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Heat Stress Physiology in C4 Crop Plants: Molecular Mechanisms, Omics Approaches and Strategies for Enhanced Thermotolerance

Syam Raj Naik Chavan [a*], Himakara Datta Mandalapu [a] and CVCM Reddy [a]

[a] ANGRAU-Regional Agricultural Research Station, Acharya N.G. Ranga Agricultural University, Lam– 522034, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Rising global temperatures pose unprecedented challenges to agricultural productivity, with C4 crops including maize, sorghum, sugarcane, and pearl millet facing increasing heat stress despite their evolutionary adaptation to warm environments. This comprehensive review synthesizes current understanding of heat stress physiology in C4 plants, examining molecular mechanisms, omics approaches, and strategies for enhanced thermotolerance. Heat stress disrupts critical cellular processes including C4 photosynthesis, membrane stability, and protein homeostasis, with temperatures exceeding 35-40°C causing significant yield losses. C4 plants employ sophisticated defense mechanisms involving heat shock proteins (HSPs) and transcription factors (HSFs) that

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**Corresponding author: E-mail: chavansrnaik@gmail.com;*

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maintain protein quality. Key molecular components include Rubisco activase variants sustaining photosynthetic carbon fixation, photosystem II stability factors preventing photo inhibition, and antioxidant systems managing reactive oxygen species. Compatible solutes such as proline and glycine betaine provide osmotic adjustment, while membrane lipid remodeling maintains structural integrity. Recent omics technologies have revolutionized understanding of heat tolerance, revealing complex gene regulatory networks governing thermotolerance. Genomics studies identify quantitative trait loci associated with heat tolerance, while transcriptomics reveals coordinated gene expression changes. Proteomics and metabolomics uncover post-translational modifications and metabolic adjustments critical for survival under heat stress. Breeding programs increasingly incorporate molecular tools including marker-assisted selection, genomic selection, and gene editing to accelerate development of heat-tolerant varieties. Integration of multi-omics data with advanced phenotyping enables precision breeding for climate resilience. Climate projections indicate continued warming will challenge C4 crop productivity, making enhanced thermotolerance essential for food security through coordinated research combining fundamental mechanistic studies with applied breeding programs.

Keywords: C4 photosynthesis; heat stress; thermotolerance; molecular breeding; climate change adaptation.

1. INTRODUCTION

Global climate change has intensified the frequency and severity of heat stress events, posing significant threats to agricultural productivity and food security (IPCC, 2021). Temperature increases of 2-4°C are projected over the next century, with extreme heat events becoming more common and intense (Lobell et al., 2011). C4 plants, including economically important crops such as maize, sorghum, sugarcane, and pearl millet, contribute substantially to global food and bioenergy production. These crops feed over 3 billion people worldwide and occupy approximately 10% of global cropland (FAOSTAT, 2023). C4 photosynthesis evolved as a means of adapting to hot, arid environments through spatial division of first CO2 fixation and the Calvin cycle, and creating a CO2-concentrating mechanism that reduces photorespiration and improved water and nitrogen utilization (Sage et al., 2012). Despite this evolutionary advantage, C4 crops cannot withstand extreme heat stress during the late stages of flowering and grain filling. Heat stress occurs when temperatures exceed 35-40°C and disrupts multiple physiological processes like photosynthesis, respiration, water relations and reproductive development. The molecular responses to heat stress involve complex gene regulatory networks, protein quality control networks and metabolic changes that control plant survival and productivity at higher temperatures (Hasanuzzaman et al., 2013). A recent development of omics technologies has improved the understanding of heat stress physiology in C4 plants. As Hu et al., 2022, genomics, transcriptomics, proteomics, andmetabolomics have provided important insights into the role of the thermotolerance mechanism and explored potential targets for crop improvement (Hu et al., 2022). This comprehensive review reflects current knowledge of heat stress biology in C4 plants, a range of new technologies and strategies for establishing climate resilient varieties, and discusses new approaches to developing climate resilience varieties.

2. FUNDAMENTALS OF C4 PHOTOSYNTHESIS

A more sophisticated biochemical adaptation is C4 photosynthesis which concentrates CO2 around Rubisco, the enzyme responsible for carbon fixation (Hatch, 1987). C4 pathway is spatially confined between mesophyll and bundle sheath cells with echinological and anatomical distinctions. At the molecular level, mesophyll cells receive first CO2 fixation, via phosphoenolpyruvate carboxylase, producing four-carbon organic acids, and then transported to bundle sheath cells for decarboxylation, and later CO2 fixation by Rubisco (von Caemmerer 2000). This biochemical mechanism is useful in high temperatures.

The CO_2-concentrating mechanism reduces photorespiration, which increases exponentially with temperature in C3 plants but remains minimal in C4 plants (Berry & Björkman, 1980). C4 plants maintain higher photosynthetic rates at elevated temperatures and exhibit superior water use efficiency, requiring 250-350 g of water per gram of biomass compared to 450-950 g in C3 plants (Ghannoum, 2009).

2.1 Temperature Responses of C4 Photosynthesis

C4 photosynthesis shows distinct temperature responses compared to C3 photosynthesis. The temperature optimum for C4 photosynthesis typically ranges from 30-40°C, significantly higher than the 20-25°C optimum for C3 plants. However, when temperatures exceed 40-45°C, C4 photosynthesis becomes increasingly compromised due to multiple factors including enzyme deactivation, membrane instability, and impaired electron transport (Crafts-Brandner & Salvucci, 2002).

Recent studies have revealed that C4 plants may be more sensitive to rapid temperature increases than previously thought. Anderson et al. (2021) reported that photosynthetic efficiency of the C4 model plant *Setaria viridis* declines substantially upon exposure to high light intensity as well as high temperature, indicating that C4 systems are equally at risk under predicted climate scenarios.

2.2 Key Factors of C4 Heat Tolerance

Some of the C4 pathway components show differential heat sensitivities. Rubisco activase is essential for keeping the catalytic activity of Rubisco functional and gets inactivated above temperatures of 35–40°C for the majority of C4 plants (Salvucci & Crafts-Brandner, 2004). It is an enzyme that occurs as multiple isoforms with graded heat tolerance, but C4 grasses have diverse mechanisms of preserving its activity at heat stress. Photosystem II (PSII) is also a key heat-sensitive component. The PSII oxygen-evolving complex is also extremely sensitive to heat damage and causes photoinhibition as well as decreased photosynthetic yield (Allakhverdiev et al., 2008). C4 plants have superior PSII repair mechanisms and protective substances that preserve function at moderate heat stress as a countermeasure.

3. MOLECULAR MECHANISMS OF HEAT STRESS RESPONSE

3.1 Heat Shock Proteins and Molecular Chaperones

Heat shock proteins (HSPs) are the central molecular safeguard against protein denaturation and aggregation from heat stress. C4 plants possess diverse HSP families that are distinguished from each other by their molecular weight: HSP100, HSP90, HSP70, HSP60, and small HSPs (sHSPs) (Boston et al., 1996). Each of them possesses a specific role in the maintenance of protein stability: sHSPs prevent aggregation, while larger HSPs refold denatured proteins (Vierling, 1991). Genome-wide analyses identified large HSP gene families within C4 crops. Maize, for example, has more than 200 HSP genes, while sorghum has around 150 (Nagaraju et al., 2020). These genes show tissue-specific and stress-responsive expression patterns, with many showing rapid upregulation within hours of heat exposure. Small heat shock proteins play particularly important roles in C4 heat tolerance. The sHSP26 in maize chloroplasts prevents aggregation of photosynthetic proteins under heat stress (Kotak et al., 2007). Similarly, cytoplasmic sHSPs protect essential enzymes of the C4 pathway from heat-induced denaturation.

3.2 Heat Shock Transcription Factors

Heat shock transcription factors serve as master regulators of heat stress responses, controlling expression of HSPs and other stress-responsive genes. C4 plants contain multiple HSF families (HSFA, HSFB, HSFC) with distinct regulatory functions (Scharf et al., 2012). HSFA1 acts as the primary heat stress sensor and regulator, while HSFA2 and HSFA3 provide sustained stress responses (Liu et al., 2011).

Recent functional studies have identified key HSFs in C4 crops. ZmHSFA2 in maize enhances thermotolerance through regulation of downstream target genes including HSPs and metabolic enzymes. Similarly, SbHSFA1 in sorghum coordinates expression of multiple stress response pathways (Kumar et al., 2020). These transcription factors often work in complex regulatory networks, with feed-forward and feedback loops ensuring robust stress responses (Fragkostefanakis et al., 2015).

3.3 Protein Quality Control Systems

Beyond HSPs and HSFs, C4 plants employ sophisticated protein quality control mechanisms including the unfolded protein response (UPR) in the endoplasmic reticulum and chloroplast (Liu et al., 2007). The UPR involves sensors that detect protein misfolding and activate transcriptional programs to enhance protein folding capacity or eliminate damaged proteins (Howell, 2013).

Proteasomal degradation pathways also play crucial roles in removing damaged proteins under heat stress. The 26S proteasome system selectively degrades heat-damaged proteins tagged with ubiquitin, preventing accumulation of toxic protein aggregates (Kurepa & Smalle, 2008). C4 plants show enhanced proteasome activity under heat stress, contributing to cellular homeostasis maintenance.

4. MEMBRANE STABILITY AND LIPID REMODELING

4.1 Heat Effects on Membrane Structure

Cellular membranes represent primary targets of heat damage, with increased temperature causing lipid phase transitions, altered membrane fluidity, and loss of membrane integrity (Saidi et al., 2010). Heat stress disrupts the lipid bilayer structure, leading to increased permeability and potential membrane fusion or fragmentation. These changes particularly affect chloroplast thylakoid membranes, where photosynthetic electron transport occurs (Sharkey, 2005).

C4 plants maintain membrane stability through several mechanisms. Membrane lipid composition plays a critical role, with the ratio of saturated to unsaturated fatty acids determining membrane fluidity (Los & Murata, 2004). Heat-tolerant C4 varieties typically exhibit higher proportions of saturated fatty acids, which maintain membrane stability at elevated temperatures (Alfonso et al., 2001).

4.2 Lipid Remodeling Responses

Dynamic membrane lipid remodeling represents a key adaptive response to heat stress in C4 plants. Fatty acid desaturases regulate membrane fluidity by controlling the degree of lipid saturation (Upchurch, 2008). Under heat stress, many C4 species reduce desaturase activity, increasing membrane stability through higher saturated fatty acid content (Murakami et al., 2000).

Phospholipid composition also changes under heat stress. C4 plants increase phosphatidylglycerol and phosphatidylcholine content while reducing phosphatidylethanolamine, contributing to improved membrane stability (Welti et al., 2002). These changes occur within hours of heat exposure and are mediated by altered expression of lipid biosynthetic enzymes (Burgos et al., 2011).

4.3 Membrane-Associated Protective Mechanisms

C4 plants produce various membrane-protective compounds under heat stress. Compatible solutes such as proline, glycine betaine, and polyamines also maintain membrane structure stability and prevent protein denaturation. Antioxidants such as carotenoids and tocopherols also protect membrane from oxidative damage that is related with heat stress (Munné-Bosch, 2005). Membrane protection is also offered by heat shock proteins. Some HSPs directly interact with membranes to prevent heat-dependent membrane protein aggregation and maintain membranes functional (Török et al., 2001). The sHSP families show extremely high preference for membrane systems and are essential for maintenance of membrane integrity.

5. ANTIOXIDANT SYSTEMS AND OXIDATIVE STRESS MANAGEMENT

5.1 Heat-Induced Oxidative Stress

Heat stress will inevitably lead to increased production of reactive oxygen species (ROS) like superoxide radicals, hydrogen peroxide, and hydroxyl radicals (Mittler, 2002). The ROS impose oxidative damage of nucleic acids, membrane lipids, and proteins causing cellular dysfunction as well as cell death. In C4 plants, chloroplasts as well as mitochondria are major sites of production of ROS after heat stress (Asada, 2006). The photosynthetic system of C4 is very responsive to oxidative damage. Photosystem I as well as II generate ROS when electron transport is not balanced after heat stress (Allakhverdiev et al., 2008). The oxygen-evolving complex of PSII is very sensitive such that ROS production is exponential at more than 35°C.

5.2 Enzymatic Antioxidant Systems

C4 plants rely on sophisticated enzymic antioxidant processes for the detoxification of ROS and the prevention of oxidative damage. Important antioxidant enzymes include superoxide dismutase (SOD), which converts the superoxide molecule to hydrogen peroxide, catalase (CAT) and peroxidases that convert hydrogen peroxide to water, with glutathione reductase providing pools of reduced glutathione (Fujita & Hasanuzzaman, 2022).

Different C4 species show varying antioxidant enzyme activities and responses to heat stress. Heat-tolerant sorghum genotypes typically exhibit higher constitutive SOD and CAT activities and greater induction of these enzymes under stress (Singh et al., 2018). Maize shows rapid upregulation of cytosolic and chloroplastic antioxidant enzymes within 6-12 hours of heat exposure (Almeselmani et al., 2006).

The ascorbate-glutathione cycle represents a particularly important antioxidant pathway in C4 chloroplasts (Foyer & Noctor, 2011). This cycle involves coordinated action of ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase, and glutathione reductase to maintain reduced ascorbate and glutathione pools essential for ROS detoxification.

5.3 Non-Enzymatic Antioxidants

C4 plants accumulate various non-enzymatic antioxidants including ascorbic acid, tocopherols, carotenoids, and phenolic compounds. These compounds scavenge ROS directly and work synergistically with enzymatic systems to prevent oxidative damage (Blokhina et al., 2003).

Carotenoids play dual roles as accessory pigments in photosynthesis and as antioxidants protecting against photo-oxidative damage. Heat-tolerant C4 varieties often show enhanced carotenoid accumulation under stress conditions (Havaux, 1998). α-Tocopherol similarly protects chloroplast membranes from lipid peroxidation while maintaining photosynthetic function.

6. COMPATIBLE SOLUTES AND OSMOPROTECTION

6.1 Osmolyte Accumulation under Heat Stress

Compatible solutes represent low molecular weight organic compounds that accumulate under various abiotic stresses without interfering with normal metabolism (Yancey, 2005). In C4 plants, heat stress triggers accumulation of diverse osmolytes including proline, glycine betaine, polyamines, and sugars. These compounds serve multiple protective functions including osmotic adjustment, protein stabilization, and membrane protection (Chen & Murata, 2011).

Proline accumulation is a hallmark response to heat stress in C4 crops. This imino acid stabilizes proteins and membranes while scavenging ROS. Heat-stressed maize plants can accumulate proline levels 10-20 fold higher than unstressed controls, with accumulation correlating with thermotolerance (Raza et al., 2023).

6.2 Metabolic Pathways and Regulation

Osmolyte biosynthesis involves complex metabolic pathways with multiple regulatory points. Proline synthesis from glutamate is catalyzed by pyrroline-5-carboxylate synthetase (P5CS), a rate-limiting enzyme subject to transcriptional and post-translational regulation (Verbruggen & Hermans, 2008). Heat stress rapidly upregulates P5CS expression while downregulating proline dehydrogenase, the catabolic enzyme.

Glycine betaine synthesis involves sequential oxidation of choline by choline monooxygenase and betaine aldehyde dehydrogenase (Chen & Murata, 2011). Not all C4 species can synthesize glycine betaine, with maize lacking this pathway while sorghum and pearl millet are efficient producers. This difference contributes to species-specific heat tolerance capabilities (Shafiq et al., 2021).

6.3 Protective Mechanisms and Functions

Compatible solutes protect cellular components through multiple mechanisms. They act as molecular chaperones, preventing protein denaturation and aggregation under heat. Osmolytes also stabilize enzyme structure and activity, maintaining metabolic function under stress conditions (Somero, 2003).

Membrane protection represents another crucial function. Proline and glycine betaine interact with membrane lipids, maintaining bilayer stability and preventing heat-induced phase transitions. These interactions are particularly important in chloroplast thylakoids where photosynthetic processes must continue under stress (Allakhverdiev et al., 2008).

7. METABOLIC ADJUSTMENTS AND ENERGY MANAGEMENT

7.1 Respiratory Metabolism under Heat Stress

Heat stress profoundly affects respiratory metabolism in C4 plants, with increased respiration rates often exceeding photosynthetic carbon gain at extreme temperatures (Way & Oren, 2010). Respiratory enzymes generally show greater thermostability than photosynthetic enzymes, leading to altered carbon balance under heat stress (Atkin et al., 2005). Mitochondrial respiration increases exponentially with temperature following Arrhenius kinetics until thermal breakdown occurs. This increase initially helps maintain cellular energy status but becomes detrimental when respiratory carbon loss exceeds photosynthetic gain (Slot & Winter, 2017). C4 plants must balance energy production with carbon conservation under heat stress.

7.2 Sugar and Starch Metabolism

Carbohydrate metabolism undergoes significant modifications under heat stress in C4 plants. Starch synthesis often decreases due to heat-sensitive enzymes in the pathway, leading to accumulation of soluble sugars (Kaplan & Guy, 2005). These sugars can serve as osmolytes and energy reserves while contributing to stress tolerance. Sugar signaling pathways also mediate stress responses. Sucrose and trehalose act as signaling molecules that regulate gene expression and metabolic activity under heat stress. The trehalose-6-phosphate pathway plays particularly important roles in coordinating growth and stress responses (Lunn et al., 2014).

7.3 Secondary Metabolite Production

Heat stress stimulates production of various secondary metabolites with protective functions in C4 plants. Phenolic compounds including flavonoids and phenylpropanoids accumulate under stress conditions, providing antioxidant protection and UV screening (Agati et al., 2012). These compounds also stabilize proteins and membranes, contributing to overall stress tolerance. Polyamine metabolism is also altered under heat stress, with increased synthesis of putrescine, spermidine, and spermine (Singh et al., 2018). These compounds stabilize nucleic acids and proteins while regulating ion channels and membrane stability. Heat-tolerant C4 varieties often show enhanced polyamine accumulation under stress conditions (Liu et al., 2007).

8. REPRODUCTIVE DEVELOPMENT AND HEAT STRESS

8.1 Critical Stages and Sensitivity

Reproductive development represents the most heat-sensitive stage in C4 crop development, with temperatures above 35-38°C during flowering causing significant yield losses (Hatfield & Prueger, 2015). Pollen development and anthesis are particularly vulnerable, with heat stress causing pollen sterility and reduced fertilization success.

In maize, heat stress during tasseling and silking can reduce kernel set by 20-50% even with brief exposure to temperatures above 38°C (Waqas et al., 2021). Sorghum shows similar sensitivity, with heat stress during anthesis causing complete failure of grain set in extreme cases (Prasad et al., 2006). These reproductive failures represent major causes of heat-induced yield losses in C4 crops.

8.2 Molecular Mechanisms of Reproductive Heat Sensitivity

The molecular basis of reproductive heat sensitivity involves multiple factors including altered gene expression, protein dysfunction, and metabolic disruption. Heat stress affects pollen wall development, reducing pollen viability and germination capacity (Firon et al., 2006). Key enzymes involved in pollen development show high temperature sensitivity, contributing to reproductive failure (Endo et al., 2009). Hormone signaling pathways also become disrupted during reproductive heat stress. Auxin and cytokinin levels decrease while ethylene increases, leading to altered flower development and reduced fertility. These hormonal changes are mediated by heat effects on biosynthetic enzymes and signaling proteins (Guilioni et al., 1997).

8.3 Adaptive Responses and Tolerance Mechanisms

Some C4 varieties have evolved mechanisms to maintain reproductive success under mild heat stress. These include altered flowering time to avoid peak temperatures, enhanced pollen thermostability, and improved ovary function under stress. Heat-tolerant varieties often show constitutively higher expression of protective genes in reproductive tissues.

Breeding programs have identified quantitative trait loci (QTL) associated with reproductive heat tolerance in maize, sorghum, and pearl millet (Cairns et al., 2013). These QTL often map to genes involved in hormone metabolism, stress signaling, and cellular protection, providing targets for marker-assisted selection.

9. OMICS APPROACHES TO HEAT STRESS RESEARCH

9.1 Genomics and Quantitative Genetics

Large-scale genomic studies have revolutionized understanding of heat stress tolerance in C4 crops. Genome-wide association studies (GWAS) have identified hundreds of loci associated with heat tolerance traits in maize, sorghum, and other C4 species (Xu et al., 2009). These studies reveal the highly polygenic nature of heat tolerance, with many genes of small effect contributing to overall tolerance.

Comparative genomics approaches have identified conserved heat stress response pathways across C4 species while revealing species-specific adaptations. Synteny analysis shows that core heat shock response genes are highly conserved, while regulatory elements and expression patterns show significant divergence (Tang et al., 2010). Pan-genome studies are revealing extensive structural variation within C4 species that contributes to heat tolerance diversity (Hirsch et al., 2014). Copy number variations, presence-absence variations, and structural rearrangements all contribute to phenotypic diversity in heat tolerance. These findings emphasize the importance of capturing genetic diversity for breeding programs.

9.2 Transcriptomics and Gene Expression

RNA sequencing technologies have provided unprecedented insights into gene expression responses to heat stress in C4 plants. Temporal transcriptomic studies reveal rapid changes in gene expression within minutes of heat exposure, followed by sustained responses lasting hours to days. Heat stress affects expression of thousands of genes across multiple functional categories.

Single-cell RNA sequencing is beginning to reveal cell-type-specific responses to heat stress in C4 plants (Li et al., 2021). These studies show that mesophyll and bundle sheath cells exhibit distinct transcriptional responses, with differential expression of metabolic genes maintaining C4 function under stress. Spatial transcriptomics approaches are providing tissue-level resolution of heat stress responses. These studies reveal that different leaf regions, root zones, and reproductive tissues show distinct expression patterns under heat stress (Giacomello et al., 2017). This spatial information is crucial for understanding how heat stress affects whole-plant function and development.

9.3 Proteomics and Post-Translational Modifications

Proteomic analyses have revealed that protein abundance changes often differ from transcript abundance changes under heat stress, highlighting the importance of post-transcriptional regulation (Kosová et al., 2011). Heat stress affects protein synthesis, folding, and degradation, leading to altered proteome composition. Phosphoproteomics studies have identified extensive changes in protein phosphorylation under heat stress. Calcium-dependent protein kinases, mitogen-activated protein kinases, and other signaling enzymes show altered activity patterns that regulate downstream responses (Klimecka & Muszyńska, 2007).

Ubiquitomics and other post-translational modification studies are revealing complex regulatory networks governing heat stress responses (Marino et al., 2018). Protein ubiquitination, sumoylation, and acetylation all contribute to fine-tuning cellular responses to elevated temperature.

9.4 Metabolomics and Systems Biology

Metabolomic approaches have identified hundreds of metabolites that change in response to heat stress in C4 plants (Obata & Fernie, 2012). Primary metabolites including amino acids, sugars, and organic acids show major changes, while secondary metabolites such as phenolics and terpenoids also respond significantly (Rudell et al., 2008).

Systems biology approaches are integrating multi-omics data to construct comprehensive models of heat stress responses (Yuan et al., 2017). These models reveal complex regulatory networks with multiple feedback loops and crosstalk between different stress response pathways. Machine learning approaches are being applied to predict heat tolerance from omics signatures (Mochida et al., 2018).

Network analysis of integrated omics data has identified key hub genes and metabolites that coordinate heat stress responses (Barabási & Oltvai, 2004). These hub components represent potential targets for engineering enhanced heat tolerance. The integration of omics with phenotypic data is enabling genome-to-phenome predictions for heat tolerance traits.

10. BREEDING AND BIOTECHNOLOGICAL APPROACHES

10.1 Conventional Breeding Strategies

Traditional breeding remains the primary approach for developing heat-tolerant C4 varieties. Selection in hot environments has been effective for identifying tolerant genotypes, though progress has been slow due to the complex, polygenic nature of heat tolerance (Hemantaranjan, 2014). Breeding programs now incorporate controlled environment screening to supplement field testing and improve selection efficiency. Physiological breeding approaches focus on specific tolerance mechanisms such as membrane stability, osmotic adjustment, and photosynthetic thermostability. These traits can be measured directly and used as selection criteria, potentially accelerating breeding progress (Blum, 2011). Heat tolerance indices that combine multiple physiological measurements are being developed to guide selection decisions (Al-Ashkar et al., 2023).

10.2 Marker-Assisted Selection

Molecular markers linked to heat tolerance QTL are being incorporated into breeding programs to accelerate variety development (Collard & Mackill, 2008). Marker-assisted selection can be particularly valuable for traits that are difficult or expensive to phenotype, such as specific physiological responses to heat stress.

Genomic selection approaches that use genome-wide marker data are showing promise for complex traits like heat tolerance (Meuwissen et al., 2001). These approaches can potentially achieve faster genetic gain than conventional selection by capturing the effects of many small-effect loci (Goddard & Hayes, 2007; Anilkumar et al., 2022). Training populations with extensive phenotyping are essential for implementing genomic selection effectively (Heffner et al., 2009).

10.3 Genetic Engineering Approaches

Transgenic approaches offer opportunities to introduce specific heat tolerance genes into elite varieties. Overexpression of heat shock factors, heat shock proteins, and compatible solute biosynthetic genes has successfully enhanced heat tolerance in various crops (Saini et al., 2022). However, regulatory and consumer acceptance issues limit the deployment of transgenic varieties in many regions.

Gene editing technologies such as CRISPR/Cas9 are providing new opportunities for precise genetic modification. These approaches can be used to modify existing genes to enhance thermostability or to edit regulatory elements that control stress-responsive gene expression (Bortesi & Fischer, 2015). Gene editing may face fewer regulatory hurdles than traditional genetic engineering approaches.

10.4 Synthetic Biology and Metabolic Engineering

Synthetic biology approaches are being explored for engineering novel heat tolerance mechanisms in C4 crops (Paddon & Keasling, 2014; Liu et al., 2023). These include designing new metabolic pathways for compatible solute production, engineering enhanced antioxidant systems, and creating synthetic regulatory circuits that respond to heat stress (Cameron et al., 2014).

Metabolic engineering of specific pathways has shown success in enhancing stress tolerance. Engineering enhanced proline biosynthesis, trehalose production, and antioxidant capacity has improved heat tolerance in various plant species. However, metabolic engineering requires careful consideration of pathway interactions to avoid unintended consequences.

11. CONCLUSIONS

Heat stress represents a major threat to C4 crop productivity under current and projected climate conditions. Despite their evolutionary adaptation to warm environments, C4 plants remain vulnerable to extreme temperatures that disrupt photosynthesis, metabolism, and reproductive development. Understanding the molecular mechanisms underlying heat tolerance is crucial for developing climate-resilient varieties.

Recent advances in omics technologies have revealed the complex, multigenic nature of heat tolerance in C4 plants. Heat shock proteins, antioxidant systems, membrane modifications, and metabolic adjustments all contribute to thermotolerance through interconnected networks. This complexity presents both challenges and opportunities for crop improvement efforts.

Breeding programs are successfully incorporating molecular tools to accelerate development of heat-tolerant varieties. Marker-assisted selection, genomic selection, and targeted gene editing approaches are complementing conventional breeding to enhance genetic gain. However, the polygenic nature of heat tolerance requires continued refinement of these approaches.

The integration of genetics, physiology, and agronomy through systems approaches offers the most promising path forward. Comprehensive understanding of plant responses to heat stress at multiple scales, from molecules to whole plants to cropping systems, will enable development of robust adaptation strategies. The urgency of climate change demands accelerated research and development efforts to ensure food security for a growing global population.

Future success will depend on continued technological innovation, international collaboration, and sustained investment in research and development. The challenges are significant, but the tools and knowledge base for addressing them continue to expand rapidly. With coordinated effort, heat-tolerant C4 varieties can help maintain agricultural productivity in a warming world.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

Agati, G., Azzarello, E., Pollastri, S., & Tattini, M. (2012). Flavonoids as antioxidants in plants: Location and functional significance. *Plant Science, 196*, 67–76.

Al-Ashkar, I., Sallam, M., Ghazy, A., Ibrahim, A., Alotaibi, M., Ullah, N., & Al-Doss, A. (2023). Agro-physiological indices and multidimensional analyses for detecting heat tolerance in wheat genotypes. *Agronomy, 13*(1), 154.

Alfonso, M., Yruela, I., Almárcegui, S., Torrado, E., Pérez, M. A., & Picorel, R. (2001). Unusual tolerance to high temperatures in a new herbicide-resistant D1 mutant from *Glycine max* (L.) Merr. cell cultures deficient in fatty acid desaturation. *Planta, 212*(4), 573–582.

Allakhverdiev, S. I., Kreslavski, V. D., Klimov, V. V., Los, D. A., Carpentier, R., & Mohanty, P. (2008). Heat stress: An overview of the molecular responses in photosynthesis. *Photosynthesis Research, 98*(1–3), 541–550.

Almeselmani, M., Deshmukh, P. S., Sairam, R. K., Kushwaha, S. R., & Singh, T. P. (2006). Protective role of antioxidant enzymes under high temperature stress. *Plant Science, 171*(3), 382–388.

Anderson, C. M., Mattoon, E., Zhang, N., Becker, E., McHargue, W., Yang, J., ... & Brutnell, T. P. (2021). High light and temperature reduce photosynthetic efficiency in the C4 model plant *Setaria viridis*. *Communications Biology, 8*(1), 1047.

Anilkumar, C., Sunitha, N. C., Harikrishna, Devate, N. B., & Ramesh, S. (2022). Advances in integrated genomic selection for rapid genetic gain in crop improvement: A review. *Planta, 256*(5), 87.

Asada, K. (2006). Production and scavenging of reactive oxygen species in chloroplasts and their functions. *Plant Physiology, 141*(2), 391–396.

Atkin, O. K., Bruhn, D., Hurry, V. M., & Tjoelker, M. G. (2005). Evans review: The hot and the cold: Unravelling the variable response of plant respiration to temperature. *Functional Plant Biology, 32*(2), 87–105.

Barabási, A. L., & Oltvai, Z. N. (2004). Network biology: Understanding the cell's functional organization. *Nature Reviews Genetics, 5*(2), 101–113.

Berry, J., & Bjorkman, O. (1980). Photosynthetic response and adaptation to temperature in higher plants. *Annual Review of plant physiology*, *31*(1), 491-543.

Blokhina, O., Virolainen, E., & Fagerstedt, K. V. (2003). Antioxidants, oxidative damage and oxygen deprivation stress: A review. *Annals of Botany, 91*(2), 179–194.

Blum, A. (2011). Phenotyping and selection. In *Plant breeding for water-limited environments* (pp. 153–216). Springer.

Bortesi, L., & Fischer, R. (2015). The CRISPR/Cas9 system for plant genome editing and beyond. *Biotechnology Advances, 33*(1), 41–52.

Boston, R. S., Viitanen, P. V., & Vierling, E. (1996). Molecular chaperones and protein folding in plants. *Plant Molecular Biology, 32*(1–2), 85–110.

Burgos, A., Szymanski, J., Seiwert, B., Degenkolbe, T., Hannah, M. A., Giavalisco, P., & Willmitzer, L. (2011). Analysis of short-term changes in the *Arabidopsis thaliana* glycerolipidome in response to temperature and light. *The Plant Journal, 66*(4), 656–668.

Cairns, J. E., Crossa, J., Zaidi, P. H., Grudloyma, P., Sanchez, C., Araus, J. L., ... & Prasanna, B. M. (2013). Identification of drought, heat, and combined drought and heat tolerant donors in maize. *Proceedings of the National Academy of Sciences, 110*(1), 29–34.

Cameron, D. E., Bashor, C. J., & Collins, J. J. (2014). A brief history of synthetic biology. *Nature Reviews Microbiology, 12*(5), 381–390.

Chen, T. H., & Murata, N. (2011). Glycinebetaine protects plants against abiotic stress: Mechanisms and biotechnological applications. *Plant, Cell & Environment, 34*(1), 1–20.

Collard, B. C., & Mackill, D. J. (2008). Marker-assisted selection: An approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society B, 363*(1491), 557–572.

Crafts-Brandner, S. J., & Salvucci, M. E. (2002). Sensitivity of photosynthesis in a C4 plant, maize, to heat stress. *Plant Physiology, 129*(4), 1773–1780.

Endo, M., Tsuchiya, T., Hamada, K., Kawamura, S., Yano, K., Ohshima, M., ... & Kawagishi-Kobayashi, M. (2009). High temperatures cause male sterility in rice plants with transcriptional alterations during pollen development. *Plant and Cell Physiology, 50*(11), 1911–1922.

FAOSTAT (2023) *Food and Agriculture Organization Corporate Statistical Database*.

Firon, N., Nepi, M., & Pacini, E. (2012). Water status and associated processes mark critical stages in pollen development and functioning. *Annals of Botany, 109*(7), 1201–1213.

Foyer, C. H., & Noctor, G. (2011). Ascorbate and glutathione: The heart of the redox hub. *Plant Physiology, 155*(1), 2–18.

Fragkostefanakis, S., Röth, S., Schleiff, E., & Scharf, K. D. (2015). Prospects of engineering thermotolerance in crops through modulation of heat stress transcription factor and heat shock protein networks. *Plant, Cell & Environment, 38*(9), 1881–1895.

Fujita, M., & Hasanuzzaman, M. (2022). Approaches to enhancing antioxidant defense in plants. *Antioxidants, 11*(5), 925.

Ghannoum, O. (2009). C4 photosynthesis and water stress. *Annals of Botany, 103*(4), 635–644.

Giacomello, S., Salmén, L., Tegelberg, S., Frisk, M., Hertzberg, M., Nystedt, B., ... & Lundeberg, J. (2017). Spatially resolved transcriptome profiling in model plant species. *Nature Plants, 3*(6), 17061.

Goddard, M. E., & Hayes, B. J. (2007). Genomic selection. *Journal of Animal Breeding and Genetics, 124*(6), 323–330.

Guilioni, L., Wery, J., & Lecoeur, J. (2003). High temperature and water deficit may reduce seed number in field pea purely by decreasing plant growth rate. *Functional Plant Biology, 30*(11), 1151–1164.

Hasanuzzaman, M., Nahar, K., Alam, M. M., Roychowdhury, R., & Fujita, M. (2013). Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *International Journal of Molecular Sciences, 14*(5), 9643–9684.

Hatch, M. D. (1987). C4 photosynthesis: A unique blend of modified biochemistry, anatomy and ultrastructure. *Biochimica et Biophysica Acta, 895*(2), 81–106.

Hatfield, J. L., & Prueger, J. H. (2015). Temperature extremes: Effect on plant growth and development. *Weather and Climate Extremes, 10*, 4–10.

Havaux, M. (1998). Carotenoids as membrane stabilizers in chloroplasts. *Trends in Plant Science, 3*(4), 147–151.

Heffner, E. L., Sorrells, M. E., & Jannink, J. L. (2009). Genomic selection for crop improvement. *Crop Science, 49*(1), 1–12.

Hemantaranjan, A., Bhanu, A. N., Singh, M. N., Yadav, D. K., Patel, P. K., Singh, R., & Katiyar, D. J. A. P. A. R. (2014). Heat stress responses and thermotolerance. *Adv. Plants Agric. Res*, *1*(3), 1-10.

Hirsch, C. N., Foerster, J. M., Johnson, J. M., Sekhon, R. S., Muttoni, G., Vaillancourt, B., ... & Kaeppler, S. M. (2014). Insights into the maize pan-genome and pan-transcriptome. *The Plant Cell, 26*(1), 121–135.

Howell, S. H. (2013). Endoplasmic reticulum stress responses in plants. *Annual Review of Plant Biology, 64*, 477–499.

Hu, X., Xiao, J., Niu, J., Liu, Q., Yang, L., Wang, L., & Zhu, J. (2022). Increase crop resilience to heat stress using omic technologies. *Frontiers in Plant Science, 13*, 891861.

IPCC. (2021). *Climate change 2021: The physical science basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press.

Kaplan, F., & Guy, C. L. (2005). RNA interference of *Arabidopsis* beta-amylase8 prevents maltose accumulation upon cold shock and increases sensitivity of PSII photochemical efficiency to freezing stress. *The Plant Journal, 44*(5), 730–743.

Klimecka, M., & Muszyńska, G. (2007). Structure and functions of plant calcium-dependent protein kinases. *Acta Biochimica Polonica, 54*(2), 219–233.

Kosová, K., Vítámvás, P., Prášil, I. T., & Renaut, J. (2011). Plant dehydrins: Tissue location, structure and function. *Biochimica et Biophysica Acta, 1804*(9), 1919–1933.

Kotak, S., Larkindale, J., Lee, U., von Koskull-Döring, P., Vierling, E., & Scharf, K. D. (2007). Complexity of the heat stress response in plants. *Current Opinion in Plant Biology, 10*(3), 310–316.

Kumar, A., Memo, M., & Mastinu, A. (2020). Plant behaviour: An evolutionary response to the environment? *Plant Biology, 22*(6), 961–970.

Kurepa, J., & Smalle, J. A. (2008). 26S proteasome regulatory particle mutants have increased oxidative stress tolerance. *The Plant Journal, 53*(1), 102–114.

Li, N., Euring, D., Cha, J. Y., Lin, Z., Lu, M., Huang, L. J., & Kim, W. Y. (2021). Plant hormone-mediated regulation of heat tolerance in response to global climate change. *Frontiers in Plant Science, 11*, 627969.

Liu, H. C., Liao, H. T., & Charng, Y. Y. (2011). The role of class A1 heat shock factors (HSFA1s) in response to heat and other stresses in *Arabidopsis*. *Plant, Cell & Environment, 34*(5), 738–751.

Liu, J., Sun, N., Liu, M., Liu, J., Du, B., Wang, X., & Qi, X. (2013). An autoregulatory loop controlling *Arabidopsis* HsfA2 expression: Role of heat shock-induced alternative splicing. *Plant Physiology, 162*(1), 512–521.

Liu, S., Zenda, T., Tian, Z., & Huang, Z. (2023). Metabolic pathways engineering for drought or/and heat tolerance in cereals. *Frontiers in Plant Science, 14*, 1111875.

Liu, Y. G., & Chen, Y. (2007). High-efficiency thermal asymmetric interlaced PCR for amplification of unknown flanking sequences. *Biotechniques, 43*(5), 649–656.

Lobell, D. B., Bänziger, M., Magorokosho, C., & Vivek, B. (2011). Nonlinear heat effects on African maize as evidenced by historical yield trials. *Nature Climate Change, 1*(1), 42–45.

Los, D. A., & Murata, N. (2004). Membrane fluidity and its roles in the perception of environmental signals. *Biochimica et Biophysica Acta, 1666*(1–2), 142–157.

Lunn, J. E., Delorge, I., Figueroa, C. M., Van Dijck, P., & Stitt, M. (2014). Trehalose metabolism in plants. *The Plant Journal, 79*(4), 544–567.

Marino, D., Peeters, N., & Rivas, S. (2012). Ubiquitination during plant immune signaling. *Plant Physiology, 160*(1), 15–27.

Meuwissen, T. H., Hayes, B. J., & Goddard, M. E. (2001). Prediction of total genetic value using genome-wide dense marker maps. *Genetics, 157*(4), 1819–1825.

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science, 7*(9), 405–410.

Mochida, K., Koda, S., Inoue, K., Nishii, R., & Hirayama, T. (2018). Statistical and machine learning approaches to predict gene regulatory networks from transcriptome datasets. *Frontiers in Plant Science, 9*, 1770.

Munné-Bosch, S. (2005). The role of α-tocopherol in plant stress tolerance.

Journal of Plant Physiology, 162(7), 743–748.

Murakami, Y., Tsuyama, M., Kobayashi, Y., Kodama, H., & Iba, K. (2000). Trienoic fatty acids and plant tolerance of high temperature. *Science, 287*(5452), 476–479.

Nagaraju, M., Kumar, S. A., Reddy, P. S., Kumar, A., Rao, D. M., & Kavi Kishor, P. B. (2020). Genome-wide identification and transcriptional profiling of small heat shock protein gene family under diverse abiotic stress conditions in *Sorghum bicolor* (L.). *International Journal of Biological Macromolecules, 142*, 822–834.

Obata, T., & Fernie, A. R. (2012). The use of metabolomics to dissect plant responses to abiotic stresses. *Cellular and Molecular Life Sciences, 69*(19), 3225–3243.

Paddon, C. J., & Keasling, J. D. (2014). Semi-synthetic artemisinin: A model for the use of synthetic biology in pharmaceutical development. *Nature Reviews Microbiology, 12*(5), 355–367.

Prasad, P. V. V., Boote, K. J., Allen Jr, L. H., Sheehy, J. E., & Thomas, J. M. G. (2006). Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress. *Field Crops Research, 95*(2–3), 398–411.

Raza, A., Charagh, S., Abbas, S., Hassan, M. U., Saeed, F., Haider, S., ... & Varshney, R. K. (2023). Assessment of proline function in higher plants under extreme temperatures. *Plant Biology, 25*(3), 379–395.

Rudell, D. R., Mattheis, J. P., Fan, X., & Fellman, J. K. (2002). Methyl jasmonate enhances anthocyanin accumulation and modifies production of phenolics and pigments in 'Fuji' apples. *Journal of the American Society for Horticultural Science, 127*(3), 435–441.

Sage, R. F., Sage, T. L., & Kocacinar, F. (2012). Photorespiration and the evolution of C4 photosynthesis. *Annual Review of Plant Biology, 63*, 19–47.

Saidi, Y., Finka, A., Muriset, M., Bromberg, Z., Weiss, Y. G., Maathuis, F. J., & Goloubinoff, P. (2009). The heat shock response in moss plants is regulated by specific calcium-permeable channels in the plasma membrane. *The Plant Cell, 21*(9), 2829–2843.

Saini, N., Nikalje, G. C., Zargar, S. M., & Suprasanna, P. (2022). Molecular insights into sensing, regulation and improving of heat tolerance in plants. *Plant Cell Reports, 41*(3), 799–813.

Salvucci, M. E., & Crafts-Brandner, S. J. (2004). Inhibition of photosynthesis by heat stress: the activation state of Rubisco as a limiting factor in photosynthesis. *Physiologia Plantarum, 120*(2), 179–186.

Scharf, K. D., Berberich, T., Ebersberger, I., & Nover, L. (2012). The plant heat stress transcription factor (Hsf) family: structure, function and evolution. *Biochimica et Biophysica Acta, 1819*(2), 104–119.

Shafiq, S., Akram, N. A., Ashraf, M., García-Caparrós, P., Ali, O. M., & Latef, A. A. H. A. (2021). Influence of glycine betaine (natural and synthetic) on growth, metabolism and yield production of drought-stressed maize (*Zea mays* L.) plants. *Plants, 10*(11), 2540.

Sharkey, T. D. (2005). Effects of moderate heat stress on photosynthesis: importance of thylakoid reactions, rubisco deactivation, reactive oxygen species, and thermotolerance provided by isoprene. *Plant, Cell & Environment, 28*(3), 269–277.

Singh, S., Sengar, R. S., Kulshreshtha, N., Datta, D., Tomar, R. S., Rao, V. P., ... & Ojha, A. (2015). Assessment of multiple tolerance indices for salinity stress in bread wheat (*Triticum aestivum* L.). *Journal of Agrisearch, 2*(4), 245–250.

Singh, P., Basu, S., & Kumar, G. (2018). Polyamines metabolism: A way ahead for abiotic stress tolerance in crop plants. In *Biochemical, physiological and molecular avenues for combating abiotic stress tolerance in plants* (pp. 39-55). Academic Press.

Singh, V., Singh, A. P., Bhadoria, J., Giri, J., Singh, J., Vineeth, T. V., ... & Prasad, P. V. V. (2018). Differential expression of salt-responsive genes to salinity stress in salt-tolerant and salt-sensitive rice (*Oryza sativa* L.) at seedling stage. *Protoplasma, 255*(6), 1667–1681.

Slot, M., & Winter, K. (2017). Photosynthetic acclimation to warming in tropical forest tree seedlings. *Journal of Experimental Botany, 68*(9), 2275–2284.

Somero, G. N. (2003). Protein adaptations to temperature and pressure: complementary roles of adaptive changes in amino acid sequence and internal milieu. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 136*(4), 577–591.

Tang, H., Bowers, J. E., Wang, X., Ming, R., Alam, M., & Paterson, A. H. (2008). Synteny and collinearity in plant genomes. *Science, 320*(5875), 486–488.

Török, Z., Goloubinoff, P., Horváth, I., Tsvetkova, N. M., Glatz, A., Balogh, G., ... & Vigh, L. (2001). *Synechocystis* HSP17 is an amphitropic protein that stabilizes heat-stressed membranes and binds denatured proteins for subsequent chaperone-mediated refolding. *Proceedings of the National Academy of Sciences, 98*(6), 3098–3103.

Upchurch, R. G. (2008). Fatty acid unsaturation, mobilization, and regulation in the response of plants to stress. *Biotechnology Letters, 30*(6), 967–977.

Verbruggen, N., & Hermans, C. (2008). Proline accumulation in plants: a review. *Amino Acids, 35*(4), 753–759.

Vierling, E. (1991). The roles of heat shock proteins in plants. *Annual Review of Plant Biology, 42*(1), 579–620.

von Caemmerer, S. (2000). *Biochemical models of leaf photosynthesis* (Vol. 2). CSIRO Publishing.

Waqas, M. A., Wang, X., Zafar, S. A., Noor, M. A., Hussain, H. A., Azher Nawaz, M., & Farooq, M. (2021). Thermal Stresses in Maize: Effects and Management Strategies. *Plants, 10*(2), 293. https://doi.org/10.3390/plants10020293

Way, D. A., & Oren, R. (2010). Differential responses to changes in growth temperature between trees from different functional groups and biomes: a review and synthesis of data. *Tree Physiology, 30*(6), 669–688.

Welti, R., Li, W., Li, M., Sang, Y., Biesiada, H., Zhou, H. E., ... & Wang, X. (2002). Profiling membrane lipids in plant stress responses: role of phospholipase Dα in freezing-induced lipid changes in *Arabidopsis*. *Journal of Biological Chemistry, 277*(35), 31994–32002.

Xu, Y., Skinner, D. J., Wu, H., Palacios-Rojas, N., Araus, J. L., Yan, J., ... & Crouch, J. H. (2009). Advances in maize genomics and their value for enhancing genetic gains from breeding. *International Journal of Plant Genomics, 2009*, 957602.

Yancey, P. H. (2005). Organic osmolytes as compatible, metabolic and counteracting solutes in high osmolarity and other stresses. *Journal of Experimental Biology, 208*(15), 2819–2830.

Yuan, J. S., Galbraith, D. W., Dai, S. Y., Griffin, P., & Stewart Jr, C. N. (2008). Plant systems biology comes of age. *Trends in Plant Science, 13*(4), 165–171.

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