CABI Reviews

REVIEW

The mechanisms of melatonin action in shielding photosynthesis during heat stress

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Abstract

The escalating global climate shifts lead to several negative impacts, including a continuous increase in average temperatures, imposing significant consequences on plant physiology, biochemical dynamics, and molecular processes. Central to these ramifications is the process of photosynthesis, pivotal for carbohydrate synthesis and essential for sustaining growth amidst normal or stressful environmental conditions. Exposure to heat stress (HS) negatively affects the photosynthetic potential, primarily due to the increased synthesis of reactive oxygen species, disrupting the optimal functionality of the photosynthetic apparatus. Melatonin (MLT) has emerged as a multifaceted signaling molecule exhibiting promising capabilities in mitigating the adverse effects of abiotic stress including HS on plants. While a substantial body of literature addresses the enhancement of plant resilience to various environmental stresses using MLT, the present review specifically focuses on the impact of MLT on photosynthesis during HS. It emphasizes MLT's role in regulating chlorophyll synthesis and degradation, regulating stomatal movement, safeguarding photosynthetic proteins, and sustaining the photosynthetic process under HS. This could be achieved by various mechanisms including enhancing the transcription of photosystem genes, activating antioxidant defenses, promoting the xanthophyll cycle, and modulating enzymes involved in photosynthesis and carbohydrate metabolism. Consequently, this review aims to enhance our understanding of how MLT mediates the protection of the photosynthesis process under HS, offering insights for the development of heat-tolerant crops.

Keywords: photosynthetic efficiency, heat acclimation, biostimulant, light and dark reaction, crosstalk, antioxidants

Introduction

Climate change is anticipated to elevate global temperatures by 1.8–4°C by the end of the century, raising concerns for food security due to its impact on agricultural productivity (Iturbide *et al*., 2020). Heat stress (HS) threatens plant growth and development, negatively impacting various cellular, physiological, and molecular processes. It leads to membrane damage, protein denaturation, nucleotide impairment, and the accumulation of reactive oxygen species (ROS). Additionally, HS increases the fluidity of the lipid membrane, this also affects microtubule organization, which is crucial for cell division and potentially trigger programmed cell death (Raza *et al*., 2022a, 2024). Photosynthesis, crucial for carbon cycling (Ferguson *et al*., 2021), is significantly impaired by extreme temperatures, limiting crop productivity essential for plant growth and development. Chloroplasts serve as the metabolic hub for photosynthesis and play a pivotal role in detecting high temperatures and triggering adequate responses via retrograde signaling (Hu *et al*., 2020; Pollastri *et al*., 2021). HS-induced damage to chloroplasts is primarily due to excessive generation of ROS, which compromises the integrity of the thylakoid membrane, disrupting both photosystem I (PSI) and photosystem II (PSII) along with their associated proteins. This disruption leads to a suppression of light energy absorption and distribution, electron

transfer, photophosphorylation, and the Calvin cycle metabolism due to elevated temperatures (Jahan *et al*., 2021a; Xu *et al*., 2023; Zahra *et al*., 2023). Moreover, chlorophyll levels decrease due to the degradation of chlorophyll synthase under HS (Raza *et al*., 2024). HS reduced the activity and expression of Rubisco enzyme as well as the inactivation of the Rubisco activase (Ristic *et al*., 2009; Alvi *et al*., 2024), resulting in decreased photosynthetic efficiency (Gautam *et al*., 2022a; Zahra *et al*., 2023; Alvi *et al*., 2024; Raza *et al*., 2024). In addition to this, HS also disrupts plant carbohydrate metabolism by modifying the activity of enzymes and expression of genes associated with this process; thus reducing photosynthetic performance and ultimately diminishing agricultural yields (Xalxo *et al*., 2020; Iqbal *et al*., 2021).

Plant hormones or growth regulators play a significant role in enhancing plant growth and productivity, particularly under optimal and HS conditions (Sehar *et al*., 2023). Melatonin (N-acetyl-5-methoxy tryptamine), first identified in plants in 1995 (Dubbels *et al*., 1995; Kolar *et al*., 1995) and found to be constitutively in various plant organs like roots, stems, leaves, fruits, flowers, and seeds, contributes substantially to plant growth, maturation, and resilience by affecting physiological processes (Raza *et al*., 2022a). Biosynthesis of MLT in plants usually occurs within chloroplasts. Recognized as a pleiotropic signaling molecule, MLT

serves as a potential growth regulator, aiding in defense against diverse environmental challenges (Arnao and Hernández-Ruiz, 2018; Raza *et al*., 2022a). Evidence suggests that MLT acts as a broad-spectrum antioxidant, effectively scavenging ROS (Arnao and Hernández-Ruiz, 2021; Raza *et al*., 2022a). It also influences the expression of stress-responsive genes and heat shock proteins (HSPs), enhances the accumulation of amino acids, osmoprotectants, secondary metabolites, and modulates the synthesis of endogenous MLT and other growth regulators (Arnao and Hernández-Ruiz, 2021; Hassan *et al*., 2022; Raza *et al*., 2022b; Karumannil *et al*., 2023). MLT interacts with well-established phytohormones like auxins (IAA), gibberellins (GA), cytokinin (CK), ethylene (ET), brassinosteroids (BRs), salicylic acid (SA), jasmonic acid (JA) as well newly discovered signaling molecules like hydrogen sulfide (H_2S), nitric oxide (NO), polyamines (PAs) to mitigate various abiotic stress (Raza *et al*., 2022b). For instance, MLT suppressed the HS-induced leaf senescence by altering the expression of ABA, GA, and CK genes (Zhang *et al*., 2017; Jahan *et al*., 2021b). Also, by affecting IAA content MLT improved the content of photosynthetic pigment (Jia *et al*., 2020). The synthesis of MLT likely occurs within the chloroplast, positioning it ideally to serve as a regulator of photosynthesis. Numerous studies have presented convincing proof that MLT improves photosynthetic efficiency and contributes to the consistent production of photosynthates, especially under different abiotic stress (Jahan *et al*., 2020; Iqbal *et al*., 2021; Khan *et al*., 2022; Altaf *et al*., 2022). A meta-analysis study highlights MLT's significant role in enhancing wheat resilience to abiotic stress by improving photosynthetic parameters, chlorophyll pigments, and leaf water status, with its effectiveness influenced by environmental conditions, temperature ranges, and application methods (Muhammad *et al*., 2024). Moreover, MLT affects cell layer permeability mediated through ion transporters, thereby regulating stomatal opening and closing in plants. By enhancing the expression of chlorophyll-synthesis-related genes and photosynthesis-related proteins while inhibiting chlorophyll degradation-associated genes, MLT improves the photosynthetic capacity of plants, enhances the activity of PS II and PSI, increases nitrogen and chlorophyll content, and boosts the levels of soluble proteins and Rubisco (Jahan *et al*., 2021a; Khan *et al*., 2022; Yang *et al*., 2022a). This review provides a comprehensive examination of the protective role of MLT in preserving photosynthetic machinery under HS conditions. By doing so, it will not only shed light on the mechanisms by which MLT positively impacts photosynthesis but also provide valuable insights for the development of crops with enhanced photosynthetic efficiency, thereby contributing to improved plant maintenance and increased crop yield.

Review methodology

We conducted a comprehensive search for relevant literature using three primary academic databases: Google Scholar, Dimensions.ai, and Web of Science. The following keywords were employed to ensure a thorough exploration of the topic: 'heat stress', 'photosynthesis', 'melatonin', 'photosystems', 'photosynthetic pigments', 'carbon fixation', 'Calvin cycle enzymes', 'protein degradation', 'chloroplastic heat shock proteins', 'electron transport'. Combinations of these words were used using 'AND' and 'OR'. Relevant papers including original research, and review papers were added.

Impact of heat stress on photosynthesis

Among the intricate physiological processes affected, photosynthesis stands out as particularly heat-sensitive. This includes key mechanisms like the fluidity of thylakoid membranes, photosynthetic pigment metabolism, electron transport, photochemical reactions, and the Calvin cycle. Studies have outlined the repercussions of HS on photosynthesis, which are summarized in Table 1.

CHLOROPLAST

Under HS conditions, cellular organelles, particularly chloroplasts, undergo notable ultrastructural deformations illustrated in Fig. 1. Chloroplasts exhibit dilation, roundness, and dissociation from the cell wall, with degraded chloroplast envelopes, inflated thylakoids, loosely arranged grana lamellae or loss of grana and stroma thylakoids, increased cytoplasmic vacuolization is evident in HS cells, and the presence of osmiophilic particles within chloroplasts increases in both number and size, with some chloroplasts expanding into spherical or oblate shapes, and the inner membrane system of certain chloroplasts is extensively damaged, resulting in hollow chloroplasts (Zou *et al*., 2017; Zhang *et al*., 2023; Li *et al*., 2024). Additionally, HS induces a significant increase in the number and size of plastoglobules in both *SGRi* (*STAYEGREEN RNAi)* lines and wild-type plants, accompanied by severe degradation of chloroplast and thylakoid membranes, and reduced stacking of granum thylakoids in *SGRi* lines (Zhang *et al*., 2022).

PHOTOSYNTHETIC PIGMENTS

Chlorophyll (Chl) serves as a vital pigment crucial for absorbing light energy and facilitating the process of photosynthesis in plants. There exists a direct correlation between photosynthetic activity and the content of Chl in plants. The level of chlorophyll in plants is governed by a delicate equilibrium between its production and degradation processes (Hu *et al*., 2020). Several studies have demonstrated that exposure to HS leads to a decrease in levels of Chl a, Chl b, total Chl, the ratio of Chl a/Chl b, and carotenoid content (Xu *et al*., 2020; Fatma *et al*., 2021; Fan *et al*., 2023). Under HS, the increased activity of chlorophyll-degrading enzymes like chlorophyllase and peroxidase leads to a notable decrease in chlorophyll levels (Wang *et al*., 2018). Additionally, the first enzyme in pyrrole production, 5-aminolevulinate dehydratase, becomes less active in elevated temperatures (Mathur *et al*., 2014).
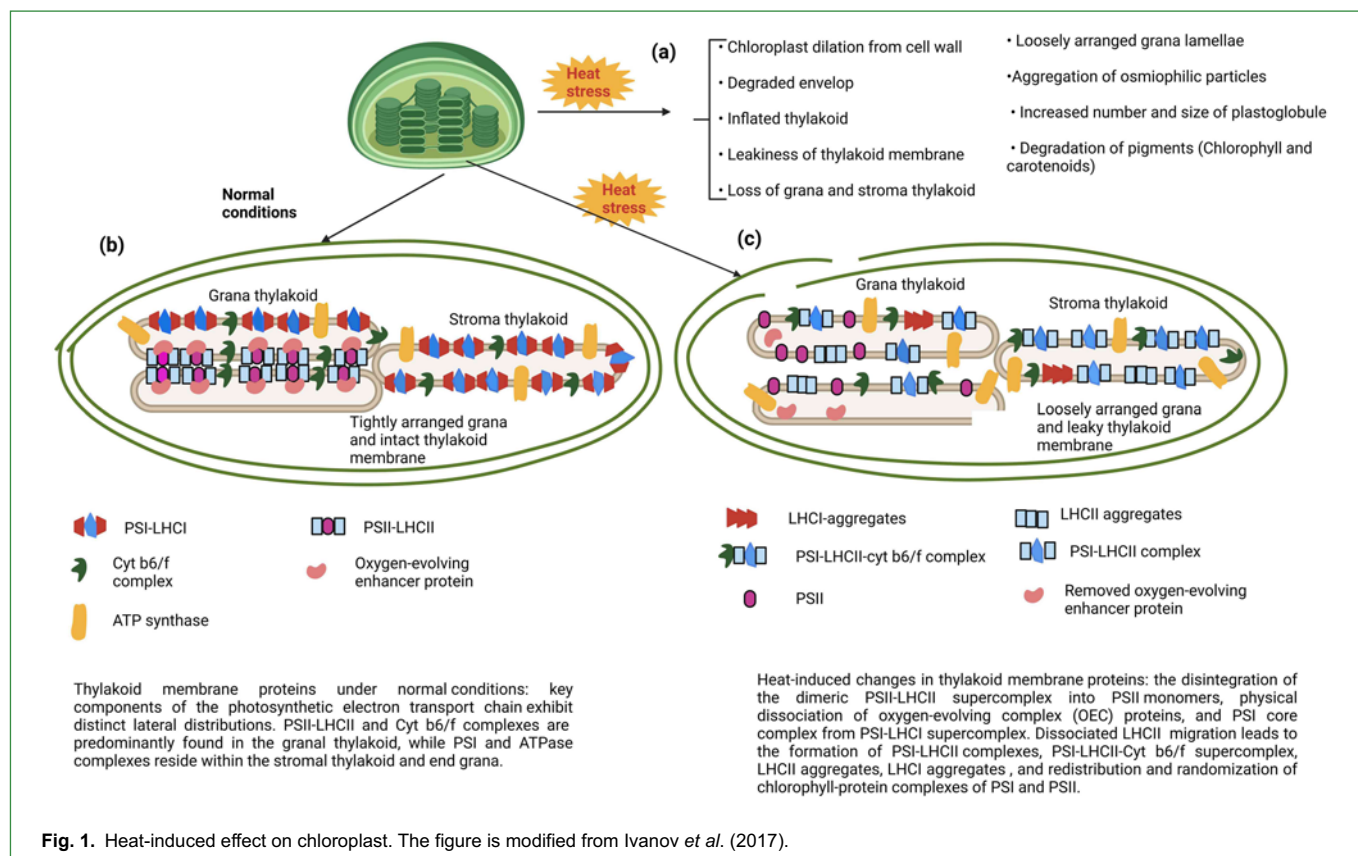
PHOTOSYSTEMS AND ELECTRON TRANSPORT

Studies have indicated that while both PSI and PSII are vital components of the photosynthetic process, PSI exhibits greater resilience to HS compared to PSII. HS caused a significant reduction in the efficiency of open PSII center (Fv/Fm), actual photochemical efficiency of PSII (ΦPSII), the electron transfer rate (ETR), photochemical quenching (qP), OJIP curve shape, PSII performance indicator Plabs effective quantum efficiency and maximum fluorescence of PSI but increase in nonphotochemical quenching was recorded in HS plants (Jahan *et al*., 2021b; Luo *et al*., 2023).

The reduction observed in various components of the photosystem under HS is due to the disruption of its structural integrity, which includes disruption in ordered α-helix structures causing D1 protein content reduction, removal of oxygen-evolving enhancer proteins, and separation of LHCII complexes from the PSII core complex, leading to decreased quantum yield of PSII photochemistry and reduction in PSII-generated electron flow (Ivanov *et al*., 2017). Membrane damage induced by HS results in the breaking of the reaction center-binding D1 protein of PSII, clustering of D1 protein with neighboring polypeptides D2 and CP43, and removal of extrinsic proteins PsbO, PsbP, and PsbQ. The underlying mechanism involves heat-induced lipid peroxidation generating singlet oxygen ($^1O_2$) at the acceptor side of PSII, damaging the DE-loop of the D1 protein and causing cleavage and aggregation. Destabilization of the manganese-calcium cluster at the donor side of PSII by heat leads to hydrogen peroxide (H_2O_2) generation, which, in turn, reacts with transition metal ions to produce hydroxyl radicals (OH·), resulting in protein damage, including D1 and D2, and the extrinsic PsbO and PsbQ proteins (Yamashita *et al*., 2008). Studies have demonstrated that HS decreases the expression of genes encoding key proteins of the photosynthetic system, such as *PsbA* (D1 protein)*, PsbB* (CP47), and *PsbC* (CP43) (Fatma *et al*., 2021; Gautam *et al*., 2022b).

Table 1. Effect of heat stress on photosynthesis in various plant species.

Plant	Heat stress	Effect on photosynthesis	References
Agrostis stolonifera	38°C	Chlorophyll (Chl) degrading enzyme peroxidase, chlorophyllase, and pheophytinase increases, chl content decreased	Rossi *et al*. (2017)
Ipomoea aquatica	30, 35, 40, 45°C	Net photosynthesis, stomatal conductance, intercellular CO_2 concentration, transpiration rate, stomatal aperture, Chl a, Chl b, carotenoid content, maximum quantum yield (Fv/Fm), effective quantum efficiencies (Y(II)), photochemical quenching coefficient (qP) decreased, the non-photochemical quenching (NPQ) increased, the chloroplast number declined and organelle sections clumped together, the thylakoids were loose and damaged, less starch grains, leaf anatomy showed damage to the palisade tissue	Wang *et al*. (2023)
Medicago sativa	38°C	Electrolyte leakage, Fj (implying the inhibition of electron transport beyond primary quinine of PSII) increases; decrease in chl. a, chl. B content, low RC/ABS (reaction center-to-PS II antenna absorption ratio), PIABS and PI total, Fv/Fm,	Wassie *et al*. (2020)
	32°C	Carotenoid, lutein violaxanthin, Fv/Fm decreases	Xu *et al*. (2020)
Miscathus sinensis	42°C	Phosphoribulokinase (PRK), sedoheptulose1,7-bisphosphatase (SBPase) decrease	Sharmin *et al*. (2013)
Oryza sativa	40°C	Net photosynthesis, stomatal conductance, intercellular CO_2, rubisco activity and gene expression of *rbcS, rbcL*, and stomatal aperture, decreases and chloroplast structure is disrupted, whereas activity and expression of carbonic anhydrase gene *βCA1* increases	Alvi *et al*. (2024)
	40°C	Net photosynthesis, stomatal conductance, intercellular CO_2, rubisco, FBPase activity, expression of *psbA* and *psbB* decreases, activity of sucrose phosphate synthase, ADP-glucose pyrophosphorylase decreases while sucrose synthase and soluble acid invertase increases	Gautam *et al*. (2022a)
Populus simonii	42°C	Net photosynthesis, stomatal conductance, intercellular CO_2 concentration, transpiration rate also reduced ETR, qP decrease…	Song *et al*. (2014)
Quercus pubescens	45°C	Net photosynthesis and stomatal conductance decreased $Φ_{PSII}$, F_v'/F_m', qp and rubisco activity reduced	Haldimann and Feller (2004)
Solanum *lycopersicum*	42°C	The transcript level of chlorophyll catabolic genes; nonyellow coloring (*NYC1*), pheide a oxidase (*PAO*), and senescence-inducible chloroplast stay-green protein (*SGR*) upregulated, rubisco activity and FBPase activity, the parameters of chl fluorescence like Fv′/Fm′, ΦPSII, ETR, and qP reduced.	Jahan *et al*. (2021a)
	42°C	Electrolyte leakage increased, Fo, Fv/Fm Y(II), Y(NO), NPQ, chl a, chl b, carotenoid, WUE decreases	Jahan *et al*. (2019)
	45°C	Gas exchange parameters, PSII efficiency quantum yield, and photochemical efficiency decreased and increased non-photochemical quenching (NPQ) levels, transcript level of *PSIP680* and *RCA* (Rubisco activase) downregulate	Raja *et al*. (2020)
Sorghum bicolor	48°C	Oxygen evolving complex, PSII both get damaged, photosynthetic electron donor to PSI gets sharply reduced, irreversible damage to PSII.	Yan *et al*. (2013)
Spinacia oleracea	40°C	Oxygen evolving activity decreased, aggregation of CP43 and D1 protein leads to photoinhibition of PSII	Komayama *et al*. (2007)
Triticum aestivum	40°C	Rubisco activity, chlorophyll content, gas-exchange parameters, maximum activity of PSII decreased, activity of ADP-glucose phosphorylase, sucrose synthase, soluble acid invertase decreased, activity of sucrose phosphate synthase increased	Iqbal *et al*. (2021)
	38°C	Chl, RWC, Fv/Fm, qP, ΦPSII, PSII-LHCII complex, PsbQ, D1 protein decrease	Chen *et al*. (2017)
	42°C	Expression level of *psbA, psbB,* and *psbC* genes, RC/ABS (reaction center-to-PS II antenna absorption ratio), PI (performance index) decrease	Fatma *et al*. (2021)
	42°C	Reduction in gas exchange parameters, Fv/Fm, ΦPSII, NPQ, and qP, increase NPQ, activity and expression of rubisco, aldolase, FBPase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase decreases, D1 protein reduced	Li *et al*. (2021)
Zea mays	38°C	Rubisco activity, net CO_2 assimilation rate decreased	Perdomo *et al*. (2017)

Fig. 1. Heat-induced effect on chloroplast. The figure is modified from Ivanov *et al*. (2017).

HS-induced reductions in carbon-fixing reactions consequently decrease the demand for photosynthetic electrons needed for CO_2 fixation. This scenario imposes limitations at the acceptor site of PSI, leading to increased excitation pressure over PSII and a predominantly reduced PQ (plastoquinone) pool. Subsequent lateral movement of heat-induced dissociated LHCII complexes facilitates the formation of PSI–LHCII complexes, enhancing the efficiency of P700 photo-oxidation. Furthermore, heat-induced randomization of PSII, PSI, and Cyt b6/f complexes promotes the formation of PSI–LHCII–Cyt b6/f supercomplexes, thereby up-regulating two partially redundant PSI-dependent cyclic electron flow (CEF) pathways: NAD(P)H dehydrogenase-dependent cyclic electron flow and ferredoxin-quinone oxidoreductase-dependent cyclic electron flow (Ivanov *et al*., 2017).

Under stress, increased CEF I increases oxidized P700 ($P700^+$), reducing the likelihood of electron leakage to oxygen to produce ROS and mitigate the risk of PSI photoinhibition (Takagi *et al*., 2017). Another study reported that heat-induced inhibition of PSII and upregulation of CEF work synergistically to safeguard PSI from ROS damage by moderately down-regulating the flow of photosynthetic electrons from PSII to PSI (Jiang *et al*., 2021). However, a reduction was observed in *RhPGR5A* expression associated with the PGR5/PGRL1-mediated pathway of CEF (ferredoxin-quinone oxidoreductase-dependent) around PSI, ultimately reducing PSI activity under HS (Xu *et al*., 2023). Similarly, low CEF activity in young leaves may accelerate PSI photoinhibition under fluctuating light conditions at high temperatures (Tan *et al*., 2020).

Understanding how PSII and PSI respond differently to HS provides crucial insights for enhancing plant resilience. Future research should prioritize unraveling the molecular mechanisms and regulatory networks to develop strategies for breeding heat-tolerant crops and improving agricultural productivity under changing climate conditions. Figure 2 depicts the effect of HS on photosystem structure, electron transport, and carbon fixation.

CARBON FIXATION MACHINERY

Rubisco, serving as the rate-limiting enzyme for RuBP carboxylation, stands out as a central hub under diverse stress conditions. Research indicates that increased temperatures impede photosynthesis through Rubisco, primarily by diminishing its specificity for CO_2 over O_2, as the rates of CO_2 solubility decline compared to O_2 as temperature escalates (Sharkey, 2005). Furthermore, the functionality of Rubisco activase, vital for Rubisco modulation, is severely hampered under HS conditions, indicating its susceptibility to heat (Crafts-Brandner and Law, 2000).

Studies elucidate the downregulation of various genes implicated in photosynthetic carbon fixation such as *RUBISCO ACTIVASE (RCA), RUBISCO (rbcL, rbcS), PHOSPHOENOLPYRUVATE CARBOXYLASE 4 (PPC4), FRUCTOSE-1,6-BISPHOSPHATASE (FBPase), SEDOHEPTULOSE-BIPHOSPHATASE (SBPase), RIBOSE-5-PHOSPHATE ISOMERASE 2 (RPI2), GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE (GAPDH), PHOSPHOGLYCERATE KINASE (PGK)*, alongside an augmentation in carbonic anhydrase (CA) activity and the expression of the CA encoding gene *βCA1* under HS conditions (Jahan *et al*., 2021a; Li *et al*., 2021; Alvi *et al*., 2024; Cheng *et al*., 2024).

Furthermore, enzymes associated with carbohydrate metabolism, which also affect the photosynthesis process, exhibit modulation under stress conditions. For instance, in *Rhododendron × pulchrum* Sweet, HS leads to the downregulation of *BETA GLUCOSIDASE 32 (BGLU32) and STARCH SYNTHASE 2 (SS2)*, while *BETA GLUCOSIDASE 2 (BGLU2)* and *UDP-GLUCOSE PYROPHOSPHORYLASE 2 (UGP2)* are upregulated (Cheng *et al*., 2024). Moreover, in HS wheat plants, there is an increase in sucrose phosphate synthase (SPS) activity, while sucrose synthase (SuSy), soluble acid invertase (INV), and ADP-glucose phosphorylase (AGPase) activities decrease. Conversely, in rice plants under HS, SPS and AGPase activities decrease, while SuSy and INV activities increase (Iqbal *et al*., 2021; Gautam *et al*., 2022a). These contrasting enzyme activity patterns underscore the species-specific strategies employed by plants to cope with HS, highlighting the complexity of plant stress responses and the importance of considering species-specific adaptations in agricultural and ecological contexts.

HS disrupts photosynthesis through intricate interactions among chloroplast structure, pigment composition, and carbon fixation,
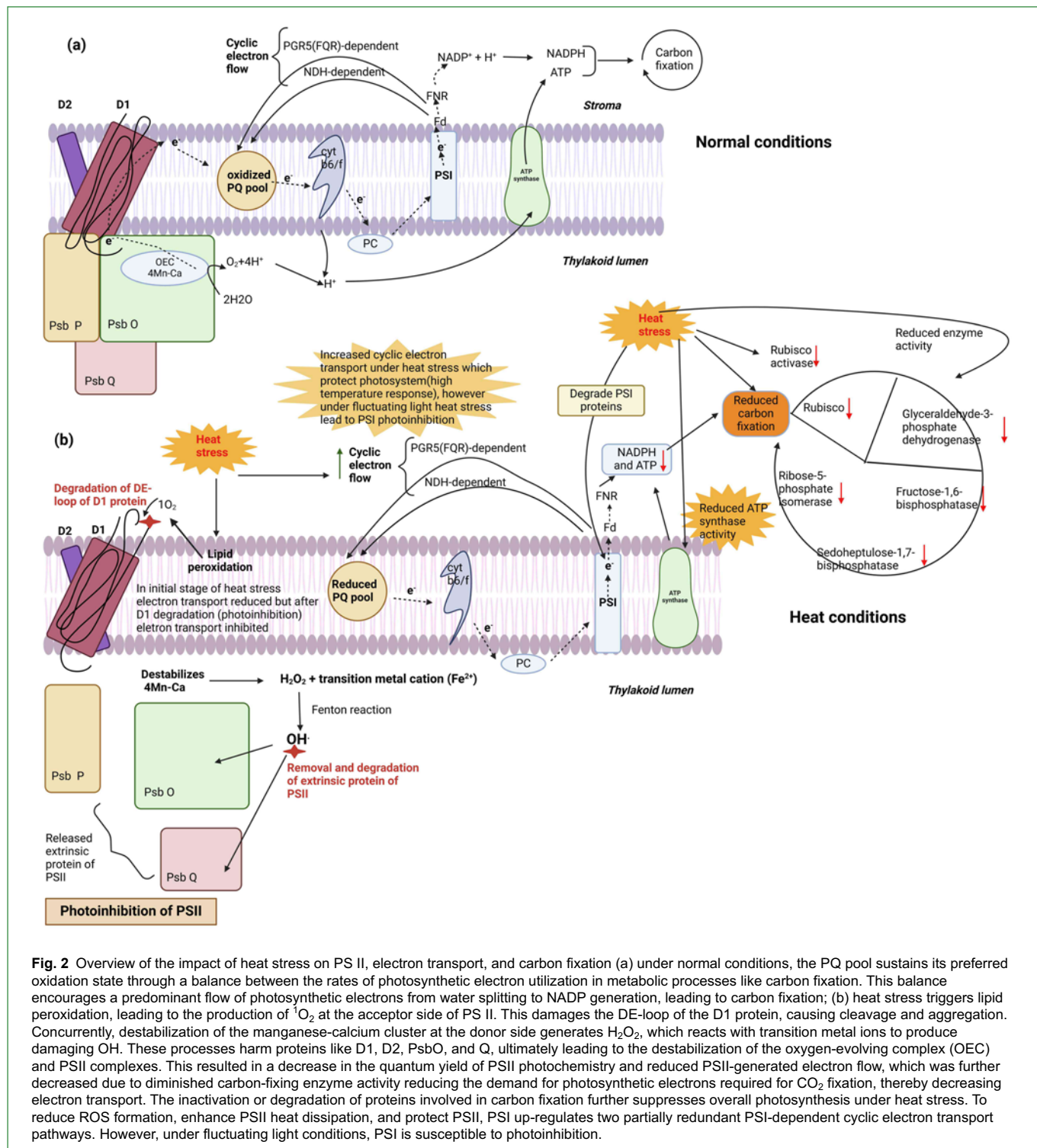
Fig. 2 Overview of the impact of heat stress on PS II, electron transport, and carbon fixation (a) under normal conditions, the PQ pool sustains its preferred oxidation state through a balance between the rates of photosynthetic electron utilization in metabolic processes like carbon fixation. This balance encourages a predominant flow of photosynthetic electrons from water splitting to NADP generation, leading to carbon fixation; (b) heat stress triggers lipid peroxidation, leading to the production of $^1O_2$ at the acceptor side of PS II. This damages the DE-loop of the D1 protein, causing cleavage and aggregation. Concurrently, destabilization of the manganese-calcium cluster at the donor side generates H_2O_2, which reacts with transition metal ions to produce damaging OH. These processes harm proteins like D1, D2, PsbO, and Q, ultimately leading to the destabilization of the oxygen-evolving complex (OEC) and PSII complexes. This resulted in a decrease in the quantum yield of PSII photochemistry and reduced PSII-generated electron flow, which was further decreased due to diminished carbon-fixing enzyme activity reducing the demand for photosynthetic electrons required for CO_2 fixation, thereby decreasing electron transport. The inactivation or degradation of proteins involved in carbon fixation further suppresses overall photosynthesis under heat stress. To reduce ROS formation, enhance PSII heat dissipation, and protect PSII, PSI up-regulates two partially redundant PSI-dependent cyclic electron transport pathways. However, under fluctuating light conditions, PSI is susceptible to photoinhibition.
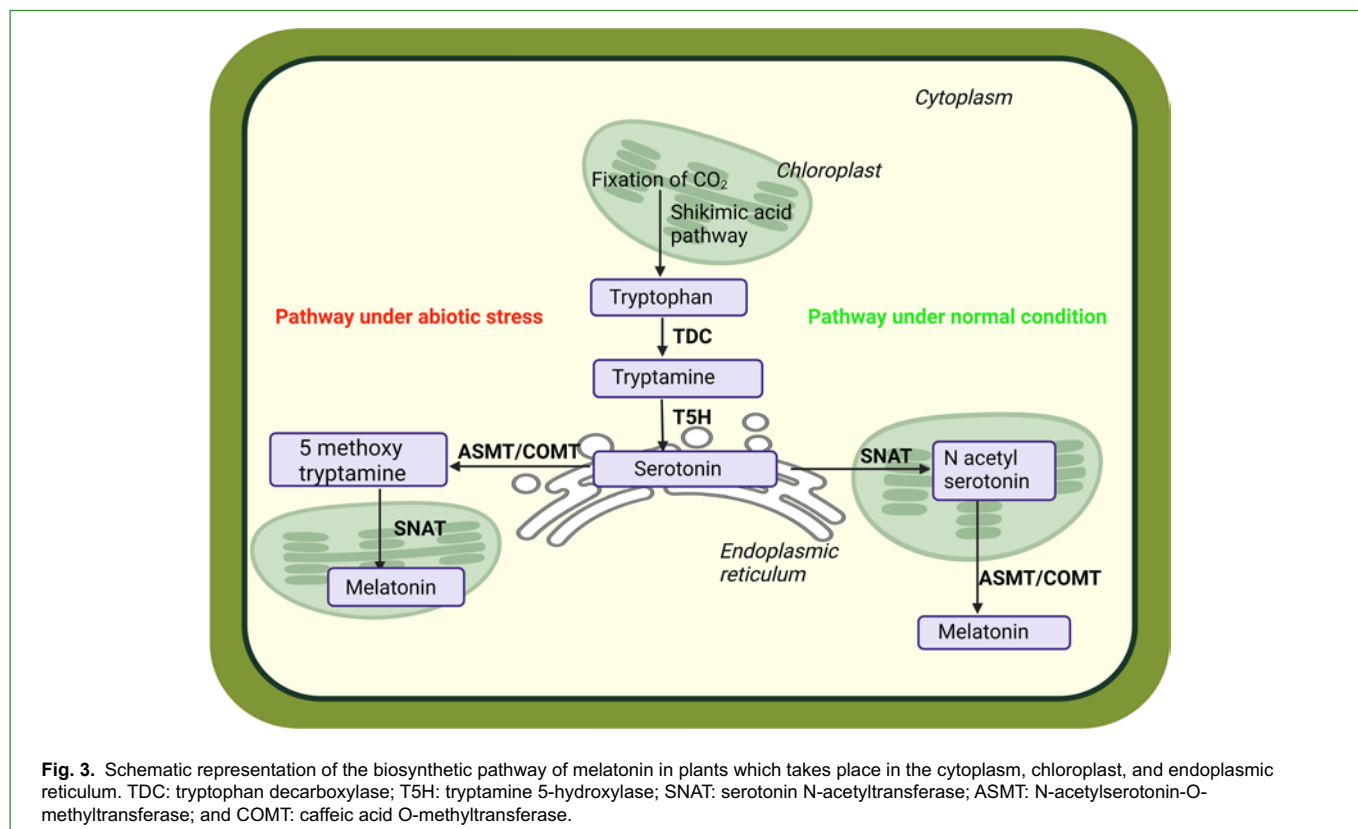
emphasizing its pivotal role in plant survival and ecosystem stability. Implementing diverse strategies is crucial to counteract HS and uphold photosynthetic efficiency for sustainable agriculture and ecological resilience amidst climate change.

Melatonin biosynthesis

MLT, originating from tryptophan, is a fundamental biomolecule synthesized across a broad spectrum of living organisms, encompassing both animals and plants. Figure 3 illustrates the biosynthesis of MLT in plants. The MLT biosynthetic pathway in plants differs from the pathway in animals. The detailed biosynthetic pathway of MLT is comprehensively explained by Raza *et al.* (2022a) and Zeng *et al.* (2022).

Action of melatonin on photosynthetic protection under heat stress

MLT serves a dual role in plants, acting as both a growth regulator and a potent protector against abiotic stresses due to its antioxidant properties. Its ability to regulate gene expression related to photosynthesis further enhances its potential as a versatile biostimulant for agricultural use, offering opportunities to enhance plant resilience and optimize photosynthetic efficiency across various growth stages (Karumannil *et al.*, 2023). MLT safeguards photosynthesis by modulating ROS homeostasis, influencing the expression of genes and proteins associated with chloroplast function, pigments, and both light and dark reactions. Studies have demonstrated MLT's role in protecting photosynthesis under HS,

Fig. 3. Schematic representation of the biosynthetic pathway of melatonin in plants which takes place in the cytoplasm, chloroplast, and endoplasmic reticulum. TDC: tryptophan decarboxylase; T5H: tryptamine 5-hydroxylase; SNAT: serotonin N-acetyltransferase; ASMT: N-acetylserotonin-O-methyltransferase; and COMT: caffeic acid O-methyltransferase.

summarized in Table 2, and Fig. 4 illustrates how MLT mediates photosynthetic protection under HS.

CHLOROPLAST INTEGRITY AND STRESS RESPONSE

MLT, synthesized in chloroplasts, acts as a direct antioxidant and induces the expression and activity of other antioxidants enzymes (SOD, CAT, POD, APX, GR), HSPs, and HSFs under HS (Zeng et al., 2022; Sehar et al., 2023). Furthermore, it regulates the non-enzymatic antioxidant system such as it influences the ascorbate-glutathione cycle by enhancing the content of ascorbate and reduced glutathione and by modulating associated enzymes such as monodehydroascorbate reductase, dehydroascorbate reductase, glutathione reductase, and glutathione-S-transferase (Liang et al., 2018; Jahan et al., 2019). By augmenting the activity of the antioxidant system, MLT helps maintain redox balance and mitigates lipid peroxidation in chloroplasts, thus preserving the functionality of the photosynthetic system.

A recent study showed the role of calcium ions and ROS, generated with the participation of NADPH oxidase, as signaling mediators in the MLT-induced antioxidant system activation and HS resistance of wheat seedlings (Kolupaev et al., 2024). It was recorded that administering MLT externally resulted in the increased expression of genes encoding enzymes such as *PYRUVATE KINASE, PHOSPHOFRUCTOKINASE-1,* and *PHSPHOGLYCERATE KINASE*, which play roles in the pyruvate synthesis pathway. Conversely, the gene expression of enzymes associated with the tricarboxylic acid cycle, namely *ACONITATE HYDRATASE, ISOCITRATE DEHYDROGENASE,* and *MALATE DEHYDROGENASE* decreased with MLT application under HS in *Apium* plants. This alteration in gene expression patterns led to the accumulation of pyruvate within the cells, which involved in scavenging of heat-induced ROS (Li et al., 2022), thus protecting plants from oxidative damage, and protecting proteins, the membrane of plants. However, detailed exploration of calcium ion and NADPH oxidase signaling pathways could optimize melatonin use for enhancing plant heat stress resilience.

MLT induces the expression of HSPs and HSFs under HS, crucial for stabilizing proteins and preventing denaturation. While evidence

of chloroplast-specific HSP/HSFs activation under HS is limited, a few studies have shown the induction of them in leaves under HS. Therefore, it is plausible to consider that they could directly or indirectly impact chloroplast function via protein stability/folding or also interact with other stress response pathways regulating ROS levels. Thus, further investigation is needed to understand the impact of MLT-mediated HSP/HSF activation under HS specifically on chloroplast function and maintenance of the photosynthetic apparatus.

In this review, we provide a summary of studies highlighting the involvement of MLT in HSPs and HSFs-mediated thermotolerance. MLT plays a role in chloroplast protein quality control, involving chloroplast heat shock proteins (CpHSPs) and caseinolytic protease (Clp), crucial for the correct folding of imported proteins from the cytoplasm (Yang et al., 2022a). It was observed that the expression of *ClpR1, ClpR4, ClpP1, CpHSP70.1,* and *CpHSP70.2* was inhibited in the *snat1* mutant (serotonin N-acetyltransferase-knockout), but this effect was reversed with the application of exogenous MLT (Lee and Back, 2021). In Arabidopsis plants overexpressing MLT, there was a minor decrease in ClpR1 protein levels, suggesting a potential role of MLT in chloroplast protein quality control. But the induction of CpHSPs and Clps and their specific function under HS further needs to be investigated.

In Arabidopsis, exposure to HS, as well as the application of external MLT, led to a notable increase in the transcript levels of *class A1 heat-shock factors (HSFA1s)*. These HSFA1s act as pivotal regulators in orchestrating the plant's response to HS. Moreover, the increased activity of HSFA1s resulted in the activation of transcripts associated with heat-responsive genes such as *HSFA2, HSA32, HSP90,* and *HSP101*. This suggests that MLT treatment may play a role in enhancing thermotolerance by facilitating the activation of these heat-responsive genes through the regulation of HSFA1s (Shi et al., 2015). Similarly, another study showed MLT upregulated the 10 HSPs, comprising seven *HSP70* and three *sHSPs*, indicating their involvement in MLT-induced thermotolerance enhancement in Kiwifruit, however, further investigation of their specific roles is required (Xia et al., 2021).

Table 2 Effect of melatonin on photosynthesis under heat stress in various plant species.

Plant species	Heat stress	Melatonin concentration	Effect on photosynthesis	Reference
Arabidopsis thaliana snat1 and *snat2* mutant (melatonin biosynthesis enzyme)	High light – 1000 $\mu mol\ m^{-2}\ s^{-1}$ for 3h	100$\mu mol\ L^{-1}$	Increased gas-exchange attributes, chlorophyll and carotenoid content and PSII activity, improved the level of photosystem II protein namely D1. D2, CP43, PsbS, Lhcb2/3/4/5/6 in *snat1* and *snat2* mutant plant and photosystem I protein Lhca1, Lhca2 and Lhca3 in *snat2* under high light	Yang *et al*. (2021)
Brassica juncea	40°C	100 µM	Treatment with MLT plus excess-S increased antioxidant enzyme activity, photosynthetic-S use efficiency (p-SUE), Rubisco activity, gas-exchange parameters and photosynthesis	Iqbal *et al*. (2023)
Chrysanthemum	40 /30°C	200 µM	MLT regulated the genes of chlorophyll metabolism (*HEMA* and *PORA*), flavonoid biosynthesis (*CHS, DFR*, and *FNS*), and carotenoid biosynthesis (*DXPS, GGDP,* and *PSY*), starch and sucrose metabolism (*EDGL, BGLU, SuS*, and *SPS*) and heat shock transcription factors (HSFs) and heat shock proteins (HSPs)	Xing *et al*. (2021)
Dianthus caryophyllus	42°C	1, 5, and 10 mM	Increased chlorophyll content, increased activity of SOD, POD, and CAT. In addition, the expression of HSPs and HSF was recorded.	Elmongy and Abd El-Baset (2024)
	42/35 °C (day/night)	100 µM	The MLT treatment effectively increased the Fv/Fm, Y(II), qP, Fv/Fo, Fm, and PI$_{abs}$ values and decreased the NPQ and Fo values, thus mitigating the damage of PS II in the leaves of carnation under heat stress, energy fluxes (per RC) parameters: decreased the ABS/RC, DIo/RC, and TRo/RC and increased the ETo/RC value indicating that MLT could stabilize the structure of the photosynthetic system and optimize the energy allocation of PS II, the content of Chl a, Chl b, total Chl and carotenoid increased	Hu *et al*. (2023)
Festuca arundinacea	42°C	1 mM and 50 mM	Increased Chl, total protein, and antioxidant enzyme activities	Alam *et al*. (2018)
	44 °C	100 $\mu mol\ L^{-1}$	MLT weakened the electron transfer efficiency of PS II per light reaction center at donor-side and receptor-side, while increased the number of RC per unit cross-sectional area, increased photochemical quenching, Y(II), decreased NPQ and encouraged reverse reaction of the xanthophyll cycle.	Wang *et al*. (2022a)
Fragaria × ananassa (strawberry)	35°C and 40°C	50 and 100 µM	Increased Fv/Fm ratio and expression of HSPs and Hsf	Manafi *et al*. (2022)
Ipomoea batatas	42°C	10, 25, 50 and 100 $\mu mol\ L^{-1}$	Increased antioxidants, chlorophyll content, stomatal traits (length, width and number), PSII efficiency	Kumar *et al*. (2024)
Lolium perenne	38°C	20 µM	Reduced leaf senescence and enhanced chlorophyll, photochemical efficiency, net photosynthesis rate, and cell membrane stability	Zhang *et al*. (2017)
Oryza sativa	38°C	200 $\mu mol\ L^{-1}$	Brighter green appearance of leaves, increased activity of PSII, promotes optimization of amylopectin synthesis during grain filling stage	Fan *et al*. (2022)
Pinellia ternata	35/30°C	100 µM	Increased chlorophyll content, chlorophyll a fluorescence transient curve (OJIP curve), and *LHC1* expression downregulated	Ma *et al*. (2020)
Raphanus sativus	35 /30 °C (day/night)	11.6, 17.4, 29.0, 34.8, 67.0 mg L^{-1}	Increased content of chlorophyll and carotenoid and activity of Rubisco	Jia *et al*. (2020)
Rhododendron	42/35°C (14 h/10 h)	200 $\mu mol\ L^{-1}$	The upregulated gene of ATP synthase: *RhATPB;* proton gradient regulation: *RhPGR5A* for improved cyclic electron flow around PSI; Light harvesting: *RhLHCB3*; and rubisco: *RhRbsA*	Xu *et al*. (2023)

Continued

Table 2. Continued.

Plant species	Heat stress	Melatonin concentration	Effect on photosynthesis	Reference
Solanum lycopersicum	38/28°C (16/8 h)	100 µM	*Rbohs* gene, chlorophyll catabolic genes, and senescence-associated gene expression levels were significantly suppressed leading to reduced leaf senescence and increased Fv/Fm ratio	Jahan *et al.* (2021b)
	40°C	50 mM	Enhanced stomatal aperture, stomatal conductance, intercellular CO_2 concentration, net photosynthesis, chlorophyll content, effect on parameters related to PSII : *Fv/Fm* and the performance index on an absorption basis (PI abs), density of reaction centers per cross-section *(RC/CS$_m$)*, absorbed energy flux per cross-section *(ABS/CS$_m$)*, trapped energy flux per cross-section *(TR/CS$_m$)*, electron transport flux per cross-section *(ET/CS$_m$)*, expression of PSII subunit genes (*PsbA*, *PsbB*, *PsbC* and *PsbP*) increased, effect on parameters related to PSI: the maximum oxidation state of PSI (Pm), the quantum yield of PSI photochemistry [Y(I)], the quantum yield of PSI non-photochemical energy dissipation due to donor-side limitation [Y(ND)], the quantum yield of PSI non-photochemical energy due to acceptor-side limitation [Y(NA)], expression of PSI subunit genes (*PsaB*, *PsaC*, *PsaD*, and *PsaL*) enhanced, MLT alleviated the oxidative damage of PSII by balancing the electron transfer of the donor side, reaction center, and receptor side, thus protecting photosynthesis	Sun *et al.* (2023)
	38/30°C in combination with drought	1.05 mM	Stomatal opening is promoted by the inhibition of ROS signaling. MLT increases stomatal conductance in combined heat and drought stress, but the effect is not mediated through ABA signaling.	Jensen *et al.* (2023)
	55°C	Overexpression of *SlSNAT* (melatonin biosynthesis enzyme)	HSP40 binding with *SlSNAT* in chloroplasts resulted in elevated MLT levels, thereby reducing ROS and boosting maximum PSII efficiency and rubisco activity.	Wang *et al.* (2020)
Solanum tuberosum	42°C	50, 100, 150, and 200 µM	Upregulation of photosynthesis-related gene and antioxidant enzymes, increased photochemical quenching, electron transport rate chlorophyll content and transpiration rate	Qiulan *et al.* (2023)
[Q09] *Triticum aestivum*	42°C	100 µM	Enhanced expression and activity of SOD, POD and CAT, activating the ascorbate-glutathione cycle reduce oxidative stress. Stable photosynthetic machinery by increasing chlorophyll content.	Buttar *et al.* (2020)
	40°C	100 µM	Reduced oxidative stress by increasing the activity of CAT, GR, APX, and SOD. Enhances gas-exchange parameters thus increasing photosynthesis and carbohydrate metabolism.	Iqbal *et al.* (2021)
Triticum aestivum	40°C	100 µM	MLT enhances net photosynthesis, stomatal conductance, intercellular CO_2 concentration, maximum PSII efficiency, rubisco activity, and expression of *psbA* and *psbB*	Sehar *et al.* (2023)

Proteins, vital for cell structure and stress response, suffer oxidation, misfolding, and denaturing under HS due to ROS, thus impairing the structural organization of chloroplast, and inhibiting biosynthesis and enzyme activity. Without proper removal, accumulated misfolded proteins become toxic (Salvucci and Crafts-Brandner, 2004; Xu *et al.*, 2016). A study showed the foliar application of MLT or overexpression of MLT biosynthesis gene *ASMT* alleviated heat-induced photoinhibition and electrolyte leakage and enhanced thermotolerance in tomatoes by reducing insoluble and ubiquitinated proteins, while enhancing HSP expression for protein refolding. It also induced *ATG* (autophagy-related) gene expression and autophagosome formation, aiding in aggregated protein degradation under HS (Xu *et al.*, 2016). A recent study demonstrated that MLT enhances autophagic activity

in apples under HS promoting *WRKY33*-mediated transcriptional activation of *ATG18a*, aiding in the degradation of deformed proteins, and alleviating HS (Gao *et al.*, 2024). Moreover, *SlSNAT* overexpression line of tomato showed high MLT concentration resulting in ROS scavenging, preserving PSII function, content of Rubisco and Rubisco activase enzyme, and upregulating the response of heat transcription factor and HSPs under HS. In addition, HSP40, a DnaJ-type chaperone, interacted with *SlSNAT* in chloroplasts, with its downregulation resulting in lower MLT synthesis under HS, suggesting a pivotal role for HSP40 in safeguarding the SNAT enzyme during MLT synthesis and contributing to MLT-related thermotolerance in tomato plants (Wang *et al.*, 2020). MLT enhances plant thermotolerance and indirectly safeguards photosynthesis by increasing HSPs, promoting ATG
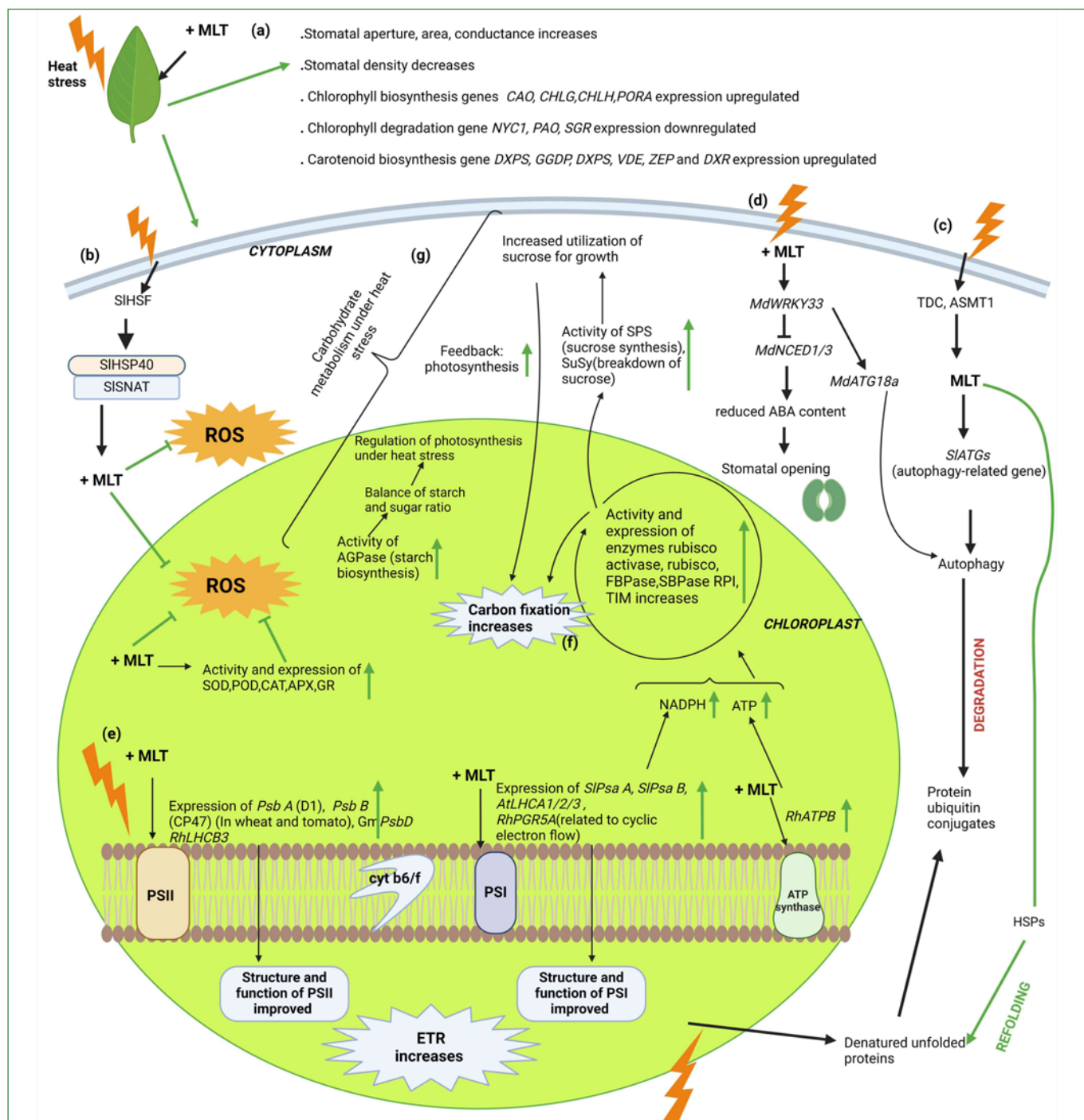
Fig. 4. Mechanism of melatonin-mediated protection of photosynthesis under heat stress: (a) Melatonin (MLT) enhances stomatal characteristics and chlorophyll and carotenoid content; (b) Under heat stress, the accumulation of Heat Shock Factor (HSF) stimulates the transcription of SISNAT. Simultaneously, the interaction of heat-induced HSP40 with SNAT, prevents SNAT degradation under heat stress, facilitating MLT biosynthesis, this MLT, acting either directly as a ROS scavenger or by stimulating antioxidant enzymes, helps to preserve the structure and function of PSII and the activity of rubisco and other enzymes; (c) heat stress triggers the accumulation of endogenous MLT by upregulating the expression of MLT biosynthetic genes. Exogenous MLT application, as well as increasing endogenous levels, promotes the refolding of denatured proteins and degrades insoluble proteins induced by heat stress by inducing HSPs expression and promoting the formation of autophagy respectively; (d) MLT via WRKY33 enhances the autophagic activity and regulates ABA content to facilitate stomatal opening, thereby protecting against heat stress; (e) MLT promotes the expression of genes associated with PSII, PSI, and ATPase, thus protecting structure and function which leads to improved electron transport, enhances photosynthesis under heat stress; (f) MLT increased the expression and activity of enzymes involved in carbon fixation, promoting photosynthesis under heat stress; and (g) Improved carbohydrate metabolism enhances the photosynthesis under heat stress. CAO: Chlorophyllide A oxygenase; NYC1: Non-yellow coloring; PAO: Pheide A oxidase; SGR: Stay green; CHL G: Chlorophyll synthase; CHLH: Magnesium-chelatase subunit; POR: Protochlorophyllide oxidoreductase; DXPS: 1-deoxy-D-xylulose-5-phosphate synthase; GGDP: Gernylgeranyl diphosphate synthase; PSY: Phytoene synthase; VDE: Violaxanthin de-epoxidase; ZEP: Zeaxanthin epoxidase; DXR: 1-deoxy-d-xyluluose-5-phosphate; TDC: Tryptophan decarboxylase; SNAT: Serotonin N-acetyltransferase; ASMT: N-acetylserotonin-O-methyltransferase; FBPase: fructose 1,6- bisphosphatase; SBPase: sedoheptulose-1,7-bisphosphatase; TIM: Triosephosphate isomerase; RPI: Ribose 5-phosphate isomerase; SPS: Sucrose phosphate synthase; SuSy: Sucrose synthase; and AGPase: ADP-glucose phosphorylase.

expression, and facilitating autophagosome formation. However, further research is needed to determine if these mechanisms are consistent across different crop species and to understand the long-term physiological impacts.

PHOTOSYNTHETIC PIGMENT

Chlorophyll, pivotal for light absorption during photosynthesis, studies have demonstrated that MLT enhances the levels of Chl a, Chl b, total chl, and carotenoid contents in HS-exposed

tomato seedlings by suppressing the heat-induced expression of chlorophyll catabolic genes, such as *NON-YELLOW COLORING (NYC1), PHEIDE A OXIDASE (PAO)*, and senescence-inducible chloroplast *STAY GREEN (SGR)*, while promoting the expression of chlorophyll biosynthesis genes like *CHLOROPHYLL SYNTHASE (CHL G), CHLOROPHYLLIDE A OXYGENASE (CAO)*, and *PROTOCHLOROPHYLLIDE OXIDOREDUCTASE (POR)* (Jahan *et al.*, 2021b).

Research findings indicated that MLT under HS, decreased the transcript levels of senescence-related genes *SAG12.1, h36*, and cell death-related gene *PDCD,* while concurrently enhancing the expression of genes involved in cytokinin synthesis and signaling. Moreover, MLT downregulated the synthesis and signaling pathways of abscisic acid, contributing to the suppression of heat-induced leaf senescence (Zhang *et al.*, 2017; Liu *et al.*, 2022). Another study reported that the synergistic action of *Rhizobium inoculation* and MLT improved the nitrogen content, which is an integral part of chlorophyll, thus having higher chlorophyll content in HS-threated *Medicago truncatula* (Irshad *et al.*, 2022).

Carotenoids, known for their antioxidant properties, can counteract free radicals, safeguard against lipid peroxidation, and stabilize membrane structure. MLT treatment significantly boosts carotenoid content by upregulation of key carotenoid synthesis genes, *PHYTOENE DESATURASE (PDS)* and *ZETA-CAROTENE DESATURASE (ZDS)* (Xia *et al.*, 2021). Gene silencing of *CAFFEIC ACID O-METHYLTRANSFERASE 1 (COMT1)* in tomato under HS showed reduced MLT content leading to a significant decrease in Chl a, Chl b, and carotenoid content (Ahammed *et al.*, 2018).

The transcriptomic study showed chlorophyll biosynthesis (*GLUTAMYL – tRNA REDUCTASE (HEMA), COPROPORPHYRINOGEN III OXIDASE (HEMF), MAGNESIUM-CHELATASE SUBUNIT (CHLH),* and *PROTOCHLOROPHYLLIDE OXIDOREDUCTASE (PORA)*), chlorophyll cycle (*CHLOROPHYLLIDE A OXYGENASE (NYC1/NOL)* and *HYDROXYMETHYL CHLOROPHYLL A REDUCTASE (HCAR)),* and nine carotenoid biosynthesis gene (*1-DEOXY-D-XYLULOSE-5-PHOSPHATE SYNTHASE (DXPS), GERANYLGERANYL DIPHOSPHATE SYNTHASE (GGDP), PHYTOENE SYNTHASE (PSY), VIOLAXANTHIN DE-EPOXIDASE (VDE), ZEAXANTHIN EPOXIDASE (ZEP)*, and *1-DEOXY-D-XYLULOSE-5-PHOSPHATE REDUCTOISOMERASE (DXR)*) were significantly upregulated on the application of MLT in HS chrysanthemum seedlings (Xing *et al.*, 2021). Thus, MLT prolongs leaf lifespan by regulating chlorophyll and carotenoid metabolism and senescence genes. Carotenoids shield plants by quenching singlet oxygen and absorbing excess energy from chlorophyll, safeguarding photosynthesis. Yet, gaps persist in comprehending MLT's interactions with hormones, mineral nutrients like N and Mg, and its synergies with Rhizobium and other microbes to boost chlorophyll and carotenoid levels under heat stress.

LIGHT HARVESTING AND ELECTRON TRANSPORT

Reduction in the maximum quantum yield of PSII (Fv/Fm), the effective quantum efficiencies of PSII (Y(II)), coupled with an increase in specific energy flux parameters indicated a compromised PSII performance under HS. This could be due to an imbalanced energy transfer from the light-harvesting complex to the chlorophyll reaction center within the photosystem. Analysis of PSII electron transport efficiency fluctuates the efficiency/probability for electron transport (ψEo), and quantum yield for electron transport (ϕEo) suggested that electron transport at the acceptor side of PSII is the limiting hub of HS, as indicated by the increases in non-regulated (Y(NO)) and regulated energy dissipation (Y(NPQ)) (Jahan *et al.*, 2021b). The application of MLT enhances Fv/Fm and Y(II), indicating a positive impact on photoprotection and effective mitigation of heat-induced photoinhibition (Jahan *et al.*, 2021b; Wang *et al.*, 2022a; Hu *et al.*, 2023).

Additionally, MLT treatment reduces Y(NO) and Y(NPQ), indicating enhanced utilization of excess light energy, simultaneously

encouraging the reverse reaction of the xanthophyll cycle thereby safeguarding the photosynthetic apparatus (Jahan *et al.*, 2021a; Wang *et al.*, 2022b). It was recorded that MLT reduced oxidative damage to PSII by balancing electron transfer on the donor, reaction center, and acceptor sides (Sun *et al.*, 2023). Another study suggested that MLT decreased the electron transfer efficiency of PS II per light reaction center at both the donor and receptor sides, while simultaneously increasing the number of reaction centers per unit cross-sectional area. Consequently, this led to enhanced photochemical quenching. It was hypothesized that the inefficient yet more reaction center induced by MLT serves as a protective mechanism for PSII against oxidative damage under HS (Wang *et al.*, 2022a). Studies have demonstrated that MLT influences energy flux parameters and improves electron transport efficiency, thereby enhancing photoprotection. However, further research is required to uncover the molecular mechanisms and long-term effects of MLT on PSII function in diverse plant species.

HS also reduces the maximum fluorescence of PSI (Pm) and the effective quantum efficiency of PSI (Y(I)), primarily due to increased donor-side limitation of PSI, as indicated by elevated oxidation of PSI donor side (Y(ND)). However, MLT-treated seedlings show a decline in Y(ND) values following HS, suggesting alleviation of donor-side limitation. Furthermore, MLT ameliorates HS-induced acceptor-side limitation of PSI, evidenced by a reduction in the status value of the PSI acceptor side (Y(NA)), indicating increased restriction on the acceptor side (Jahan *et al.*, 2021b). Thus, MLT alleviates both donor and acceptor-side limitations of PSI, highlighting its role in balancing energy absorption and utilization in the photosynthetic electron transport chain. Investigating the molecular mechanisms underlying PSI protection by MLT could enhance photosynthetic resilience.

A reduction in Fv/Fm along with an increase in NPQ was observed in the *cand2* mutant under photooxidative stress, suggesting a potential involvement of CAND2/PMTR1-mediated signaling pathway of MLT in photosynthetic physiology (Wei *et al.*, 2018; Bychkov *et al.*, 2021). Similarly, silencing the *COMT1* gene reduces various photosynthetic parameters in HS tomato. This includes light absorption flux, trapped energy flux, energy dissipation, active reaction center density per PSII cross-section, ETR, and Fv/Fm. However, applying exogenous MLT alleviates heat-induced photosynthetic inhibition in both silenced and non-silenced plants, indicating its critical role in maintaining photosynthetic capacity during stress (Ahammed *et al.*, 2018).

Gene expression analysis showed increased expression of PSII (*PsbB:* CP47 protein) and PSI (*PsaA* and *PsaB*) in HS tomato plants, thus MLT regulates photosynthesis efficiency by modulating the expression of photosynthesis-associated genes (Jahan *et al.*, 2021a). Similarly, it was demonstrated that MLT alone and in combination with methyl-jasmonate upregulated the expression of *Psb A* (D1 protein) and *Psb B* gene in HS wheat plants (Sehar *et al.*, 2023). Another study showed that MLT treatment led to dose-dependent and time-dependent upregulation of 64 differentially expressed genes associated with photosynthetic complexes in tea plants, under unstressed conditions. Genes such as *PsbS/P/Q/R/Y/27/28* and *PetF* (vital for photosynthetic electron transport) were significantly upregulated (Yang *et al.*, 2022b). HS induces osmotic stress in plants through increased evaporation and transpiration, causing substantial water loss and dehydration. Studies showed that under osmotic stress MLT maintains photosynthetic proteins by primarily boosting the transcription of genes like *PsbA*, *PsbB, PsbC, PsbD*, and *PsbO*, which encode core PSII proteins (Alyammahi and Gururani 2020). Future studies should delve into the expression of these genes under HS and transcriptional networks through which MLT mediates these gene regulatory effects.

In plants, the majority of chlorophyll is associated with the LHCII complex, comprising three major proteins (LHCB1-3) and three minor proteins (LHCB4-6) (Pietrzykowska *et al.*, 2014). A study identified *RhLHCB3* as a homolog of *AtLHCB3*, which has been

shown to influence the macrostructure of PSII, and the rate of state transitions in Arabidopsis was found to be upregulated with MLT application under HS in rhododendron, suggesting *RhLHCB3* is important for MLT-induced plant responses to HS (Xu *et al*., 2023). This upregulation potentially contributes to enhanced photoprotection and efficient energy utilization during photosynthesis.

Osmotic stress often accompanies HS as a secondary stressor, a report showed that reversible phosphorylation of thylakoid membrane proteins is crucial in plant responses to environmental stresses, including state transitions and PSII repair. Under osmotic stress, wheat exhibits strong phosphorylation of the PSII reaction center and LHCII proteins. However, MLT application notably increases PSII (LHCII, CP43, D1) dephosphorylation, possibly by enhancing the activity of chloroplast enzymes like protein phosphatases (including PPH1) and PSII core phosphatase (PBCP). This acceleration of the PSII repair cycle by MLT helps maintain the excitation energy balance between PSI and PSII under stress conditions (Lin *et al*., 2022). Also, western blot analysis of PSII proteins demonstrated that under osmotic stress conditions, melatonin increased the levels of D1, Lhcb5, Lhcb6, PsbQ, and PsbS proteins in wheat (Lin *et al*., 2022). HS and high light stress disrupt photosynthesis via oxidative stress and electron transport disruption. MLT application under high light conditions improved the level of Lhcb2/3/4/5/6 and Lhca1/2/3, indicating potential resilience enhancement across stress conditions (Yang *et al*., 2021). MLT consistently enhances photosynthesis by upregulating critical photosynthetic genes, proteins, and components of the LHC complex and PSII repair, ensuring optimal photochemical efficiency. However, additional research is necessary to fully elucidate the specific transcriptional networks and molecular mechanisms by which MLT regulates photosynthesis across various plant species and stress environments. This is crucial for broadening its applicability and fully realizing its potential in enhancing plant resilience.

Furthermore, the application of exogenous MLT improved PSII performance, and ETR and mitigated the increase in variable fluorescence levels under HS suggesting an increase in the energy demand of the Calvin cycle. This possibility is supported by the result in which melatonin promotes ATP production by up-regulating the expression of ATP synthase gene *RhATPB* in rhododendrons under HS (Xu *et al*., 2023). HS significantly reduced the expression of *RhPGR5A* (proton gradient regulation (PGR)5), a key regulator of CEF, leading to decreased PSI activity and overall electron transport rate. However, MLT treatment mitigated these decreases by increasing *RhPGR5A* expression, thereby enhancing PGR5-mediated CEF, PSI activity, and overall ETR under HS (Xu *et al*., 2023). There remains a need to further explore the specific molecular mechanisms through which MLT regulates PGR5 expression and its downstream effects on electron transport dynamics and plant performance. Additionally, investigating how MLT affects ATP production and its impact on the Calvin cycle across various stress levels and plant species is crucial. Understanding these mechanisms could refine MLT application strategies, enhancing crop resilience and productivity in challenging environmental conditions.

Another study reported that increased atmospheric carbon dioxide (eCO$_2$) levels stimulate MLT production in tomatoes, via chloroplast pathways, enhancing plant tolerance to HS in wild-type and *COMT1*-overexpressing plants. Overexpression of *COMT1* significantly increases chloroplastic MLT concentrations, especially under eCO$_2$ conditions, this endogenous MLT boosts the transcript levels of chloroplast and nuclear-encoded photosynthetic genes, such as *rbcL, rbcS, rbcA, psaD, petB*, and *atpA*, along with the upregulation of some upstream regulators that control the transcript level of photosynthetic genes such as *sigF*(sigma factor), *CRM* (intron splicing RNA binding factor), and *GBF1* (G-box binding factor), mainly in *COMT1*-overexpressing

plants (Hasan *et al*., 2023). This finding underscores the pivotal role of optimal endogenous MLT levels induced by elevated CO$_2$ in enhancing photoprotection. Further research is needed to clarify how CO$_2$ stimulates MLT via chloroplast pathways and its specific effects on photosynthesis.

CARBON ASSIMILATION AND SUGAR PRODUCTION

Many reports demonstrated the positive effect of MLT on gas exchange parameters including photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO$_2$ concentration (Ci), and transpiration rate (Tr) (Jahan *et al*., 2021a; Sehar *et al*., 2023; Kumar *et al*., 2024). A study showed MLT affects the stomatal characteristics of tea plants in a dose-dependent manner. Stomatal density was negatively correlated with the stomatal area, suggesting that plants adopted an ecological equilibrium strategy at the stomatal level to adapt to environmental cues. It is hypothesized that MLT could enhance photosynthesis in tea plants by short-term regulation of Gs and long-term regulation of stomatal development (Yang *et al*., 2022b). Exogenous MLT increases stomatal number and stomatal conductance (increased pore width, pore area, and stomata width/length ratio) in tomato seedlings subjected to combined heat and drought stress, and this was mediated through impacts on the ROS metabolism not by ABA signaling, which is considered as key regulator of stomatal regulation (Jensen *et al*., 2023; Mumithrakamatchi *et al*., 2024). However opposite to a recent study application of MLT and overexpressing *ASMT9* reduced ABA accumulation by promoting *WRKY33*-mediated transcriptional inhibition of *NCED1* and *NCED3*, which in turn induces stomatal opening for improved heat dissipation in apples (Gao *et al*., 2024), therefore, it needs to be further investigated under HS. Given that HS can induce osmotic stress as a secondary effect, understanding the interaction between MLT signaling and stomatal regulation under these conditions is crucial. Wang *et al*. (2022a) found that overexpression of maize *PMTR1* in Arabidopsis rescued defects in MLT-induced stomatal closure in the *cand2-1* mutant, thereby reducing water loss under osmotic stress. Also, mutant plants lacking PMTR1 exhibit disrupted diurnal stomatal closure, with stomata staying open both day and night (Li *et al*., 2020). Wei *et al*. (2018) noted that MLT regulated stomatal closure through the CAND2/PMTR1 receptor, modulating H$_2$O$_2$ and Ca^{2+} signals in Arabidopsis. Therefore, it is reasonable to hypothesize that similar regulatory mechanisms may be at play under HS. However, further investigation is warranted to elucidate the signaling of MLT on stomatal regulation in HS.

Photosynthetic enzymes, including Rubisco, are highly susceptible to elevated temperatures and are recognized as crucial components in response to stress. Reports showed that MLT application notably enhances the activity of Rubisco and FBPase along with elevating the expression of pivotal photosynthesis-related genes such as *RCA*, *rbcS, rbcL*, *FBPase*, and *SBPase* which experience significant reduction under HS in tomato seedlings. Additionally, MLT supplementation increases CO$_2$ assimilation, as indicated by enhanced J$_{max}$: maximum electron transport rate and Vc$_{max}$: leaf level maximum carboxylation rate (following Farquhar's model), consequently stabilizing gas exchange parameters in HS tomato plants (Jahan *et al*., 2021b). A genetic study by silencing the MLT biosynthesis gene *COMT1* revealed a decrease in the light-saturated rate of CO$_2$ assimilation which was attributed to reduced stomatal conductance and transpiration rate in HS tomato (Ahammed *et al*., 2018). Therefore, MLT enhances the activity of crucial photosynthetic enzymes and upregulates key genes, improving CO$_2$ assimilation and stabilizing gas exchange under HS. However, further research is needed to elucidate the underlying mechanisms and regulatory pathways and validate these effects across various plant species and stress conditions.

In the intricate network of plant responses to HS, the modulation of carbohydrate metabolism emerges as a pivotal determinant of photosynthetic performance. A transcriptomic investigation revealed

that MLT exerted a regulatory influence on HS chrysanthemum plants by upregulating the expression of *TRIOSEPHOSPHATE ISOMERASE (TIM), RIBOSE 5-PHOSPHATE ISOMERASE A (RPI),* and *PHOSPHOENOLPYRUVATE CARBOXYKINASE (PCK)* genes (Xing *et al*., 2021). This upregulation significantly impacted carbon fixation and improved photosynthetic processes. Furthermore, studies have shown that sugars are involved in temperature stress mitigation in plants (Raza *et al*., 2023). Stress induction accelerates the conversion of starch to sugars, thereby, playing a protective role as it contributes to osmotic regulation and a quick energy supply (Dong and Beckles, 2019), MLT counters these effects through adjustment of the starch-to-sucrose ratio. A study by Iqbal *et al*. (2023), demonstrated that crosstalk of MLT and H_2S determines photosynthetic performance by regulation of carbohydrate metabolism in wheat under HS. It showed a notable decrease in starch accumulation coupled with an increase in sucrose content under HS. This shift in carbohydrate allocation was corroborated by reductions in the activity of AGPase (involved in starch synthesis) and SuSy (involved in cleaving sucrose), alongside an elevation in SPS (involved in sucrose synthesis) activity. Notably, enhanced SPS activity facilitated the redirection of carbon flux from starch toward sucrose synthesis, a mechanism crucial for heat tolerance. Moreover, MLT treatment during HS amplified SPS activity, albeit leading to a decrease in sucrose content compared to HS plants. This contrasting effect may be attributed to MLT's mediated increased activity of SuSy and INV, and AGPase indicating its regulatory role in carbohydrate metabolism under HS, thus, promoting utilization of sucrose for metabolic demands and indicating its regulatory role in carbohydrate metabolism under HS. However, another study recorded that MLT involves the suppression of *SuSy* and the stimulation of *SPS* genes, and induces the accumulation of starch and balancing the content of sugars, thus orchestrating metabolic adjustments critical for bolstering HS tolerance in chrysanthemum plants (Xing *et al*., 2021). MLT plays a crucial role in regulating carbohydrate metabolism by modulating key genes and enzymes, improving carbon assimilation, balancing the starch-to-sucrose ratio, and enhancing photosynthesis under HS. However, further research is needed to elucidate MLT's precise mechanisms, verify its consistency across species, and resolve contradictory findings on enzyme activities to optimize its use for improving plant resilience to HS.

Crosstalk of melatonin with other molecules in regulating photosynthesis under heat stress

Researchers have reported the interactions of MLT with various phytohormones, underlining its significant role in enhancing plant stress tolerance and developmental regulation. Despite these findings, specific investigations into the crosstalk between MLT and other hormones in improving photosynthesis under HS remain sparse. For instance, a study by Jia *et al*. (2020) reported that MLT application enhances not only chlorophyll and carotenoid content but also boosts antioxidant activity and IAA levels, thereby protecting photosynthesis under HS. The crosstalk between MLT and IAA highlights a synergistic relationship where MLT-induced IAA production supports plant growth and stress resilience, especially during the early growth stages. This intricate interplay underscores the potential of MLT as a major stress mitigator and an endogenous signaling molecule for developing climate-resilient crop cultivars.

Under HS, MLT treatment significantly reduces ABA levels by inhibiting ABA biosynthetic genes (*ZEP, NCED1, NCED2, AAO3)* and signaling genes (*ABI3, ABI5*), while upregulating ABA catabolism genes (*CYP707A1, CYP707A2*), thus revealing antagonistic crosstalk between MLT and ABA in regulating senescence (Zhang *et al*., 2017; Jahan *et al*., 2021a). Additionally, MLT enhances endogenous GA levels by inducing

GA biosynthetic genes (*GA20ox1, GA20ox2*) and inhibiting GA signaling suppressor gene *GAI* and GA catabolic genes (*GA2ox1, GA2ox2*), ultimately enhancing heat tolerance and suppressing heat-induced leaf senescence in tomato seedlings (Jahan *et al*., 2021b). Furthermore, MLT supplementation during HS stimulates the expression of genes involved in cytokinin (CK) biosynthesis (*LpIPT2, LpOG1*) and CK signaling pathway B-type ARR genes (*LpARR1, LpARR10*), while suppressing A-type ARR genes (*LpARR5, LpARR17*), thus demonstrating an interplay between MLT and CK in regulating leaf senescence (Zhang *et al*., 2017). Similarly, a recent study by Wei *et al*. (2023) found that arbuscular mycorrhizal symbiosis and MLT application in heat-stressed perennial ryegrass enhances photosynthesis and reduces leaf senescence by increasing GA and CK levels through the regulation of their biosynthesis genes and signal transduction factors while downregulating ABA-related genes and reducing ABA levels. This hormonal modulation improves turf quality and plant growth and reduces membrane lipid peroxidation and the expression of chlorophyll catabolic and senescence-associated genes. In chrysanthemum under HS, MLT increased the expression of ABA receptor *PYL4*, suggesting enhanced ABA signaling, which is known to improve stress tolerance. Additionally, MLT reduced the transcription of auxin-related genes *TIR1* and *Aux/IAA*, which are typically upregulated under HS, thereby potentially improving HS resistance through modulation of ROS metabolism (Xing *et al*., 2021). MLT also influences GA signaling by promoting the expression of GA biosynthesis genes and maintaining GA levels, which are otherwise reduced under HS (Xing *et al*., 2021).

Moreover, MLT downregulates the ET signaling genes *EBF2* (EIN3-Binding F-Box protein) and *ERF25* (ethylene-responsive transcription factor 25). This suggests that MLT orchestrates a complex hormonal response, integrating multiple signaling pathways to enhance the plant's resilience to HS. The alterations in hormone-related genes imply a broader regulatory adjustment that fine-tunes plant stress responses, indirectly supporting photosynthetic efficiency (Xing *et al*., 2021). A study demonstrated MLT's role in modulating MeJA-induced protection of wheat photosynthesis under HS by regulating the ET-synthesis gene (*1-aminocyclopropane-1-carboxylic acid (ACC) synthase*) and antioxidant metabolism. This research highlighted a synergistic interaction between MLT and MeJA (Sehar *et al*., 2023). MLT, combined with excess sulfur (2 mM SO_4^{2-}), enhances photosynthesis in heat-stressed mustard by increasing sulfur assimilation and modulating ET levels. This treatment boosts enzyme activities, cysteine, and methionine content, while reducing ethylene sensitivity, leading to improved antioxidant activity, and photosynthetic efficiency. ET inhibition using NBD (norbornadiene; an ET action inhibitor) negates these benefits, highlighting its critical role in MLT-mediated heat stress relief, though the exact interaction remains unclear (Iqbal *et al*., 2023).

Jahan *et al*. (2019) found that MLT alleviates thermal oxidative stress in heat-stressed tomatoes by interacting with PA and NO biosynthesis pathways. In MLT-pretreated seedlings, the expression of PA biosynthesis genes *ADC1/2, SAMDC1/2, SPMS,* and *SPDS1/2/3/5/6* increased, while *PAO1/2* decreased, enhancing Put, Spd, and Spm production. MLT also boosted NO content, NR (nitrate reductase) activity, NOS (nitric oxide synthase) – like activity, and related gene expression. This coordination with PA- and NO-mediated pathways helped mitigate HS. Additionally, MLT elevated endogenous PA and SA levels upregulated the SA biosynthesis gene *PAL2*, and reduced ABA levels (Imran *et al*., 2021), this study concluded that MLT reduces HS-induced oxidative damage by coordinating with PA and SA biosynthesis pathways to detoxify excess ROS.

Reports have shown the interaction of MLT with signaling molecules like ROS and H_2S under abiotic stress. For instance, RBOH-derived apoplastic ROS acts downstream of MLT to mediate stress tolerance, as evidenced on inhibiting RBOH or scavenging H_2O_2 significantly diminished MLT-induced defenses,

including decreased expression of stress-related genes and reduced antioxidative enzyme activity and PSII efficiency. These findings highlight a new mechanism where RBOH activity and H_2O_2 signaling play pivotal roles in MLT-induced drought, heat, and cold stress tolerance (Gong *et al.*, 2017; Ahammed *et al.*, 2024). Also, H_2S-mediated MLT signaling mitigates the adverse effects of HS by reducing TBARS and H_2O_2 content, boosting antioxidative enzyme activity, and enhancing photosynthetic processes and carbohydrate metabolism, which supply energy and carbon skeletons to the stressed plant (Iqbal *et al.*, 2021).

A study found a paradoxical role for MLT in abiotic stress responses. The suppression of the *SEROTONIN N-ACETYLTRANSFERASE 2* (*snat2*) gene, crucial for MLT biosynthesis, led to decreased levels of both MLT and BR in rice. This resulted in a semi-dwarf phenotype with erect leaves, characteristic of BR deficiency. Notably, *snat2* rice displayed enhanced tolerance to multiple stress conditions, including cadmium, salt, cold, and heat, indicated by lower MDA levels and higher chlorophyll content. In contrast, *SNAT2* overexpression lines were less stress-tolerant than wild-type plants. Further analysis revealed that other MLT-deficient mutants with suppressed BR synthesis also showed increased stress tolerance, whereas mutants without reduced BR levels did not exhibit this trait. This suggests that the observed stress tolerance is not solely due to MLT deficiency but is mediated through a decrease in BR levels. Thus, it is tempting to speculate that MLT exerts physiological functions in a BR-dependent or -independent manner in plants, although the detailed mechanism remains to be determined (Hwang and Back 2019).

MLT and SA showed synergistic interaction in activating antioxidant enzymes in *Mentha × piperita* and *Mentha arvensis* in response to heat stress (Haydari *et al.*, 2019), thus quenching ROS. These findings highlight MLT's potential in alleviating HS effects, but additional research is needed to comprehensively understand the mechanisms and the potential of these interactions in enhancing plant photosynthetic efficiency under such conditions.

Conclusion and future perspectives

In conclusion, MLT plays a crucial role in regulating photosynthesis under stress conditions through various mechanisms. It directly combats heat-induced oxidative stress by enhancing both enzymatic and non-enzymatic antioxidants, protecting membranes, proteins, and metabolic pathways. It also influences stomatal movement, improving gas exchange and chloroplast integrity by modulating HSPs, and HSFs. Additionally, it regulates gene expression related to chlorophyll and carotenoid biosynthesis, crucial for light capture during photosynthesis. MLT's gene expression and activity of photosystem and enzymatic processes in both light and dark phases. Furthermore, it enhances carbohydrate metabolism, promoting photosynthetic rates and growth during HS.

However, certain aspects remain unclear, such as whether MLT regulates stomatal movement through the CAND2/PMTR1-H_2O_2 pathway under HS, the specific induction patterns of HSPs HSFs within chloroplasts, and their functional significance in maintaining photosynthesis-related proteins. Further elucidation of the molecular mechanisms and hormonal crosstalk of MLT is warranted to comprehensively understand its impact on both the light and dark reactions of photosynthesis. Overall, the findings underscore MLT's pivotal role as a key regulator in plant responses to HS, particularly in the realm of photosynthesis and its potential applications in enhancing crop resilience to HS.

CONFLICT OF INTEREST

The authors have no conflict of interest.

AUTHOR CONTRIBUTIONS

SK, SS, and AS contributed to the text writing and visualizations, NAK conceived the idea and prepared the final draft of the manuscript.

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