as model plant species, Arabidopsis and agriculturally important crops, maize, rice, wheat, peanut, soybean, tobacco, potato, and tomato, the review captures both universal and species-specific heat stress responses. This approach ensures the findings are applicable to global agricultural challenges and highlights strategies that can be adapted across different crops. Additionally, a dedicated section highlights candidate genes that have been functionally validated for thermotolerance, serving as a valuable resource for targeted breeding programs.

HEAT STRESS AND GENERAL HEAT STRESS RESPONSES IN PLANTS

Generally, a temperature of 10–15 ℃ above the optimum is causing a heat shock or heat stress [9]. The heat stress threshold depends on several factors, such as the geographic zone of the crop [7]. For instance, tropical crops have a higher temperature threshold than temperate crops [10], given that tropical zones generally experience higher temperatures where heat stress becomes a major limiting factor. The thermal lethal temperatures of aquatic, temperate, and desert plants are 38–42 ℃, 45–55 ℃, and 60–65 ℃ respectively [11]. Other factors influencing the threshold include the developmental stage and even the different genotypes of the same species. As an example, heat stress has a greater impact on the photosynthetic activity of younger leaves of corn than that of mature leaves [12].

Heat stress is defined as “the rise in temperature beyond a threshold level for a period of time sufficient to cause irreversible damage to plant growth and development”, usually about 10–15°C above the optimum temperature [7]. The level of heat stress is influenced by factors such as intensity, duration, and the rate of temperature increment. For example, long-term exposure to higher temperatures can result in the loss of seed vigor for germination and hinder the germination process. Additionally, studies have shown that a rapid increase in temperature has more severe effects on metabolic activities like photosynthesis compared to a gradual temperature increase [13]. The rate at which temperature increases significantly influences the heat stress responses in crops, affecting their growth, development, and survival. Rapid temperature spikes often leave less time for crops to activate protective mechanisms such as heat shock protein (HSP) production, antioxidant enzyme activity, and osmolyte accumulation, resulting in greater cellular damage and reduced resilience. Conversely, gradual temperature increases may allow crops to acclimate, triggering stress-responsive pathways that mitigate damage [14]. This dynamic underscores the importance of understanding temperature fluctuations in the context of global climate change. Studies have shown that a slower rate of temperature increase enhances the expression of heat stress-responsive genes, improving crop tolerance. For example, research on rice demonstrates that gradual heat exposure can enhance their photosynthetic efficiency and reproductive success under high temperatures, compared to abrupt stress conditions [15]. Similarly, Gradual acclimation to heat stress in *Arabidopsis thaliana* (increasing from 22 ℃ to 45 ℃ over 6 hours) was shown to be more effective in enhancing survival and inducing significant transcriptomic changes compared to stepwise acclimation (a pre-treatment at 38 ℃ followed by a return to 22 ℃ and then exposure to 45 ℃) [14].

Generally, under heat stress, plants show visible symptoms like leaves scorching, sunburn on tissues, senescence, abscission, fruit discolorations, and overall plant growth inhibition by reducing dry mass and internodal lengths, thus decreasing crop plant size [11]. In addition, heat stress can alter metabolic and biochemical processes in plants, such as induction a production of ROS including H_2O_2 and O_2^-, as well as the accumulation of thermotolerant compounds including sugars and polyols, leading to changes in carbohydrate and nitrogen metabolism. Heat stress significantly influences reproductive metabolism by impairing pollen viability, tube growth, or germination, affecting microsporogenesis and fertilization, and also causes a loss of ovule viability and fruit set. At the cell level, heat stress causes lose cell organizational integrity through changes in membrane fluidity, protein denaturation, and the accumulation of toxic compounds, and ultimately causes cell death and through inactivating or denaturing enzymes, particularly those associated with mitochondria and chloroplasts, and altering cytoskeleton organization.

PLANT PHYSIOLOGICAL RESPONSES TO HEAT STRESS

Photosynthesis

Chloroplast, the organelle of photosynthesis in plants, is highly sensitive to heat (Figure 1) [9]. The primary sites vulnerable to heat damage are stroma and thylakoids in chloroplasts, leading to several structural and physiological changes such as the loss of grana stacking, alterations in the shape of chloroplasts, and clump formation in the vacuole [16]. Heat stress influences both light reaction and Calvin cycle. Photosystem II (PSII) and its Oxygen Evolving Complex (OEC) in the thylakoid membranes are more susceptible to degradation under higher temperatures compared to Photosystem I (PSI). This is because the electron transportation system collapses due to the dissociation of OEC, denaturation of reaction center proteins like D1 and D2 proteins, and denaturation of manganese-stabilizing protein present in PSII [16]. Ultimately, this leads to the cessation of oxygen emission from water, a phenomenon referred to as the heat-induced inhibition of oxygen evolution in PS II operation [7]. Additionally, heat stress causes the degradation of photosynthetic pigments, including chlorophyll a and b, leading to a reduction in net photosynthesis [16]. However, carotenoids play a crucial role in thermotolerance, as well as in other abiotic stress tolerances. There are several types of carotenoids, such as carotene and xanthophyll, both known for their antioxidant properties, offering protection against oxidative stress specifically caused by ROS generated during heat stress. Furthermore, carotenoids provide substrates to the Abscisic acid (ABA) biosynthesis pathway, contributing to a thermotolerant mechanism [17]. Additionally, carotenoids act as accessory pigments that can dissipate excess light energy, safeguarding tissues from photo-oxidation. Some plants, such as tomato (*Lycopersicon esculentum*) and sugarcane (*Saccharum* spp.), exhibit thermotolerance traits, including a higher chlorophyll a:b ratio and a lower ratio of chlorophyll to carotenoids, indicating a higher content of carotenoids [7].

Higher temperatures also can affect diminishing the activity of key enzymes involved in starch synthesis and the Calvin cycle, such as sucrose phosphate synthase, ADP-glucose pyrophosphorylase, and invertase [7]. Furthermore, heat stress has a greater harmful effect on C3 plants compared to C4 plants due to Rubisco inactivation and changes in gas solubilities in C3 plant cells. A significant distinction between the carbon fixation of C3 and C4 plants lies in the presence of phosphoenolpyruvate (PEP) carboxylase in C4 plants, which efficiently seeks out CO_2 compared to the Rubisco enzyme present in C3 plants. Unlike the Rubisco enzyme, which possesses both carboxylase and oxygenase activities, PEP carboxylase lacks oxygenase activity. Additionally, Rubisco exhibits a high affinity for dissolved oxygen compared to carbon dioxide, as the oxygenase activity increases at high temperatures where there is a high O_2/CO_2 ratio in cells, leading to photorespiration and reduction in carbon fixation in C3 plants [13]. The study also suggests that the optimal temperature for photosynthesis in both C3 and C4 plants increases with a higher concentration of carbon dioxide or a lower O_2/CO_2 ratio in cells. However, despite the generally higher optimal temperature for photosynthesis in C4 than C3 plants, photosynthesis in C4 plants is inhibited at around 38 ℃ (e.g., maize) [13].

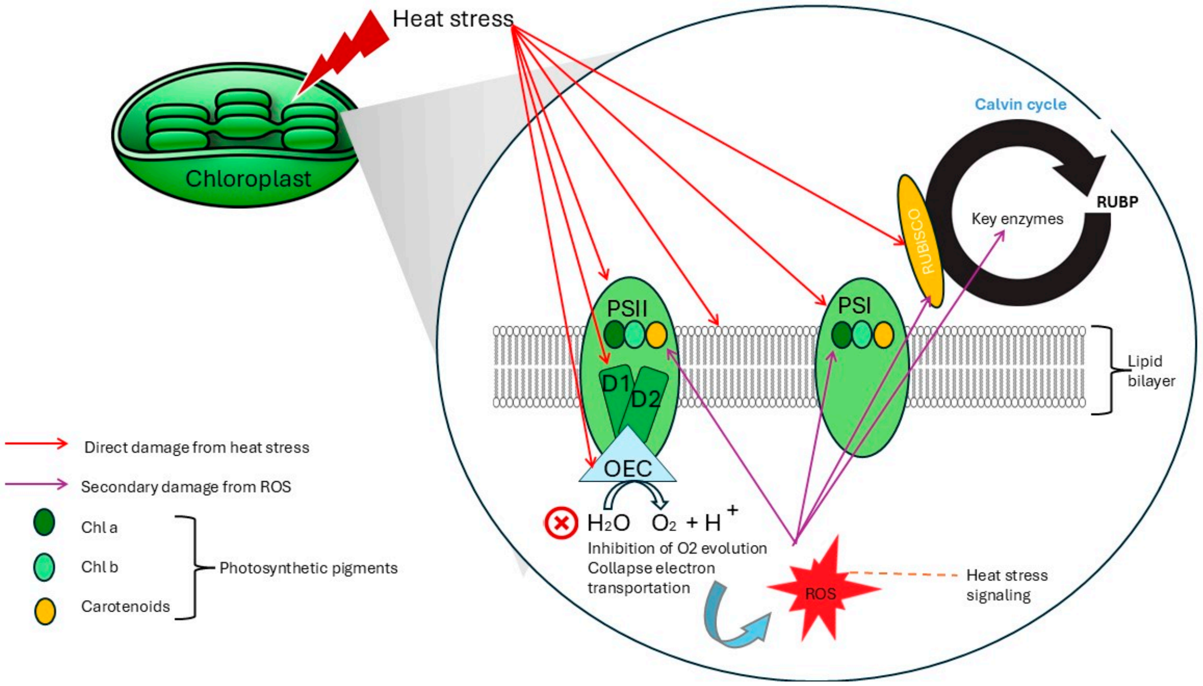

Figure 1. A schematic illustration of the components damaged in chloroplast and photosynthesis due to heat stress. Red arrows indicate direct damage of chloroplast membranes including PSII and its components (reaction center proteins and OEC), PSI, and major photosynthetic enzymes including RUBISCO. Purple arrows indicate secondary damage by heat stress caused by the generation of ROS towards photosynthetic pigments (Chl a, Chl b and carotenoids) and major photosynthetic enzymes.

Reproduction

The plant reproductive stage is the most vulnerable developmental phase for heat stress [4]. Heat stress leads to a decline in seed set, induces male sterility during anthesis, and results in pod abortion, dropping off flowers, and the abortion of opened flowers, ultimately reducing yield. Heat stress affects every stage of reproduction, including the formation of gametophores (affecting microsporogenesis and ovule viability), pollination (preventing self-pollination by elongating the style beyond the anthers), fertilization (reducing pollen tube growth), and post-fertilization (preventing the formation of the endosperm, proembryo, and embryo) [18,19]. Usually, Heat stress had a substantial negative impact on pollen development when compared to the ovule development, leading to deformed pollen and a decrease in both pollen viability and germination and pollen tube formation [18,20]. Huang et al. [6] conducted a study on heat stress related responses in pea anthers through transcriptome analysis. They demonstrated that exposing pea plants to a seven-day heat stress period at the flowering stage, with temperatures of 36/18 ℃ day/night temperatures, resulted in dysfunction in anthers, severe disruptions in pollen development, pollination, and subsequent seed set, in contrast to pea plants under control conditions of 24/18 °C in growth chamber experiments.

Interaction of Heat Stress with Drought Stress

Heat and drought stresses are interrelated abiotic stress conditions in plants. The phenological, anatomical, and metabolic changes induced by high temperatures are generally similar to the responses observed in drought stress, such as impaired carbon fixation, ROS-related cell damage, and protective mechanisms related to antioxidants and compatible solutes. However, there is a difference in the transpiration rate, as plants under drought stress tend to close stomata and reduce transpiration to conserve water inside the plant, while heat stress accelerates the transpiration rate as a cooling strategy [21]. Sometimes, this increased rate of transpiration can lead to a reduction in water level, thereby creating secondary stress and causing a negative water balance throughout the plant [11]. Moreover, numerous studies have revealed that high temperatures can alter root hydraulic conductivity, which refers to the roots' ability to transport water from the root surface to the stem's xylem, facilitated by a difference in water potential. This implies that the water potential of heat-stressed plant tissues differs from that of control tissues, even in the presence of ambient humidity and soil water [22]. Moreover, to thrive under abiotic stress conditions related to water stress, plants tend to accumulate special organic compounds with low molecular weights known as compatible osmolytes. Examples of such compounds include sugars and sugar alcohols like trehalose, fructans, and mannitol, amino acids such as proline and γ-aminobutyric acid (non-protein), as well as polyamines like glycine betaine and tertiary sulphonium compounds [7].

Hormonal Changes

Hormones and growth regulators are major signaling molecules that induce various responses in plants. However, under heat stress conditions, there are changes in hormonal balance, stability, composition, synthesis, and compartmentalization [5,20]. The accumulation of plant hormones, such as salicylic acid (SA), indole acetic acid (IAA), Jasmonic acid (JA), ABA, and cytokinin (CK) is crucial for a plant's ability to withstand heat stress during the recovery period [20,21,23]. ABA plays a crucial role in regulating the expression of numerous genes associated with thermotolerance, including the upregulation of HSPs such as HSP70. Heat stress elevates endogenous ABA levels, which contribute to thermotolerance through various mechanisms. These include increasing ROS levels and thereby enhancing antioxidant activity, acting as a thermo-priming hormone to improve stress acclimation, modulating carbohydrate levels and energy partitioning to support energetically demand activities like gene expression, protein synthesis and facilitating ROS scavenging under heat stress, [23,24]. Additionally, IAA plays a key role in structural adaptations to heat stress, such as promoting hypocotyl and stem elongation as well as leaf hyponasty, by interacting with various transcription factors like phytochrome interacting factors 4 and 7, and proteins such as PIN-LIKE auxin carriers and heat shock proteins like HSP90 [24]. As a further matter, CK application enhances flower development under heat stress, as evidenced by decreased pre-anthesis abortion in passion fruit (*Passiflora edulis*) with exogenous CK application [25]. Also, CK improves antioxidant metabolism by increasing the activity of enzymes like superoxide dismutase and ascorbate peroxidase, helping plants mitigate oxidative damage caused by high temperatures [24]. Moreover, SA is a heat stress-responsive plant hormone that plays a critical role in protecting plants from heat-induced damage through multiple mechanisms. It enhances physiological activities such as plant height, biomass, and photosynthetic efficiency while reducing membrane damage and boosting the activity of antioxidant enzymes [26]. SA stabilizes heat shock transcription factors, regulates calcium ion homeostasis, and maintains high levels of chloroplast HSP21 proteins, which aid in photosynthesis recovery after heat stress [27]. Additionally, SA promotes proline production, an adaptive antioxidant response, while contributing to systemic acquired resistance and the hypersensitive response, further enhancing plant thermotolerance [24]. The heat stress-induced transcription factor WRKY39 plays a critical role in thermotolerance by positively regulating the cooperation between SA and JA signaling pathways, enhancing the expression of key SA- and JA-responsive genes involved in heat stress defense [28]. Ethylene is known to accumulate in reproductive structures such as flowers, pedicels, and fruits in response to heat stress and the external application of ethylene-emitting substances, such as ethephon, activates various thermotolerance responses [29]. Moreover, ethylene signals to induced expression of heat shock factors (e.g., *HsfA2)* to promote thermotolerance [5]. In addition to ABA, IAA, CK, SA, JA and ethylene compounds such as thiourea, Gamma-Aminobutyric acid, and brassinosteroids cause an increase in the levels of soluble sugars, osmoprotectants, antioxidant enzymes, and gas exchange traits, thereby enhancing a plant’s tolerance to heat stress [4,5].

Membrane Fluidity Changes and Protein Denaturation

Biological membranes are responsive to temperature differences, leading to changes in their fluidity, permeability, and integrity. Since membranes consist mainly of lipids and proteins, which are temperature-sensitive macromolecules (proteins), heat stress induces membrane fluidity, causing an increase in molecular motion within the lipid bilayer [9]. The loss of tertiary and quaternary structures of membrane proteins and changes in protein folding and conformation under high temperatures result in electrolyte leakage in membranes [30]. The fluidity of membranes is primarily maintained by polyunsaturated fatty acids

(PUFA). PUFA constitutes a major target for lipid peroxidation, a chemical conversion of lipids to lipid peroxides through oxidation. This phenomenon, known as lipid peroxidation, is induced by high light or heat in plant cells. During this process, various secondary products such as lipid radicals, aldehydes, and ROS are formed. These products contribute to damaging membrane fluidity and result in protein oxidation, as well as the cleavage of protein-protein, lipid-protein, and lipid-lipid crosslinks within membranes [31]. Additionally, the increased fluidity of the thylakoid membranes in chloroplasts is a major consequence of the loss of membrane integrity due to heat stress. This leads to the inhibition of the activity of membrane-associated electron carriers and enzymes, resulting in the inactivation of PSII, a key component of the thylakoid membrane, and ultimately reducing the net photosynthesis rate [32].

Heat Shock Proteins, Heat Shock Factors, and Other Related Protein Transcription

HSPs constitute a group of molecular chaperones capable of mitigating structural changes in proteins induced by abiotic stresses, particularly heat stress. HSPs, induced by heat stress, safeguard cellular integrity and homeostasis by preventing the misfolding and aggregation of cellular proteins under such conditions, which could otherwise lead to cell death. Additionally, HSPs are considered evolutionarily conserved, underscoring their critical importance in biological systems. They are predominantly regulated at the transcriptional level by heat shock factors (HSFs) or transcription factors capable of binding to cis-elements and N-terminal binding domains, such as promoters and enhancers of heat stress response-related genes. These genes include not only HSPs but also other chaperones, ROS scavenging proteins, enzymes involved in protective metabolic pathways and osmolyte synthesis, apoptotic regulators, and various other transcription factors [33,34]. Plant HSFs belong to 18–52 large gene families which can be classified into three main classes: HSFA, HSFB, and HSFC, each comprising several subclasses [35]. Class A contains an activator motif close to the C terminal region while Class B contains a repressor motif needed for the transcriptional activation [35]. However, the function of class C is yet to be known. Among the members of class A, subclasses HSFA1, HSFA2, HSFA3 and HSFA7 stand out as major HSF crucial for acquired thermotolerance, observed in species like tomato, maize, rice and Arabidopsis [33,35]. HSF class B is known to be both repressor and activator of heat stress responses as it can suppress and activate some of the HSFAs and HSP genes.

HSPs are not confined to plants but are also found in other eukaryotic and prokaryotic organisms [36]. Examples of HSP classes, categorized by molecular size, include HSP100/ClpB, HSP90/HtpG, HSP70/DnaK, HSP60/GroEL (eukaryotic/*Escherichia coli* nomenclature), and small HSPs [6,37]. HSP100, a member of the AAA+ ATPase superfamily, plays a role in resolubilizing protein aggregates formed under high-temperature conditions [37]. This process helps to reactivate the nonfunctional aggregated proteins and degrade irreversibly damaged polypeptides. HSP60, HSP70, and HSP90 share a common role as molecular chaperones in cells [38] (Table 1). Additionally, some studies have identified HSP60 as a chaperonin, primarily associated with plastid proteins like Rubisco, aiding them in achieving their functional conformation. HSP70 functions as a chaperone crucial for heat stress responses and heat acclimation, preventing protein aggregation and misfolding until they reach their proper cellular location through a mechanism called co-translational folding. Moreover, HSP70 in chloroplasts has been found to play a role in photoprotection and repairing PSII under photoinhibition. Apart from its chaperone function, HSP90 is involved in pathogen resistance in plants by interacting with the resistance protein (receptor for the signal coming from the pathogen). As mentioned earlier, another category of HSPs is the small HSPs or sHSPs, which contain a common alpha-crystallin domain with 80–100 amino acids at the C-terminal [38]. These proteins are involved in the degradation of misfolded proteins. For example, 8.5 kDa molecular weight ubiquitin is an sHSP capable of directing proteins to proteasome-mediated degradation.

However, the exact functions of HSPs remain incompletely understood due to challenges in defining HSP functions in plants, stemming from differential expression during various stress levels and developmental stages, as well as the limited availability of T-DNA insertion lines in model plants [37].

Another characteristic of HSPs and chaperones is their interactive role with other heat stress-tolerant mechanisms, such as the production of compatible osmolytes and antioxidants [23]. Typically, HSP synthesis positively correlates with increasing temperature, and the maximum temperature at which HSP synthesis increases depends on the species-specific temperature conducive to optimum growth [36]. HSPs are considered members of a complex multi-gene superfamily, wherein not all members are regulated or involved with heat stress [36]. Some HSPs are found at significant levels in normal, non-stressed cells or are produced at particular stages of the cell cycle even in the absence of stress. Additionally, certain normal cellular proteins homologous to HSPs do not exhibit increased expression in response to high temperatures. Furthermore, other stresses, including heavy metals and glucose starvation, can affect the synthesis of some or all HSPs in many organisms.

Table 1. Major HSPs and their potential functions related to heat stress response and tolerance.

HSP family	Molecular weight (kDa)	Major members of family	Potential functions
HSP00	97–120	HSP101, HSP104	Resolubilizing protein aggregates and provide thermotolerance
HSP60	58–65	HSP60	Molecular chaperones and Stabilizes proteins by refolding them and prevents the clumping of proteins that have become denatured
HSP70	66–78	HSP72, HSP73, HSP75, HSP78	Molecular chaperones and inhibiting apoptosis or programmed cell death
HSP90	82–89	HSP90	Molecular chaperone, protein translocation and support functional conformation of proteins
sHSP	10–40	sHSP22, sHSP25, sHSP 24.4, sHSP18.1, sHSP17.7, sHSP16, sHSP8.5 (ubiquitin)	Chaperone activity, proteosome mediated degradation

Reactive Oxygen Species Generation and Production of Secondary Metabolites and Antioxidants

Other than the direct damage induced in photosynthesis by PSII, heat stress induces de novo inhibition of protein synthesis by ROS, which is again a secondary stress. Both heat stress and drought stress generate ROS such as peroxide radicals, hydrogen peroxide, superoxide radicals, and hydroxyl radicals, giving rise to oxidative stress in different cellular compartments including chloroplasts, mitochondria, peroxisomes, the cell wall, apoplast, and the plasma membrane [39]. ROS mainly disturbs photosynthetic and respiratory metabolism by oxidative damage to biomolecules such as pigments, proteins, carbohydrates, and DNA. It also causes lipid peroxidation, affecting the loss or modification of membrane selective permeability and leading to organelle and cellular damage. On the other hand, there are ROS scavenging molecules or antioxidants against oxidative damage which can be divided into two categories: enzymatic and non-enzymatic antioxidants. Some examples of enzymatic antioxidants are ascorbate peroxidase (APX), superoxide dismutase (SOD), peroxidase (POX), glutathione reductase (GR), and catalase (CAT) while ascorbate (ASC), glutathione (GSH), carotenoids and tocopherols are under non-enzymatic antioxidants [23,39].

Most secondary metabolites synthesized through phenylpropanoid, shikimic acid, mevalonic acid, and methyl erythritol phosphate pathways contribute to both biotic and abiotic stress tolerance in plants. For instance, the presence of a phenyl ring in phenolic compounds like flavonoids plays a crucial role in enhancing tolerance to environmental stress [40]. Several studies have shown that phenylalanine ammonia-lyase (PAL) is a phenolic enzyme that specifically accumulates under heat-stressed conditions [19]. Flavonoid Anthocyanin, a flavonoid compound of phenolics, exhibits decreased levels in buds and fruits, reproductive parts, under high temperatures [19]. In contrast, the accumulation of anthocyanin has been observed in vegetative parts of different species like rose and sugarcane in high temperatures. This accumulation aids in increased water uptake and reduced transpiration by lowering the osmotic potential in vegetative tissues [40]. Isoprene, a volatile secondary metabolite, shows increased biosynthesis and emission under heat stress [19]. Isoprene plays a major role in scavenging singlet oxygen, a ROS formed during heat-induced oxygenase action of Rubisco, by forming isoprene conjugate double bonds [19,41].

HEAT STRESS SENSING AND SIGNAL TRANSDUCTION

There are various mechanisms that explain how plants detect elevated temperatures, including phase separation, isomerization, subcellular translocation, RNA structure changes and formation of secondary structures, and chromatin remodeling [42]. A variety of pathways participate in the signal transduction of heat stress, with the Redox system being a major one. This system involves chemical signals such as ROS (H_2O_2), phytohormones (SA, ethylene, and ABA), and calcium ions (Ca^{2+}). Additionally, nitric oxide (NO) and various sugars serve as signaling molecules in the activation of stress-responsive genes (Figure 2) [5,43]. Another sensing mechanism involves monitoring changes in membrane fluidity. For instance, alterations in the ratio of saturated to unsaturated fatty acids in membranes influence the expression of heat shock transcription factors [11].

The increase in calcium ions in the cytosol during the recovery period after exposure to higher temperatures supports the hypothesis of the involvement of MAPK signaling in the response to heat stress (Figure 2). Many HSP expressions correlate with Ca-dependent protein kinases or calcium-modulating proteins (calmodulin) involved in signal transduction within the MAPK pathway [43]. Furthermore, higher concentrations of cytosolic calcium ions stimulate different calcium-dependent protein kinases (CDPK) and SYTA (Synaptotagmin A) transmembrane proteins to promote cytoplasmic membrane repair and the expression of HSF and HSP genes [43]. In addition to calcium ion sensing, the generation of ROS, such as H_2O_2, can induce HSP synthesis through HSFs. However, these pathways have not been comprehensively explained through experimental investigations. Only several steps and intermediates have been experimentally identified in different studies.

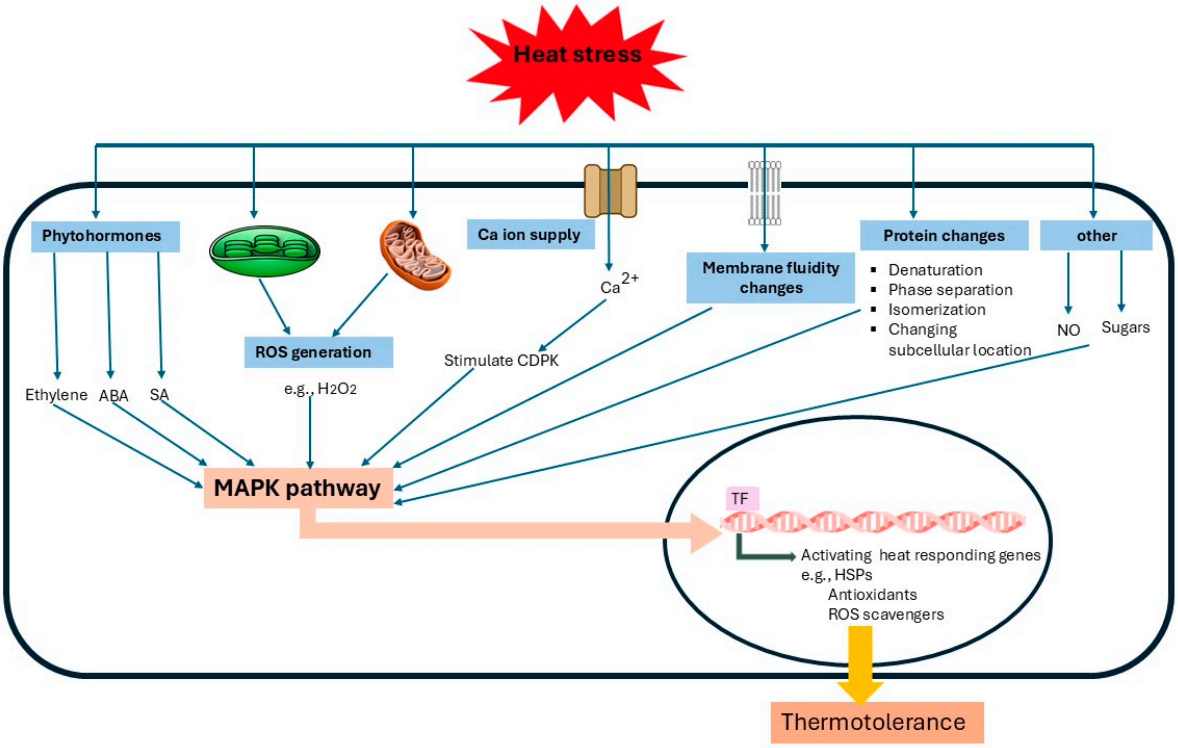

Figure 2. Illustration of the involvement of MAPK and redox signal transduction in heat stress sensing and responding. Heat stress triggers various cellular changes, including phytohormone signaling (e.g., ethylene, ABA, SA), ROS generation from chloroplasts and mitochondria (e.g., H_2O_2), calcium ion influx (Ca^{2+}), alterations in membrane fluidity, protein modifications (e.g., denaturation, phase separation, isomerization), and metabolic shifts (e.g., NO and sugars). These signals converge on the MAPK pathway, which activates transcription factors (TFs) in the nucleus. TFs induce the expression of heat-responsive genes, such as those encoding heat shock proteins (HSPs), antioxidants, and ROS scavengers, ultimately enhancing thermotolerance.

HEAT STRESS TOLERANCE IN PLANTS

Heat stress tolerance is defined as “The ability of the plant to grow and produce economic yield under high temperatures” or simply, the ability of a plant to withstand high temperatures [7]. Heat stress tolerance mainly involves modifications in physiological and biochemical processes in thermo-tolerant plants specifically under heat stress conditions. These mechanisms are highly specific even in closely related species or organs and tissues of the same plant [2]. Heat stress tolerance mechanisms activate to reduce any negative impacts after being subjected to the stress. One such example is the accumulation of special solutes, thereby adjusting the osmotic and redox balance and cellular homeostasis. Production of accessory photosynthetic pigments like carotenoids is also greatly seen in heat stress tolerant plants because it is a well-known antioxidant [43] as mentioned earlier. Also, the production of HSP and regulation of thermotolerance-related gene transcription via HSFs play major roles in heat stress tolerance in plants.

Heat Avoidance Mechanisms

Plants show various other strategies to prevent irreversible damage and maintain optimum homeostasis before or during stress conditions using temperature sensing and signal transduction pathways. Those adaptations come under heat stress avoidance mechanisms. Avoidance mechanisms primarily involve early adaptations that prevent or reduce the risk of exposure to heat stress through various phenological, morphological, and acclimation strategies [44]. Overall, these morphological adaptations and responses are known as thermos-morphogenesis which help to elevate ambient temperature in the plant [21]. Among them are changes in leaf orientation, stomatal closure, transpirational cooling, increased trichomatous densities, and larger xylem vessels [2]. These characteristics are related to preventing heat stress before it occurs. In high-temperature climates, plants often limit the absorption of solar radiation by adjusting the leaf orientation to a parallel position (paraheliotropism), rolling leaf blades, and developing a thick layer of wax, cuticle, and trichomes on leaves. Some plants have evolved to undergo leaf abscission and avoid entering their reproductive stage during hot seasons, providing clear examples of heat stress avoidance mechanisms [2,44]. Intensive transpiration, which helps maintain an optimum temperature in extreme environmental conditions, is another avoidance strategy employed by plants. However, this mechanism is limited by the availability of absorbable water in the soil [44].

CROP IMPROVEMENT FOR HEAT STRESS TOLERANCE

Mitigating Heat Stress Using Exogenous Protectants

Numerous research studies have aimed to enhance heat tolerance in crops through the external application of various molecules associated with different aspects of heat stress tolerance in plants. These molecules primarily include osmoprotectants, phytohormones, antioxidants, and signaling molecules. Osmoprotectants such as proline and glycine betaine have shown significant potential in enhancing thermotolerance. Their application promotes the accumulation of soluble sugars, maintaining osmotic balance under heat stress. Additionally, these molecules reduce the production of reactive oxygen species (ROS), particularly hydrogen peroxide (H),O and boost the synthesis of signaling molecules like calcium ions in heat-stressed tissues [30]. The external application of SA derivatives, including Suphosalacylic acid (SSA), acetyl SA, and methyl salicylate (MeSA), has demonstrated enhanced thermotolerance in several plant species in various studies. SSA effectively tolerates heat stress-induced oxidative stress by scavenging ROS, such as H_2O_2, and increasing enzyme activity associated with the degradation of H_2O_2, including CAT, glutathione peroxidase (GPX), APX, and GR [7,45]. Salicylic acid (SA) is recognized as a signaling molecule that plays a role in regulating gene expression in response to both biotic and abiotic stresses. Nonetheless, the precise mechanisms and pathways through which SA functions in plants remain not fully elucidated. Besides SA derivatives, the external application of ABA has also been found to reduce heat stress-induced oxidative stress in plants [30]. Studies have indicated that applying ABA externally improves sucrose transport and accelerates its metabolism, which helps maintain carbon balance and energy homeostasis, thereby enhancing heat tolerance in rice [46]. However, when high concentrations of exogenous ABA were applied, it reduced the heat stress tolerance of rice seedlings by boosting ROS accumulation [47]. As previously mentioned, thiourea, a plant growth regulator, acts as an effective scavenger of hydroxyl radicals, protecting plants from heat stress-induced oxidative damage and regulating growth and developmental processes. Applying exogenous thiourea (1000 mg/L) enhanced heat tolerance in camelina (Camelina sativa L.) by influencing gas exchange, antioxidant defense mechanisms, and osmoprotectant production [48]. In addition to phytohormones, antioxidants like β-carotene have been explored for their protective effects. For example, Rossi and Huang reported that β-carotene application in creeping bentgrass (*Agrostis stolonifera* cv. Penncross) suppressed heat stress-induced leaf senescence [49]. The application was found to effectively increase the activity of ROS-scavenging enzymes, including SOD, POX, and CAT, under heat stress. Recent studies related to the exogenous application of protectants to improve thermotolerance in plants are listed below (Table 2).

Table 2. Recent studies on exogenous application of protectants in various crops/plants.

Exogenous Protectant	Plant species	Observations related to thermotolerance under heat stress compared to control plants	Reference
SA	Fescue (*Festuca arundinacea*)	Elevation of photosynthetic pigments, accumulation of proline, and increasing nonenzymatic and enzymatic antioxidant activities.	[50]
	Ornamental pepper (*Capsicum annuum*)	Increased germination rate reduced oxidative damage, less water loss and maintaining high root vigor, maintaining osmotic balance decreased level of ROS and increased activity of protective enzymes.	[51]

Table 2. *Cont.*

Exogenous Protectant	**Plant species**	**Observations related to thermotolerance under heat stress compared to control plants**	**Reference**
SA	Fenugreek (*Trigonella foenum-graecum*)	Enhancement in growth, increased antioxidant enzyme activity, increased protein and chlorophyll content, reduced electrolytic leakage in leaves, reduced H_2O_2 levels and oxidative damage.	[52]
	Wheat	Improved the growth and yield of wheat crop by increasing relative water content, antioxidant activity, soluble sugars, proline, total soluble proteins, nutrient uptake and decreased electrolyte leakage, malondialdehyde (MDA) and H_2O_2.	[53]
	Alfalfa (*Medicago sativa*)	Improved plant growth, reduced electrolyte leakage and MDA level, regulated activities of antioxidant enzymes.	[54]
	Maize (*Zea mays*)	Enhanced the shoot dry weight, the activities of antioxidant enzymes, and the concentration of thermotolerance related endogenous phytohormones while decreased the MDA content.	[55]
ABA	Rice	Reduced malondialdehyde content and relative electrolytic conductivity, and decreased expression of cell death-related genes (OsKOD1, OsCP1, OsNAC4) and increased the expression of the cell death-suppressor gene OsBI1, enhanced antioxidant defense by upregulating ROS-scavenging genes and lowering ROS levels. Increased the seed-setting rate by increased trehalose content, key enzyme activities of trehalose metabolism, ATP content, and F1Fo-ATPase activity.	[46,56,57]
Glycine betaine	Chinese cabbage (*Brassica rapa*)	Transcriptomic analysis reveals activation of ABA and SA signaling pathways, antioxidant defense, heat shock response, and DNA damage repair systems.	[58]

Scaling up the exogenous application of molecules for enhancing heat stress tolerance in crops presents several challenges. Environmental persistence is a significant issue, as many compounds degrade rapidly under field conditions, particularly in high-temperature environments, necessitating frequent applications that increase costs and labor. Economic feasibility is another concern, especially for smallholder farmers, as the production, storage, and transport of these molecules can be expensive, potentially outweighing yield improvements. Additionally, the effectiveness of these applications varies across concentration, crop species, growth stages, and stress severity, limiting their universal applicability. Delivery methods, such as foliar sprays and soil applications, often result in uneven distribution or inefficiencies in absorption, further complicating their practical use. Therefore, it is important to optimize the application process in a practical manner, with precise parameters such as concentration, volume, application method, and crop species, or to explore alternative methods.

Breeding Strategies

Traditional breeding methods have been extensively used to improve heat tolerance in crops. Introgression breeding or repeated backcross to a heat stress-tolerant wild relative or any other compatible cultivar can be used to improve heat stress tolerance. For example, an introgression breeding strategy was used to improve peanut cultivars with both yield and stress tolerance in semi-arid environments through a cross between wild species (a hybrid between *Arachis duranensis* and *A. batizocoi*) and cultivated peanut (*A. hypogaea* subsp. fastigiata var. fastigiata) [59]. Additionally, Heat-treated pollen from a heat-tolerant *Gossypium barbadense* line (7456) was used in breeding to develop heat-tolerant cotton by crossing it with a heat-sensitive cultivar (Paymaster 404). Heat tolerance was observed in the F1, F2, and first and second backcross populations [60]. Nagina 22 (N22), a thermotolerant rice cultivar, was crossed with the Xieqingzao B rice line using conventional hybridization. Thermotolerance was evaluated in BC1F8 backcrossed recombinant lines with similar genomes but significantly different heat tolerance during the grain-filling stage. This led to the identification of six pairs of lines with minimal genetic differences but contrasting heat tolerance, with 703T and 704S emerging as key genetic markers for studying heat tolerance mechanisms [61]. Multi-parents advanced generation inter-cross (MAGIC) is a breeding strategy that involves crossing multiple parent lines, often more than two, and then intercrossing their offspring for multiple generations. The goal is to increase genetic diversity while maintaining high levels of recombination across the genome, which helps capture a wide range of genetic variation. This strategy is used for creating populations that can be leveraged for genetic mapping, selection, and improving traits of interest including heat stress [62].

However, developing heat-tolerant varieties through these methods is challenging due to complex genotype × environment interactions [4]. Despite their success in enhancing tolerance to various abiotic stresses in some crops, traditional breeding faces significant drawbacks, including long development times, unintended transfer of undesirable traits, potential yield reduction even with improved stress resistance, and uncertainty in obtaining beneficial gene combinations even after numerous crosses [63]. And it is difficult to develop a crop cultivar that performs well in both yield and thermotolerance.

Biotechnological and Other Strategies to Improve Heat Stress Tolerance

Omics technologies, including genomics, transcriptomics, proteomics, and metabolomics, are critical tools for improving thermotolerance in plants by identifying key genes, proteins, and metabolites involved in heat stress responses [64]. Genomics provides insights into the genetic composition of plants, helping to uncover regulatory sequences and gene functions that enhance heat stress resilience. Transcriptomics, through methods like RT-qPCR and next-generation sequencing, helps identify differentially expressed genes (DEGs) involved in stress signaling, photosynthesis, secondary metabolite production and carbohydrate metabolism under heat stress [65]. Proteomics analyzes proteins involved in cellular metabolism and heat stress tolerance, identifying HSPs and antioxidants critical for thermotolerance. Techniques like MALDI-TOF/MS and 2D PAGE have been used to study proteins in significant crops. Metabolomics examines the changes in metabolites in response to heat stress, such as those involved in glycolysis, the citric acid cycle, and antioxidant production. Metabolite profiling aids in selecting heat-resistant crop cultivars and understanding metabolic responses to stress. By combining these omics approaches with breeding techniques, researchers aim to improve crop quality and productivity under heat stress conditions.

Plant biotechnological strategies, such as quantitative trait loci (QTLs) identification, marker-assisted breeding, genetic engineering, and genome editing, are employed to enhance a variety of desirable traits including heat stress tolerance in crops. A deep understanding of molecular mechanisms, QTLs, and genes associated with the trait of interest is essential for the effective application of these approaches.

Genes and traits associated with heat tolerance, such as HSPs, compatible osmoprotectants, antioxidants, etc., have been identified as candidate genes (Table 3) to improve heat stress tolerance in crops through various techniques, including microarray analysis, transcriptome analysis, genome-wide association studies (GWAS), and RT-PCR. For instance, studies assessed the expression level of several known heat-related genes such as *NAC4, WRKY, HSP70, HSP90, HSP60, DREB2A* (dehydration-responsive element-binding 2A), *LEA4-2* (late embryogenesis abundant 4-2), *DHN1* (dehydrin) [66], functions as a chaperone, and membrane stabilizer to protect plant cells from heat-induced damages [67] and *AREB1Q* or *ABF2* (Abscisic Acid- Responsive Element-Binding Factor 2) in several heat tolerant and susceptible peanut seedlings under heat stress using RT-PCR. The results revealed that *HSP90, DREB2A*, and *LEA4-2* are upregulated significantly in tolerant genotypes among the selected genes, indicating their positive heat response.

With the development of crop-specific gene chips, microarrays have become the preferred method for assessing gene expression changes in response to abiotic stress. Affymetrix GeneChip® was employed to determine differential gene expression in wheat to identify heat-responsive transcription factors (*DREB2A, AtMBF1c* and *ERETC)*, phytohormones (*ABA-INSENSITIVE2, AREB2, AREB3 and HVA22*), HSPs, signal transduction, RNA metabolism (*STRS1, STRS2*) and etc. between heat-susceptible and heat-tolerant wheat genotypes [68]. Similarly, the Affymetrix GeneChip® Tomato Genome Array in tomato [69], the Affymetrix 22K Barley 1 Gene Chip microarray in barley [70], and the Brassica 95k EST microarray in Brassica [71] provided insights into various genes and markers including *ROF2, MBF1c, Hsa32, HvABF1, HvTPS1, HvTPS2, HvHSFA2d* and *HvSUT1* involved in heat stress tolerance.

Genotyping by sequencing (GBS) is a technique that has been used to determine single nucleotide polymorphisms (SNPs) in genomes, and it has been utilized to find QTLs related to heat stress tolerance in crops [61,72]. One of the studies have identified major QTLs related to heat stress tolerance under phenological (CaDFl_LS6.1, CaDFl_LS8.1, CaDFl_LS1.1), physiological (CaCHL_NS4.3, CaCHL_LS2.1, CaCHL_LS5.2), and yield (CaDPI_LS7.2, CaDPI_LS7.1, CaDPI_LS6.1) related traits in chickpea [72]. Similarly, another study identified TaHST1, a QTL on chr4A, associated with heat tolerance in wheat from Pakistan through GWAS [73]. GWAS is another powerful technique used to identify genetic variations associated with specific traits across the entire genome by analyzing the genetic data of a diverse population. GWAS detects single nucleotide polymorphisms (SNPs) and other markers that correlate with phenotypic traits, such as heat stress tolerance in plants. Moreover, GWAS has been used to identify genomic regions associated with heat tolerance traits in maize [74], field pea [75], sorghum [76], and rice [77]. QTL analysis or mapping is a method that utilizes molecular markers to identify genetic regions responsible for significant variation in a specific trait. While numerous QTLs associated with heat stress traits have been identified in several crops including wheat, their direct application is often hindered by their small effects and broad genomic spacing, making precise transfer and utilization challenging.

Marker-assisted breeding is another approach that developed with the improvement in genomics, QTLs, and markers that can be used to develop abiotic stress tolerance. Here, genomic selection is utilized before the phenotypic characters are developed in the seedling stage using markers. A study aimed to enhance the heat tolerance of the heat-sensitive wheat variety HD2733 by using marker-assisted backcross breeding [78]. This approach involved introgressing QTLs for early anthesis and high kernel weight from the heat-tolerant donor variety WH730, linked to the markers Xbarc186 and Xgwm190. Several studies are there to identify thermotolerance related molecular markers which help mostly in marker-assisted breeding. Xu et al. (2018) [79] identified candidate gene-based SNP markers in seven genes (*ACTIN, ATPA, CATA, CP47, HSP90, RCAB, and SS1*) linked to heat tolerance in fine fescue, revealing significant associations between specific SNP variations and physiological traits in heat-sensitive and heat-tolerant cultivars.

Integration of thermotolerant genes through genetic engineering via agrobacterium-mediated transformation or particle bombardment has also been widely used for crop heat tolerance improvement. For instance, overexpression of the *BCH* gene (specifically the *DcBCH1* gene) in *A. thaliana* increased the plant's xanthophyll cycle pool by converting beta carotene into zeaxanthin via cryptoxanthin. This enhancement enabled the plant to better endure high temperatures and intense light conditions [80].

The Modified MultiSite Gateway approach is a technique that is used to integrate several genes into a genome at once using a single vector with multiple cassettes [81]. This approach can be employed to develop abiotic stress tolerance in plants, as it is more convenient than traditional single-gene transformation, considering that abiotic stress tolerance involves many genes.

The CRISPR/Cas genome editing system presents another promising avenue for modifying the genomes of crops to exhibit desirable traits. This method has demonstrated its effectiveness in improving the resistance of numerous crop plants to abiotic stresses and its applicability in identifying related genes. For instance, a study highlighted the potential of the CRISPR/Cas genome editing system in enhancing thermotolerance in tomatoes specifically [82]. They identified *SlMAPK3*, which encodes for mitogen-activated protein kinases, as a negative regulator of thermotolerance. Through CRISPR/Cas9-mediated *SLMAPK3* mutagenesis, where *SLMAPK3* is knocked out, the mutants exhibited higher thermotolerance than the wild type.

Heat priming is another strategy that has been used to improve heat stress tolerance in plants. Priming involves pre-exposing the plant to a stress condition at an early stage, thereby enhancing tolerance to that specific stress using previous memory, which is heritable and can be passed from generation to generation [83]. This pre-exposure or priming should be at moderate and nonlethal levels during the seed stage or early vegetative stage of the plant [84]. The priming process involves epigenetic modifications, including DNA methylation and chromatin remodeling, as well as signal protein accumulation (physiological changes) and alterations in primary metabolism [85].

Table 3. Candidate genes, identified in previous studies to improve thermotolerance in different crops and functionally validated using transformation approaches.

Stress function	**Candidate gene**	**Transgenic plant/source organism**	**Approach**	**Result**	**Reference**
Improving antioxidant defense	*BcHSP70*	Tobacco (*Nicotiana tabacum*)/*Brassica campestris*	Transforming *BcHSP70* to tobacco plant to characterize *BcHSP70* in thermotolerance.	Transgenic tobaccos exhibited higher chlorophyll content, SOD and POD activities, proline and sugar content and lower MAD content than wild type when exposed to heat stress.	[85]
Enhancing HSPs and HSFs	Trehalose-6-phosphate synthase/ phosphatase (*TPSP*)	Tomatoes (cv. Joyful)/*E. coli*	Generation of TPSP overexpressing tomato lines with enhanced levels of trehalose in the seeds.	A greater germination rate in transgenic lines compared to wild type after high temperature treatment. Enhanced heat stress responsive gene expression including HsfA1, HsfA2, and HsfB1 in transgenic lines compared to wild type.	[86]

Table 3. *Cont.*

Stress function	Candidate gene	Transgenic plant/source organism	Approach	Result	Reference
Enhancing antioxidants and osmoprotectants	*CaHsp25.9*	*A. thaliana*/*Capsicum annuum*	Generation of *CaHsp25.9* transgenic *A. thaliana* lines.	Transgenic lines show higher content of proline, SOD and decreased level of malondialdehyde compared to wild type.	[87]
Enhancing HSPs, antioxidants and osmoprotectants	*HsfA1d* (heat shock factor gene)	Potato (*Solanum tuberosum*)/*A. thaliana*	Transforming potato plant with heat shock factor, *HsfA1d*, using *Agrobacterium* mediated transformation.	Transgenic lines show higher levels of HSP70, Chl a and b and proline, compared to the wild type after being subjected to heat stress.	[88]
Improving antioxidant defense	*OsMYB55*	Maize/Rice	Generation of *OsMYB55* overexpressing transgenic maize lines. And transcriptomic analysis to determine differential gene expression between wild type and transgenic maize.	Higher thermotolerance was indicated by transgenic maize lines when compared to the wild type.	[89]
Improving antioxidant defense and ROS scavenging	*OsRab7*	Rice (*O. sativa* subsp. *japonica*)/rice (*O. sativa* subsp. *Japonica* cv. Giza 177)	*OsRab7* gene was transformed into rice plants. Different physiological characteristics of the transgenic rice lines were compared with the performance of wild type.	Transgenic lines had higher survival rates, antioxidant enzymes, proline, sugar, and chlorophyll content when compared to the wild type under high temperature treatment. Several other positive responsive genes including ROS scavenging enzymes were upregulated in the transgenic lines under heat stress.	[90]

Table 3. *Cont.*

Stress function	Candidate gene	Transgenic plant/source organism	Approach	Result	Reference
Influences RNA stability, gene regulation and negative regulator of ABA response under stress	*ALKBH6* (potential RNA demethylase)	Source organism is *A. thaliana*	Generation of *ALKBH6* knockout mutants and examination for germination, survival rate and growth under different abiotic stresses.	Mutants showed downregulated ABA signaling related genes (*AB13* and *AB14*) and reduced germination, survival, and growth under heat stress.	[91]
Enhancing HSPs	*HSc70* allelic variant (the allelic variant contains a TA repeat element at the promoter region)	Potato/potato	Introduction of *HSc70* allelic variant to the heat susceptible potato cultivar using agro-infiltration and determining the expression level of *HSc70*.	Transgenic lines could perform well (higher yield) during and after high temperature treatment and they could show higher expression level of *HSc70* compared to wild type.	[92]
Enhancing HSPs	*HSP21*	Tomato/Tomato line VF36	Transforming *HSP21* for constitutive overexpression in tomato and examine the thermotolerance related to PSII.	Found that HSP21 protein protects PSII from temperature-dependent oxidative stress.	[93]
Enhancing HSPs	*Hsp101*	Rice/*A. thaliana*	Introduced *A. thaliana Hsp101* into the Pusa basmati 1 cultivar of rice (*Oryza sativa* L.) by *Agrobacterium*-mediated transformation and comparing survival of transgenic lines after exposure to heat stress with the control plants.	Transgenic rice lines showed significantly better growth performance in the recovery phase following the stress due to the overexpression of *Hsp101*.	[94]

Table 3. *Cont.*

Stress function	**Candidate gene**	**Transgenic plant/source organism**	**Approach**	**Result**	**Reference**
Enhancing HSPs	*TaMBF1c* (Multiprotein Bridging Factor1c)	Rice (cv. ZhongHua11)/wheat	*TaMBF1c* gene was transformed to rice and subjected to high temperature treatments to compare thermotolerance between transgenic lines and wild type.	*TaMBF1c* overexpressing transgenic rice plants showed higher thermotolerance than wild type plants at both seedling and reproductive stages with better growth vigor and recovery. Also, transgenic lines could over express *OsHSPs* and *OsTPSs* compared to wild type under heat stress.	[95]
Aiding photosynthetic recovery and reducing oxidative damage	*P5CR* (Pyrroline-5-carboxylate reductase gene)	Soybean/*A. thaliana*	Investigating photosynthetic responses in *P5CR* antisense and sense transgenic soybean plants and wild-type plants under drought + heat stress.	Less phenotypic symptoms like wilting and higher proline, and less dissociation of OEC in PSII were shown in sense transgenic plants compared to antisense and wild-type plants during the recovery period.	[96]
Maintaining photosynthetic efficiency	*EF-Tu* (Plastidal protein synthesis elongation factor)	Wheat (*Triticum aestivum*)/maize (*Zea mays*)	Generation of *EF-Tu* overexpressing wheat transgenic lines and assessing heat-induced protein aggregation, thylakoid membrane damage, and carbon fixation compared with the wild type.	Transgenic plants displayed reduced thermal aggregation of leaf proteins, reduced heat injury to photosynthetic membranes (thylakoids), and enhanced rate of CO_2 fixation after exposure to heat stress.	[97]

Table 3. *Cont.*

Stress function	**Candidate gene**	**Transgenic plant/source organism**	**Approach**	**Result**	**Reference**
ROS scavenging	*TaFER-5B* (ferritin gene mapped to chromosome 5B)	Wheat (cultivar Jimai5265) and *A. thaliana*/wheat ("cultivar TAM107")	*TaFER-5B* gene has been transformed to wheat and *A. thaliana* to overexpress the gene and examined the thermotolerance of transgenic lines by assessing electrolyte leakage and photosynthetic activity (F_v/F_m values).	Transgenic lines exhibited low electrolyte leakage and high F_v/F_m values compared to wild type under high temperature treatments.	[98]
Reducing oxidative damage and ROS accumulation	*TaGASR1* (GA-stimulated transcript gene family)	*A. thaliana*/wheat (TAM107 cultivar)	*TaGASR1* overexpressing *A. thaliana* transgenic lines were generated and examined ROS accumulation and electrolyte leakage compared to wild type under heat stress.	Transgenic lines showed thermotolerance under high temperature treatment by exhibiting low levels of ROS accumulation and electrolyte leakage compared to wild type.	[99]
Reducing oxidative damage and ROS accumulation	*AhVDE* (violaxanthin de-epoxidase gene)	Tobacco/peanut	*AhVDE* over expressing transgenic tobacco lines, wild-type tobacco and RNAi tobacco mutants were compared for thermotolerance under heat stress.	Overexpressing transgenic lines had better thermotolerance with lower photoinhibition of PSII, higher activities of SOD and ascorbate peroxidase and lower content of reactive oxygen species.	[100]

REMARKS

In summary, enhancing crop resilience to heat stress is critical in increasing global temperatures, as heat stress adversely impacts crop growth, yield, and quality. This review highlights the complexity of plant responses to heat stress and underscores the role of different physiological, molecular, and biotechnological approaches. Moving forward, integrating conventional breeding with advanced biotechnological tools like CRISPR/Cas genome editing and innovative priming techniques holds great promise for developing thermotolerant crop varieties. Understanding and leveraging molecular insights, such as key genes and QTLs associated with heat tolerance, will be essential for precision breeding and targeted genetic improvements. Additionally, adopting multi-gene transformation techniques, such as the modified multisite gateway approach, holds potential for a more comprehensive modification of stress-resilient traits, although it has not yet been applied to improving thermotolerance in crops. Future research should continue exploring the synergistic effects of combined biotechnological strategies and further assess field applications to bridge the gap between experimental success and practical crop improvement. By fostering interdisciplinary collaborations and accelerating advancements in genetic engineering, plant science can make significant strides toward sustainable agriculture in a warming world.

DATA AVAILABILITY

No data were generated from the study.

AUTHORS’ CONTRIBUTIONS

Conceptualization, JW; Writing—Original Draft Preparation, SMA; Writing—Review & Editing, SMA, JW, AR and HD; Supervision, JW; Funding Acquisition, JW.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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