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Literature Review

# The Role of Photorespiration in Enhancing Plant Resilience to Heat Stress

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# Abstract

Photorespiration, initiated by RuBisCO's oxygenation activity, results in the production of 2-phosphoglycolate, which must be recycled through energy-intensive reactions. These processes consume ATP and NADPH, release $CO_2$, and generate ammonia, traditionally framing photorespiration as inefficient due to the associated carbon and energy loss. Elevated temperatures exacerbate photorespiration by increasing RuBisCO's affinity for oxygen. However, under heat stress, photorespiration plays a protective role by dissipating excess energy, detoxifying harmful byproducts, and mitigating reactive oxygen species. This review highlights the adaptive functions of photorespiration during heat stress, emphasizing the natural upregulation of numerous photorespiratory genes in response to elevated temperatures. A potential strategy to enhance heat tolerance through co-overexpression of the photorespiratory genes *PGLP1* and *GOX1* in *Arabidopsis thaliana* is proposed, although this concept remains theoretical and requires experimental validation. Significant knowledge gaps persist regarding the roles of many photorespiratory genes during heat stress and the integration of the photorespiratory pathway with primary metabolism under elevated temperatures. Advancing our understanding of photorespiration through refined models and improved metabolic flux analysis holds promise for harnessing its protective potential to enhance plant resilience, reduce heat stress impacts, and safeguard crop productivity in a warming climate.

# Plain Language Summary

In a world increasingly shaped by climate change, rising temperatures and $CO_2$ levels pose significant challenges for living organisms. Unlike animals, plants cannot escape their environment; instead, they must rely on intrinsic metabolic mechanisms to adapt and mitigate environmental stressors. This review examines the role of photorespiration in enhancing plant resilience to heat stress. By exploring this adaptive mechanism, the analysis seeks to deepen our understanding of plant stress responses and guide efforts to develop climate-resilient crop varieties.

Photorespiration is a side reaction of RuBisCO, an enzyme central to photosynthesis. Through photosynthesis, plants, algae, and some bacteria convert sunlight into energy, using carbon dioxide and water to produce sugars that fuel growth and release oxygen - essential for life on Earth. However, RuBisCO has a flaw: about one in four times, it binds oxygen instead of carbon dioxide, triggering photorespiration. This process consumes energy, releases $CO_2$, and generates toxic byproducts that plants must detoxify. Historically dismissed as inefficient, recent research reveals photorespiration's critical role in stress protection. In warmer conditions, photorespiration removes harmful compounds, prevents the buildup of reactive oxygen species, and supports nutrient recycling, making it essential for plant survival in challenging environments.

As global temperatures rise, the importance of photorespiration in plant stress responses becomes increasingly evident. Many photorespiratory genes are naturally upregulated during heat stress, suggesting their critical role in helping plants cope with such conditions. To enhance heat resilience in crops, the co-overexpression of the photorespiratory genes *PGLP1* and *GOX1* has been proposed as a potential strategy. While this approach is still theoretical, it holds promise for reducing crop losses in regions affected by extreme temperatures.

However, much remains to be explored. The complexities of photorespiration, its connection to other metabolic pathways, and its full potential under heat stress conditions require deeper investigation. To accurately predict metabolic behavior and better understand how photorespiratory intermediates connect with other primary metabolic pathways, advances in metabolic flux modeling and improved resolution of

metabolic flux analysis are essential. By fully understanding and leveraging photorespiration’s protective mechanisms, we can take significant steps toward securing global food production in a rapidly warming world.

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# 1. Introduction

Climate change is recognized as one of the most pressing global challenges, with profound and escalating impacts projected for the near future. The increase in extreme weather events – such as heat waves, droughts, and floods – is directly linked to an enhanced greenhouse effect, primarily driven by elevated levels of atmospheric $CO_2$ from human activities (IPCC, 2023a). As of November 2024, the atmospheric $CO_2$ concentration stands at 422 ppm (Lan et al., 2024) with projections indicating a continued rise. In 2023, the Intergovernmental Panel on Climate Change (IPCC) released their Synthesis Report predicting climate change scenarios for the future: while the most conservative scenario (SSP1-1.9) suggests a temperature increase of 1.5 °C and net zero $CO_2$ emissions – this still increases the ppm in the air as $CO_2$ persists for centuries – by 2050, the most aggressive model (SSP5-8.5) states a 4.4 °C increase and $CO_2$ concentrations potentially exceeding 2000 ppm by 2100 (IPCC, 2023b). Regardless of the scenario, both temperature and atmospheric $CO_2$ concentrations are projected to increase. Regions near the poles are expected to experience more pronounced warming compared to areas near the equator (IPCC, 2023a). Temperature, a key factor influencing biological processes, plays a crucial role in determining species distribution across the globe (Woodward, 1987). As global temperatures rise, the atmosphere becomes drier (Ficklin & Novick, 2017), altering the thermal environment in which plants exist. Already, crop yields are decreasing due to global warming, and for wheat, crops were predicted to decline by 6% for each degree Celsius of further temperature increase (Asseng et al., 2015).

One critical physiological process affected by these environmental shifts is photosynthesis, which drives plant growth and biomass accumulation by converting light energy into chemical energy. This process depends on water, light, and carbon dioxide ($CO_2$) to produce sugars for metabolism, with oxygen ($O_2$) as a by-product. Central to photosynthesis is the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), which catalyzes the first step of the Calvin cycle during the light-dependent reactions of photosynthesis, thereby facilitating carbon fixation and biomass production. However, RuBisCO also exhibits oxygenase activity, which accounts for about 25% of the photosynthetic rate at 25°C (Sharkey, 1988). This activity involves the incorporation of $O_2$ instead of $CO_2$, resulting in the formation of 3-

phosphoglycerate (3-PGA) and 2-phosphoglycolate (2-PG). While 3-PGA can re-enter the Calvin cycle, 2-PG is a metabolite that cannot be directly utilized by the plant. If 2-PG accumulates, it exhibits toxic effects and to mitigate these, plants remove this metabolite through the energy-intensive process of photorespiration (Peterhansel et al., 2010). Each oxygenation event of RuBisCO requires 3.5 ATP and 2 NADH equivalents. In every seven RuBisCO reactions, five are carboxylation and two are oxygenation, consuming a total of 15 ATP and 10 NADH. Under normal atmospheric conditions at 25°C, 32% of ATP and 28% of NADH are allocated to these two photorespiratory reactions. These values may vary in C3 species based on environmental conditions (Walker et al., 2016). Photorespiration results in the release of pre-fixed $CO_2$ and ammonia ($NH_3$), both of which need to be re-assimilated, thereby imposing an additional metabolic burden on the plant (Peterhansel et al., 2010). At 25°C and current atmospheric $CO_2$ levels, about 30% of fixed carbon is lost to photorespiration in C3 plants, with this percentage increasing as temperatures rise (Zhu et al., 2010). This inefficiency contributes to reduced photosynthetic performance, ultimately leading to crop losses. In fact, current climate conditions account for approximately 36% of soybean and 20% of wheat production losses in the U.S., translating to a loss of 148 trillion calories annually (Walker et al., 2016).

While both carboxylation and oxygenation reaction rates increase with temperature, the oxygenase activity of RuBisCO rises more quickly than the carboxylase activity (Peterhansel et al., 2010). This imbalance results in reduced carbon fixation and increased oxygen uptake, both of which decrease photosynthetic efficiency (Brestic et al., 2014). It was also proposed that, under heat stress, crop yield loss is primarily caused by increased photorespiration, rather than direct damage to photosynthesis (Li et al., 2021). This proposition is based on the fact that the photosynthetic rate in rice was not changing over different heat treatments, while the photorespiratory rate increased. The researchers demonstrated that the combination of stable photosynthesis and increased respiration leads to inefficient energy use, where energy is diverted from growth to maintenance, reducing overall energy utilization efficiency (Li et al., 2021).

However, from an evolutionary perspective, it is rare for nature to produce a process as widespread and seemingly inefficient as photorespiration. The enzyme RuBisCO

has not always exhibited this apparent degree of inefficiency since its emergence around 3.5 billion years ago, during a period when atmospheric $CO_2$ concentrations were at least 100 times higher than they are today, while oxygen levels were 10–14 times lower (Badger et al., 2002; Kasting & Howard, 2006). During this early period, RuBisCO did not need to distinguish effectively between $CO_2$ and $O_2$, as $CO_2$ was in abundance. The advent of oxygenic photosynthesis in cyanobacteria, followed by the evolution of algae and land plants, led to significant increases in atmospheric oxygen and a concomitant decrease in $CO_2$ concentration through carbon fixation. This dramatic shift about 400 million years ago resulted in oxygen becoming the second most abundant gas in the atmosphere at 21%, while $CO_2$ was reduced to its current level of 0.04% (Badger et al., 2002). As atmospheric conditions evolved, the selective pressure on RuBisCO led to the emergence of the first $CO_2$ concentrating mechanisms in cyanobacteria (Iñiguez et al., 2020). During evolution, plants have evolved different carbon fixation mechanisms to overcome the limitations imposed by photorespiration. C4 plants, for example maize, sugarcane and sorghum, utilize an additional enzyme phosphoenolpyruvate carboxylase in mesophyll cells to capture $CO_2$ and convert it into C4 acids, which are then transported to bundle sheath cells where $CO_2$ is released, increasing local $CO_2$ concentration and minimizing photorespiration. CAM plants, such as pineapple and cactus, on the other hand, separate $CO_2$ fixation temporally, capturing $CO_2$ at night and releasing it during the day to maintain high $CO_2$ concentrations around RuBisCO, reducing photorespiration (Ehleringer & Monson, 1993).

About 85% of today's global plant population has a C3 photosynthetic pathway, with estimates varying slightly among sources. This raises the question of whether photorespiration confers some advantage to plants, particularly under abiotic stress conditions, suggesting it may play a beneficial role despite its apparent wastefulness. It was postulated that photorespiration may assist plants in managing heat stress by playing a protective role under these conditions. Although often regarded as energy-intensive, photorespiration helps divert excess energy such as ATP and NADPH, thus preventing the formation of reactive oxygen species (ROS), and thereby safeguarding the photosynthetic apparatus (Brestic et al., 2014). Additionally, this process aids in the removal of toxic metabolic intermediates, such as 2-PG, that would otherwise accumulate and be detrimental to the plant (Anderson, 1971). Photorespiration also

produces hydrogen peroxide ($H_2O_2$), a signaling molecule that contributes to programmed cell death (Heath, 2000) and assists the plant in responding to both biotic and abiotic stresses (Peterhansel et al., 2010). Furthermore, this pathway is intricately connected to the plant's primary metabolism, potentially facilitating communication about the energy state among various metabolic pathways within the cell (Nunes-Nesi et al., 2007).

This literature review examines the adaptive role of photorespiration in plants exposed to heat stress, drawing on findings from various C3 species to underscore its essential functions within cellular metabolism. Beginning with a foundational overview of photorespiration's pathway, enzymes, and metabolites, the review investigates how elevated temperatures influence this process. By analyzing the effects of photorespiration on cellular energy balance, ROS accumulation, and interactions with primary metabolic pathways, the review aims to enhance our understanding of photorespiration's benefits under heat stress. The potential to increase plant resilience through the targeted overexpression of key photorespiratory genes is also explored. Ultimately, this review highlights photorespiration's critical role in maintaining cellular stability and promoting thermotolerance in plants.

# 2. Fundamentals of Photorespiration

## 2.1 The Enzymatic Steps of the Photorespiratory Cycle

Photorespiration is a central metabolic pathway in plants that plays a role in reducing the effects of oxygenation by RuBisCO, balancing cellular energy and redox states, and supporting metabolic flexibility under stress conditions. This pathway involves a series of biochemical reactions across three key organelles: the chloroplast, peroxisome, and mitochondrion (Figure 1). This chapter provides an overview of the core components and functions of photorespiration, highlighting its significance in plant physiology.

The photorespiratory cycle begins when RuBisCO incorporates $O_2$, producing one molecule of 3-PGA and one molecule of 2-PG (Andrews et al., 1973; Peterhansel et al., 2010). The accumulation of 2-PG in high amounts can lead to the sequestration of substantial carbon and phosphorus, resulting in a depletion of the Calvin cycle's metabolite pool (Wingler et al., 2000). Furthermore, 2-PG inhibits key enzymes such as triose-phosphate isomerase and phosphofructokinase, which are crucial for chloroplast carbon metabolism, starch biosynthesis, and glycolysis (Anderson, 1971; Kelly & Latzko, 1976). To combat this, plants have evolved photorespiration as a salvage pathway to convert 2-PG into 3-PGA, which can re-enter the Calvin cycle, thus reducing its harmful effects and supporting overall photosynthetic efficiency (Peterhansel et al., 2010).

In the initial step of the photorespiratory cycle, the enzyme 2-phosphoglycolate phosphatase (PGLP) catalyzes the dephosphorylation of 2-PG into glycolate within the chloroplast (Figure 1) (Kisaki & Tolbert, 1969; Somerville & Ogren, 1979). Glycolate is transported out of the chloroplast and into the peroxisome, where it is oxidized to glyoxylate by glycolate oxidase (GO or GOX) in the presence of $O_2$, producing hydrogen peroxide ($H_2O_2$) (Kisaki & Tolbert, 1969; Tolbert et al., 1968). $H_2O_2$ is a secondary messenger involved in various abiotic and biotic stress responses (Foyer et al., 1997) which is further broken down into 2 $H_2O$ and $O_2$ by catalase, effectively decreasing the amount of ROS in the cells and thus keeping the stress response low in intact plants (Willekens, 1997). In a study on spinach, a high activity of GOX and catalase was measured only within the peroxisomes (Tolbert et al., 1968).

The previously formed glyoxylate inhibits RuBisCO activase, a regulatory protein that activates RuBisCO, if accumulated (Campbell & Ogren, 1990; Portis, 2003). Therefore, glyoxylate is transaminated with glutamate by glutamate aminotransferase (GGAT), resulting in the formation of 2-oxoglutarate (2-OG) and glycine. The 2-OG is shuttled back to the chloroplast to be used in ammonia re-assimilation, while glycine is transported to the mitochondrion (Peterhansel et al., 2010). In the mitochondrion, two molecules of glycine are converted to serine by glycine decarboxylase (GLD or GDC) and serine hydroxy methyltransferase (SHM or SHMT), producing a molecule of $CO_2$, $NH_3$ and NADH, while consuming NAD and using tetrahydrofuran (THF) for the reaction (Kisaki et al., 1971; Kisaki & Tolbert, 1970; Peterhansel et al., 2010).

At this point, $CO_2$ which was previously fixed, is now released, making photorespiration a wasteful process. However, serine and $NH_3$ are substrates for further reactions. While serine will be transformed in the peroxisome into the final substrate glycerate that re-enters the Calvin cycle, $NH_3$ will be re-assimilated in the chloroplast. The serine is transported back to the peroxisome, where it is deaminated by serine aminotransferase (SGAT) using glyoxylate as a co-substrate, yielding hydroxy pyruvate and glycine (Rehfeld & Tolbert, 1972). Glycine returns to the mitochondrion to repeat the cycle, while hydroxy pyruvate is reduced to glycerate by hydroxy pyruvate reductase (HPR) with NADH as a cofactor (Kohn & Warren, 1970; Peterhansel et al., 2010). The glycerate is then transported to the chloroplast, where it is phosphorylated to 3-PGA by D-glycerate 3-kinase (GLYK) using ATP, allowing 3-PGA to re-enter the Calvin cycle (Peterhansel et al., 2010).

## 2.2 Re-Assimilation of Ammonia During Photorespiration

As previously mentioned, during photorespiration, ammonia ($NH_3$) is released in the mitochondrion when glycine is converted to serine. This released $NH_3$ can be re-assimilated by the plant when it is transported to the chloroplast, where the GS/GOGAT cycle is involved in its incorporation (Somerville & Ogren, 1980; Wallsgrove et al., 1980). Two key enzymes facilitate this process: glutamine synthetase (GS) and ferredoxin-dependent glutamine aminotransferase (Fd-GOGAT). First, glutamine synthetase (GS) catalyzes the incorporation of ammonia into glutamate, forming glutamine in a reaction that requires ATP. In the subsequent step,

Fd-GOGAT utilizes glutamine as an amino donor for the transamination of 2-OG, which originates from the earlier transamination of glyoxylate in the peroxisome. This reaction produces two molecules of glutamate, effectively re-assimilating the ammonia for further use by the plant (Keys et al., 1978; Peterhansel et al., 2010). Theoretically, all $NH_3$ released during photorespiration is re-assimilated through the GS/GOGAT cycle. However, a portion of the $NH_3$ may evade complete fixation, resulting in excess ammonia (Peterhansel et al., 2010). Also, important to mention here are the translocators dihydroxyacetone phosphate isomerases (DiT1, DiT2) that shuttle substrates between the chloroplast and peroxisome. Photorespiratory $NH_4^+$ re-fixation in plastids via the GS/GOGAT system requires 2-OG import and glutamate export, facilitated by DiT1 and DiT2, which mediate 2-OG/malate and glutamate/malate exchanges, respectively (Eisenhut et al., 2013).

In conclusion, photorespiration serves as a complex metabolic pathway that partially reverses photosynthetic carbon fixation by releasing previously sequestered $CO_2$ while consuming ATP and NADH. Although energy intensive, this process is crucial for detoxifying the harmful compound 2-PG, which forms when oxygen, instead of $CO_2$, reacts with RuBP. Through multiple enzymatic steps, 2-PG is ultimately converted into 3-PGA, a compound that can re-enter the Calvin cycle and contribute to carbon fixation. During this conversion, several intermediates are formed, including glycolate, glyoxylate, and $H_2O_2$; these intermediates must be tightly regulated, as their accumulation can be toxic to the cell. The coordinated efforts of three organelles – the chloroplast, peroxisome, and mitochondrion – are essential for efficiently managing and completing this pathway, underscoring the importance of photorespiration for maintaining metabolic balance.

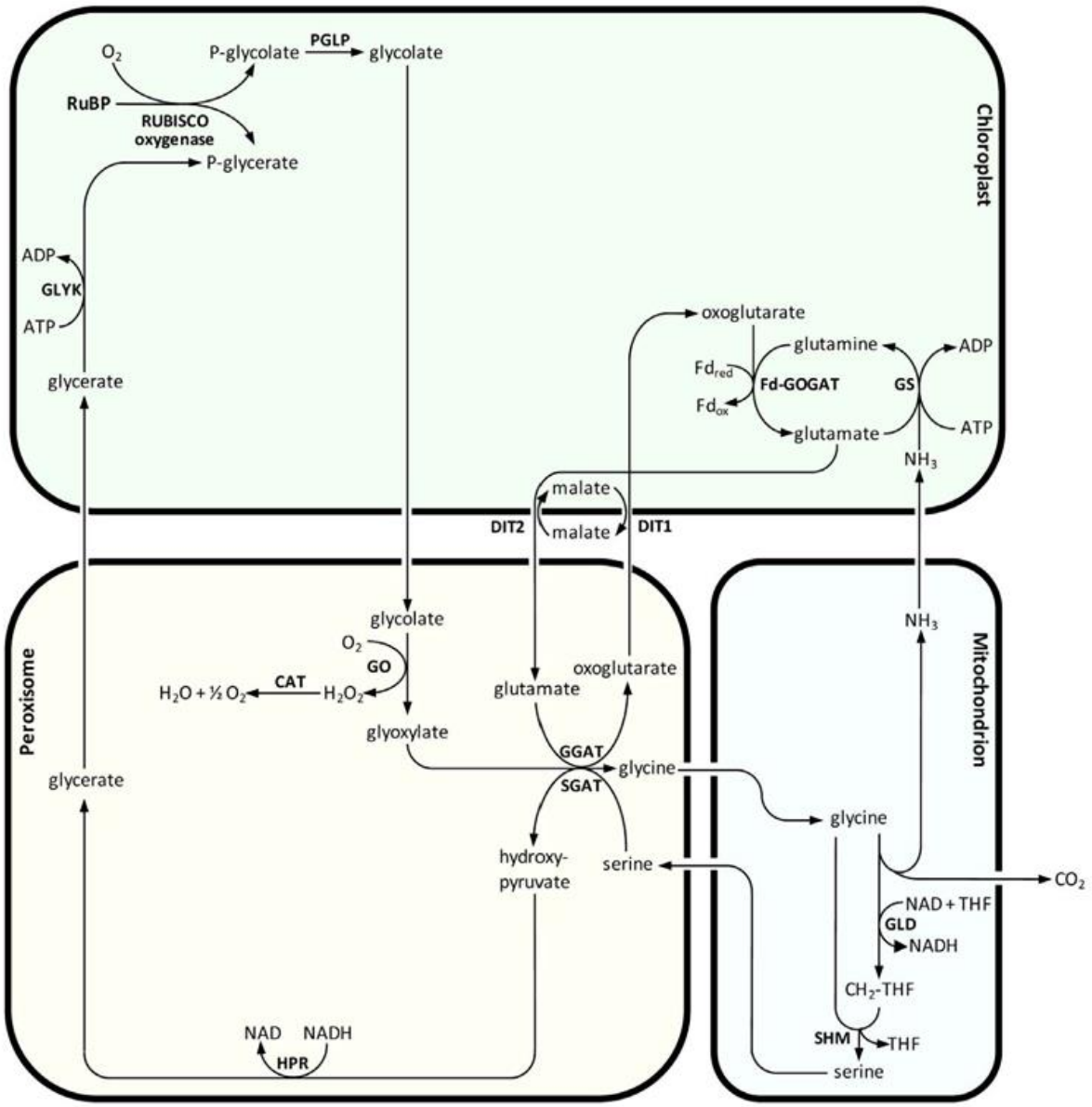


**Figure 1 | The photorespiratory pathway and ammonium reassimilation in C3 plants.** This figure provides a comprehensive overview of the photorespiratory pathway, highlighting the key enzymes and substrates involved. The pathway initiates with 2-PG (here P-glycolate), produced by the oxygenase activity of RuBisCO in the chloroplast, which is subsequently converted to 3-PGA through a series of enzymatic reactions in the photorespiratory pathway. Abbreviations: RuBP (ribulose-1,5-bisphosphate), RuBisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase), phosphoglycolate phosphatase (PGLP), glycolate oxidase (GO), catalase (CAT), glutamate aminotransferase (GGAT), serine aminotransferase (SGAT), glycine decarboxylase (GLD), serine hydroxymethyltransferase (SHM), hydroxypyruvate reductase (HPR), glycerate kinase (GLYK), glutamine synthetase (GS), ferredoxin-dependent glutamate synthetase (Fd-GOGAT), dihydroxyacetone phosphate isomerases (DiT1/DiT2). *Figure adapted from "Photorespiration," by Peterhansel et al., 2010, The Arabidopsis Book, 8, e0130. Copyright 2010 by Academic Publisher.*

# 3. The Effect of Heat Stress on Photorespiration

## 3.1 The Role of Temperature in RuBisCO Function and Photorespiration

This chapter examines the dynamics of photorespiration and its role in plant metabolism under heat stress, exploring how plants' metabolic adjustments to elevated temperatures influence their resilience and productivity. Plants have adapted to thrive across a range of environmental conditions, with their photosynthetic processes finely tuned to match the temperature profiles of their native habitats. The temperature sensitivity of key photosynthetic enzymes, particularly RuBisCO, underpins these adaptations.

The activity of RuBisCO generally increases with temperature up to a species-specific optimum, which typically aligns with the average daytime temperatures of a plant's natural environment (Berry & Björkman, 1980). Within this optimal range, increasing temperatures enhance RuBisCO carboxylase activity and, consequently, the rate of $CO_2$ assimilation (Jordan & Ogren, 1984; Laing et al., 1974). However, beyond this range, higher temperatures begin to compromise RuBisCO's efficiency, causing a shift in its affinity from $CO_2$ to $O_2$ (Monson et al., 1982). This shift leads to an increase in RuBisCO's oxygenase activity, favoring the oxygenation reaction over carboxylation due to the relatively greater solubility of $O_2$ over $CO_2$ at elevated temperatures (Farquhar et al., 1980). As RuBisCO's oxygenase activity becomes dominant, photorespiration increases, effectively competing with $CO_2$ fixation and thereby reducing photosynthetic efficiency (Figure 2), especially under heat stress conditions (Schuster & Monson, 1990). This is particularly true in low $CO_2$ environments, where the rate of $CO_2$ assimilation declines more sharply as temperatures rise, resulting in an increased photorespiration-to-photosynthesis ratio and reduced overall efficiency (Sharkey, 2005).

Figure 2 illustrates the effect of temperature on net $CO_2$ assimilation and oxygenation rates in *Gossypium barbadense* (Pima cotton) under different atmospheric $CO_2$ conditions. In this study, 190 ppm reflects $CO_2$ levels that were stable for millennia, while 370 ppm represents concentrations around the early 21st century (Lan et al., 2024; Sharkey, 2005). The data show that as leaf temperature increases, the rate of

net $CO_2$ assimilation decreases, while the photorespiratory rate rises, particularly beyond 35°C. This model suggests that at high temperatures, the metabolic costs of photorespiration may surpass the benefits of $CO_2$ fixation, especially under lower atmospheric $CO_2$ levels. These findings indicate that plants may face significant challenges in maintaining efficient photosynthesis in warmer, low-$CO_2$ environments, underscoring the importance of understanding the balance between photorespiration and $CO_2$ assimilation under varying climate conditions.

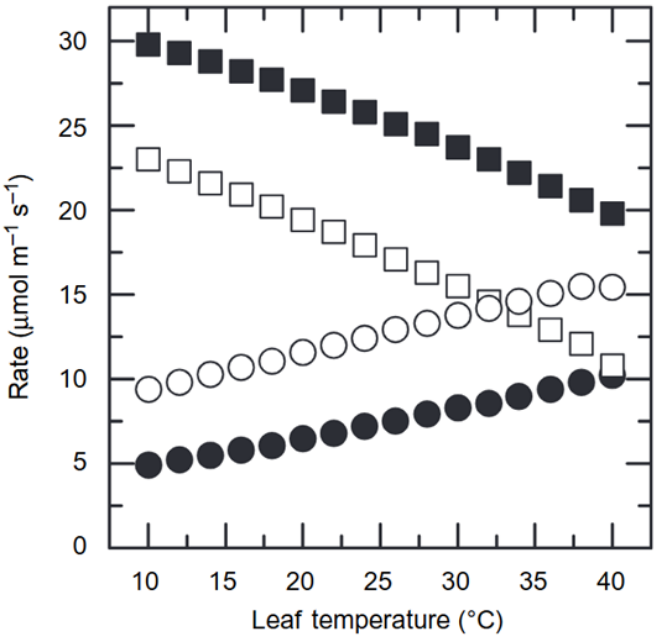


**Figure 2 | Modeled rates of photosynthesis and photorespiration as a function of temperature under two atmospheric $CO_2$ concentrations: 190 ppm (open symbols) and 370 ppm (filled symbols).** Carboxylation rates, represented by square symbols, show the efficiency of RuBisCO in $CO_2$ fixation at varying temperatures, while oxygenation rates, represented by circular symbols, indicate the rate of RuBisCO's oxygenase activity, a proxy for photorespiration. As temperature increases, a divergence between the carboxylation and oxygenation rates emerges, particularly at 190 ppm $CO_2$, where RuBisCO's preference for $O_2$ intensifies, driving an increase in photorespiration relative to photosynthesis. This model highlights how lower $CO_2$ conditions exacerbate the shift towards oxygenation at higher temperatures, suggesting increased metabolic costs for plants under warmer, low-$CO_2$ environments. *Figure adapted from "Effects of moderate heat stress on photosynthesis: Importance of thylakoid reactions, rubisco deactivation, reactive oxygen species, and thermotolerance provided by isoprene" by Sharkey T. D., 2005, Plant, Cell & Environment, 28(3), 269-277. Copyright 2005 by Academic Publisher.*

In a study on *Agropyron smithii*, the maximum observed photoinhibition reached 65% at 45°C during temperature response experiments (Monson et al., 1982). At elevated temperatures, $CO_2$ fixation is inhibited due to the inactivation of RuBisCO (Weis, 1981).

In conclusion, this section examined the thermosensitivity of RuBisCO and its impact on $CO_2$ assimilation rates as temperature increases. Elevated temperatures cause a decline in RuBisCO's affinity for $CO_2$ and favor oxygenation reactions, a shift exacerbated by the higher solubility of $O_2$ relative to $CO_2$ in aqueous solutions. This preferential binding of $O_2$ over $CO_2$ at high temperatures leads to increased rates of photorespiration, diverting energy away from carbon fixation and reducing overall photosynthetic efficiency. Notably, models of $CO_2$ assimilation demonstrate that this shift is driven not by variations in ambient $O_2$ concentration, which remained constant in the model shown in Figure 2, but by the interplay between $CO_2$ concentration and temperature. These findings underscore the vulnerability of photosynthetic efficiency to temperature fluctuations and suggest that, as global temperatures rise, understanding and potentially mitigating the metabolic costs of increased photorespiration will be critical for enhancing plant resilience and productivity.

## 3.2 Photorespiration Regulates ROS Levels and Dissipates Excess Energy During Heat Stress

Building on the impact of temperature on photorespiration, it is essential to consider how plants manage the complex disruptions in energy flow and ROS production that arise during heat stress. As temperatures increase, these imbalances intensify, challenging cellular stability and prompting the activation of defense mechanisms to mitigate oxidative damage.

During many abiotic stresses, ROS are generated through the disruption of membrane complexes involved in electron transfer chains within mitochondria and chloroplasts, disrupting redox homeostasis (Mittler et al., 2022). When temperatures rise, the photorespiratory rate increases faster, thus more ROS is generated in the peroxisomes, potentially being the first response to increasing temperatures. These ROS bursts lead to increased levels of $O_2^-$ and $H_2O_2$ that accumulate in cytosol and

nucleus (Babbar et al., 2021), activating specific stress-responsive genes and pathways but also regulating jasmonic acid (JA) and abscisic acid (ABA) hormone synthesis and signaling. However, excessive ROS can also lead to oxidative stress, causing damage to cellular components, disrupting metabolic processes, impairing signaling pathways, accelerating cellular senescence, and increasing susceptibility to some pathogens, ultimately compromising plant health and productivity (Mittler et al., 2022). In response, plants have evolved protective mechanisms to mitigate the damaging effects of ROS and excess energy to maintain cellular balance (Noctor & Foyer, 1998).

Although photorespiration generates ROS, this is done in a regulated manner as it also includes an important detoxification mechanism. Catalase catalyzes the conversion of $H_2O_2$ into 2 $H_2O$ and $O_2$, thereby mitigating the harmful effects of ROS (Mittler et al., 2022), as discussed in Chapter 2.1. A study on iron-tolerant rice cultivars (*Oryza sativa*) demonstrated that enhanced photorespiration under heat stress mitigated oxidative stress caused by iron overload, facilitating excess light energy dissipation, among others, by providing an alternative electron sink that helps to balance the energy absorbed during photosynthesis. This mechanism allows for the safe dissipation of excess light energy as heat, thereby reducing the risk of photoinhibition of photosystem II and maintaining photosynthetic efficiency. Despite similar iron level accumulation in both iron-sensitive and iron-tolerant cultivars, the latter exhibited more effective photorespiration, likely contributing to its stress resilience (De Souza et al., 2024). Similar results on two pepper cultivars – one drought-sensitive and the other drought-tolerant – showed that the drought-tolerant variety had higher photorespiratory rates (Hu et al., 2010).

Photorespiration also plays a vital role in regulating electron flow and maintaining cellular energy balance (Figure 3), particularly under suboptimal conditions, such as heat stress. During photosynthesis, light energy drives the electron transport chain, producing ATP and NAD(P)H for the Calvin cycle. However, high light intensity or limited $CO_2$ availability can disrupt this balance, leading to excess ATP and NAD(P)H. By utilizing these energy carriers, photorespiration mitigates oxidative damage and maintains redox homeostasis, thus supporting metabolic activity under stress (Voss et al., 2013). A study on barley (*Hordeum vulgare*) protoplasts further refines this

perspective, indicating that photorespiration can also enhance ATP production by coupling it with mitochondrial oxidative phosphorylation. In fractionated barley protoplasts it was demonstrated that, under photorespiratory conditions, a higher NADH:NAD ratio is observed. The NADH produced in the GDC reaction, which converts glycine to serine, is oxidized by the respiratory chain. This process leads to increased cytosolic ATP/ADP levels and a higher mitochondrial NADH:NAD ratio. Thus, photorespiration serves a dual role during heat stress: dissipating excess energy and facilitating ATP synthesis (Gardeström & Wigge, 1988).

Altogether, abiotic stresses such as heat induce ROS production due to disruptions in electron transfer chains across cellular compartments. Rising temperatures further amplify photorespiration, which becomes both a source and a sink of ROS. The $H_2O_2$ generated in the photorespiratory pathway, along with ROS from other cellular processes, is detoxified by catalase. By utilizing surplus ATP and NAD(P)H, photorespiration acts as a regulator of electron flow and maintains cellular energy balance under stress. Additionally, studies on barley protoplasts indicate that under photorespiratory conditions, an elevated NADH ratio enables further ATP production via mitochondrial oxidative phosphorylation. This suggests that, beyond its conventional role, photorespiration may also help sustain ATP levels during stress conditions.

## 3.3 Photorespiration and Its Links to Primary Metabolic Pathways under Thermal Stress

Following the previous chapter's exploration of photorespiration in energy balance and ROS degradation, this section delves into its broader integration within metabolic networks under heat stress. Photorespiration is tightly linked to primary metabolic processes (Figure 3; Timm et al., 2012), influencing plant growth, resource allocation, and stress resilience. This involves processes such as photosynthesis, the Calvin cycle, amino acid metabolism, and the TCA cycle (Hodges et al., 2016). Furthermore, photorespiration is critical in regulating nitrogen uptake and assimilation (Asensio et al., 2015).

Under heat stress conditions, increased rates of photorespiration can lead to shifts in metabolite profiles, potentially impacting plant growth and productivity (Hodges et al., 2016). As previously mentioned, the primary link between photorespiration and photosynthesis is the RuBP reaction, where $O_2$ is integrated, leading to the formation of 2-PG and 3-PGA (Figure 3). Additionally, photorespiration relies on ATP and NADPH generated by the light reactions of photosynthesis.

The amino acids glycine, serine, glutamine, and glutamate are closely linked to the photorespiratory pathway (Figure 3). Glycine is produced through two distinct reactions in the peroxisome: GGAT converts glyoxylate to glycine, while SGAT generates glycine from serine. Novitskaya et al. (2002) showed that during high photorespiratory flux rates, glycine levels significantly increased, reaching up to 40% of total amino acids in potato and wheat leaves, compared to only 1% in darkness. Conversely, serine levels declined as photorespiration rates rose. This was explained by the increasing demand for glycine and the favored reaction by SHMT1 in converting serine to glycine. Plants benefit from glycine as a nitrogen storage molecule and a key transporter within the plant, providing a readily available nitrogen source for various metabolic processes (Hartung, 2002). Additionally, glycine regulates osmotic pressure within plant cells by functioning as an osmolyte during environmental stresses (Ashraf & Foolad, 2007).

During nitrogen assimilation, glutamate acts as a key acceptor of $NH_3$ in the reaction catalyzed by GS (Figure 3). This reaction produces glutamine, which serves as an important nitrogen donor for various cellular processes. In the subsequent GOGAT reaction, glutamine transfers its amide nitrogen to 2-OG. Although increased photorespiration has been associated with enhanced nitrogen assimilation in wheat (Lawlor et al., 1987), suggesting a rise in glutamine and glutamate levels under heat stress, further studies indicate that elevated photorespiration does not directly lead to higher concentrations of these amino acids. Instead, glutamine levels appear to increase in response to net $CO_2$ uptake, independent of photorespiratory activity. Therefore, nitrogen re-assimilation seems to be more strongly influenced by the efficiency and activity of GS and GOGAT enzymes rather than by photorespiratory flux alone (Novitskaya et al., 2002).

Photorespiration is closely linked to the TCA cycle via 2-OG which is shuttled between the peroxisome and chloroplast to participate in reactions in both organelles (Figure 3). While the functional connection between photorespiration and the TCA cycle under stress conditions remains underexplored, existing studies suggest a strong relationship based on metabolite shifts. In soybean leaflets, elevated temperatures caused a 40-80% decrease in TCA cycle metabolites, including citrate, malate, malonate, fumarate, and succinate. However, under $CO_2$-enriched conditions, these declines were partially reversed. Interestingly, glycerate, a photorespiratory intermediate, decreased under $CO_2$ enrichment at both normal and elevated temperatures, indicating that the reduction in organic acids under heat stress was driven by increased photorespiration (Sicher, 2015). In maize, excess NADH levels in mitochondria were found to inhibit pyruvate dehydrogenase complex (Thelen et al., 1998), a key enzyme in the TCA cycle that converts pyruvate to acetyl-CoA. Heat stress elevates the NADH:NAD ratio through enhanced photorespiration, potentially inhibiting the TCA cycle. While research on the direct link between photorespiration and the TCA cycle is limited, this inhibition could explain the observed downregulation of TCA activity under higher photorespiratory rates.

In conclusion, while photorespiration is linked to various primary metabolic pathways, substantial research gaps remain, particularly regarding its role under heat stress. Current evidence suggests that photorespiration helps conserve resources by dissipating excess energy and ROS, potentially downregulating the TCA cycle, and producing glycine to maintain essential processes. However, the effects of heat stress and increased photorespiratory rates on various metabolic pathways, especially the connection to nitrogen assimilation and the TCA cycle, are still poorly understood. More research is needed to fully elucidate these interactions and their impact on plant resilience under stress conditions.

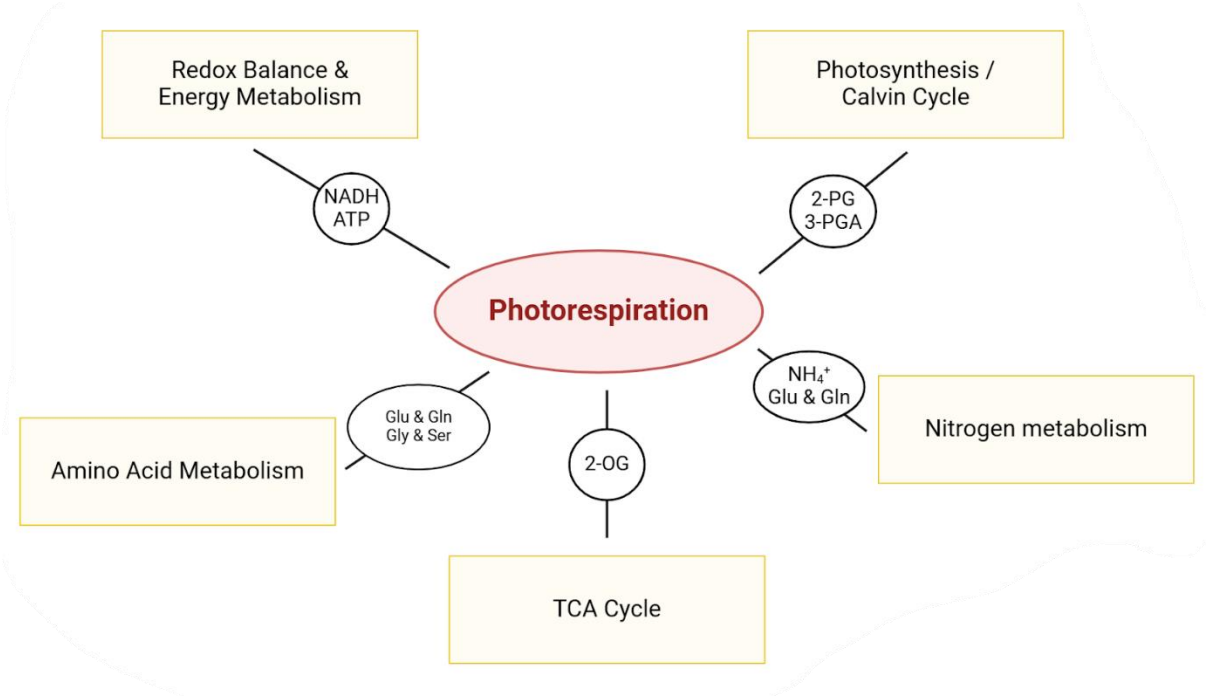


**Figure 3 | Integration of photorespiration with various metabolic pathways.** This figure depicts the interconnections between photorespiration and key metabolic pathways in plants through shared substrates and intermediates. The process begins with RuBisCO catalyzing the fixation of oxygen instead of $CO_2$, resulting in the production of 2-PG, which must be converted to 3-PGA to re-enter the Calvin cycle. The photorespiratory pathway also produces glycine and serine, linking it to amino acid metabolism. Similarly, glutamate and glutamine are involved, connecting photorespiration to nitrogen metabolism, where ammonia is released and must be re-assimilated into glutamate via 2-OG, further linking photorespiration to the TCA cycle. Additionally, photorespiration helps manage ROS by dissipating excess energy, thus preventing cellular damage. This interconnected network highlights the complex role of photorespiration in plant metabolism. *Created with BioRender.com.*

## 3.4 Boosting Plant Heat Resilience through Photorespiratory Gene Upregulation

During heat stress, photorespiration appears to shift from a seemingly wasteful process to a vital protective mechanism, dissipating excess energy and mitigating ROS accumulation. But what happens when the genes driving photorespiration malfunction? How reliant are plants on this process for survival, and could targeted upregulation of these genes enhance resilience to elevated temperatures? This chapter investigates the benefits of genetic manipulation of photorespiration under heat stress conditions. Exploring the consequences of photorespiratory gene

disruption may reveal the extent of plant reliance on this pathway, providing insights to inform strategies for enhancing resilience amid increasing environmental challenges (Table 1).

### *3.4.1 Certain photorespiratory genes, which are conditionally lethal when knocked out, may improve heat stress resilience when overexpressed*

The entry enzyme into the photorespiratory cycle is phosphoglycolate phosphatase (PGLP) which converts 2-PG into glycolate in the chloroplast. Early genetic experiments on *Arabidopsis thaliana* and barley demonstrated that mutants in these enzymatic genes are not viable under normal atmospheric $CO_2$ conditions, while the mutants could be recovered in 1% $CO_2$-enriched air, indicating conditional lethality under photorespiratory conditions (Hall et al., 1987; Somerville & Ogren, 1979). Later it was found that, contradictory to the belief that this enzyme was encoded by a single gene, two genes encode for PGLP, *PGLP1* and *PGLP2*, whereas only the knockout of *PGLP1* results in leaf PGLP deficiency. PGLP2 was thought to not contribute to the photorespiratory metabolism but to degrade other minor amounts of 2-PG in the cytosol (Schwarte & Bauwe, 2007). In *A. thaliana*, researchers found that in response to high-temperature stress (30°C), several photorespiratory genes, including *PGLP1*, were significantly upregulated in wild-type plants (Timm et al., 2019). This upregulation was also observed at the protein level. In *PGLP1* overexpression lines, where baseline mRNA and protein levels were already elevated, only minimal additional changes occurred under heat stress, and these lines showed enhanced resilience to high temperature stress. This conclusion was based on several key observations: (1) only *PGLP1* overexpression lines exhibited starch accumulation and altered sugar content during heat stress, indicating improved carbon allocation and metabolic stability; (2) photosynthetic performance was consistently higher in *PGLP1* overexpression lines under both short-term and long-term temperature stress compared to wild-type plants; and (3) while wild-type plants showed a significant increase in the expression and protein levels of other photorespiratory genes (*GGAT1*, *GDC*, *SHMT1*, *HPR1*) under heat stress, these levels remained unchanged in *PGLP1* overexpression lines. These findings suggest that *PGLP1* overexpression primes plants for temperature stress

resilience, likely due to the constitutive upregulation of other photorespiratory genes and proteins (Timm et al., 2019). These findings highlight the critical importance of rapid 2-PG removal through photorespiration. The results further suggest that upregulating *PGLP1* alone may be sufficient to confer a certain degree of tolerance to temperature stress.

Glycine decarboxylase (GDC) and serine hydroxymethyltransferase (SHMT) operate sequentially within the mitochondria to convert glycine into serine, releasing $CO_2$ and ammonia. Studies on homozygous GDC-deficient barley lines revealed early senescence and reduced photosynthetic efficiency, particularly under low $CO_2$. These mutants exhibited increased levels of glycine, glyoxylate, and a slight increase in glycolate, likely due to decreased respiratory activity. Additionally, ATP and NADPH accumulated excessively, leading to chloroplast reduction (Igamberdiev et al., 2001). In *A. thaliana,* the *shmt1* mutant displays a lethal phenotype under normal atmospheric conditions but not under $CO_2$-enriched environments (Voll et al., 2006). A *gdc-shmt1* double mutant, however, remains lethal even with $CO_2$ enrichment (Hodges et al., 2016). In tomato (*Solanum lycopersicum*), thermal *Agrostis scabra,* and heat-sensitive *Agrostis stolonifera*, *SHMT1* gene expression is slightly downregulated during heat treatment (Z. Liu et al., 2022; Xu & Huang, 2010). In *Agrostis scabra, GDC* expression also declines under heat stress, which may limit photorespiration, protecting the photosynthetic electron transport chain from over-reduction and supporting essential metabolite synthesis, such as glycine, to enhance heat tolerance (Xu & Huang, 2010). In contrast to findings in tomato and *Agrostis*, where *GDC* and *SHMT1* gene expression decreases under heat stress, later studies in *Arabidopsis* revealed an opposite response, highlighting species-specific differences likely due to varying heat adaptation mechanisms. In *Arabidopsis*, exposure to 30°C led to an upregulation of *GDC* and *SHMT1* expression for up to three days, with levels nearly returning to baseline by seven days (Timm et al., 2019). Although species-specific differences exist, no study observed sustained upregulation of these genes during heat stress. So far, it is unclear how prolonged expression of *GDC* and *SHMT1* influences the heat stress resilience.

D-glycerate-3-kinase (GLYK) is a key enzyme, catalyzing the last reaction of the photorespiratory cycle in which glycerate is phosphorylated to 3-PGA. In *Arabidopsis*,

GLYK is encoded by a single gene. GLYK-deficient mutants exhibit severe growth defects, particularly under normal atmospheric conditions, as photorespiration is exacerbated. These mutants fail to progress beyond the early cotyledon stage, but can survive in $CO_2$-enriched environments due to reduced photorespiration. However, even under these conditions, GLYK-deficient plants exhibited reduced growth and accumulate high levels of glycerate, serine, and hydroxypyruvate under $CO_2$-enriched conditions, indicating a broader role for GLYK in plant metabolism (Boldt et al., 2005). The impact of heat stress on *GLYK* activity and expression remains largely unexplored. While it is plausible that *GLYK* could be upregulated under heat stress to support energy metabolism, particularly given its ATP-dependent reaction, this hypothesis requires experimental validation. Future research should investigate the specific mechanisms by which GLYK responds to heat stress and its potential role in thermotolerance.

In chloroplasts, glutamine synthetase (GS) and the ferredoxin-dependent glutamine oxoglutarate aminotransferase (Fd-GOGAT) cooperate to re-assimilate ammonia released from photorespiratory reactions in the peroxisome. Tomato possesses six *GS* genes and two *Fd-GOGAT* genes (L. Liu et al., 2016). Barley mutants lacking GS or *Arabidopsis* mutants deficient in Fd-GOGAT are only viable under non-photorespiratory conditions, emphasizing the crucial role of these enzymes in photorespiratory nitrogen cycling (Somerville & Ogren, 1980; Wallsgrove et al., 1987). Additionally, the *fd-gogat* mutants accumulated high levels of ammonia which is toxic when in excess (Wallsgrove et al., 1987). This also indicates that leaf Fd-GOGAT is essential for the re-assimilation of ammonia generated during photorespiration, while the plant's primary nitrate reduction pathway functions independently of this enzyme (Somerville & Ogren, 1980). Furthermore, when *Fd-GOGAT* is knocked out, glutamine levels increase while glutamate levels decrease, whereas in *gs2* plants $NH_3$ production increases while glutamine decreases. Both mutants showed decreased levels of RuBisCO with even stronger effects in *fd-gogat* mutants, indicating that the decrease in photosynthetic activity may stem from nitrogen limitation, leading to reduced synthesis of essential photosynthetic enzymes in *Fd-GOGAT* impaired plants (Häusler et al., 1994). To understand the enzymes' roles in heat stress, gene expression analysis was performed under elevated temperatures, showing that *GS* and *Fd-GOGAT* expression are both upregulated to different extents in such conditions in

tomato. Several *GS* genes showed higher expression than *GOGAT* genes in response to heat stress (L. Liu et al., 2016). Upregulation of the *GS-GOGAT* pathway genes under heat stress suggests that the increased photorespiratory flux leads to more rapid release of ammonia which needs to be degraded due to its toxicity. Overexpression of *GS* and *GOGAT* has been explored to improve nitrogen use efficiency in crops. However, these enzymes are deeply integrated into metabolic networks, and their overexpression can lead to metabolic imbalances, as demonstrated in maize (Cañas et al., 2020). Since said study focused on enhancing 2-OG production for amino acid biosynthesis, no data were provided regarding potential effects under heat stress conditions. Nonetheless, it can be inferred that manipulating this component of the photorespiratory machinery may disrupt metabolic balance, potentially limiting its effectiveness under heat stress conditions.

### *3.4.2 Non-essential photorespiratory genes: knockouts with mild effects on viability but notable impact on productivity and growth*

While the previously mentioned genes are crucial for plant survival, mutations in the remaining enzymes of the photorespiratory pathway tend to have less severe impacts, allowing plants to remain viable despite disruptions. All these mutations are conditional, meaning no abnormal phenotypes are obtained in $CO_2$-enriched conditions but only in photorespiratory conditions.

Glycolate oxidase (GOX) converts glycolate and oxygen into glyoxylate in the peroxisome, with $H_2O_2$ as a byproduct. *gox1-gox2* double mutants, for example, show inhibited photorespiration that negatively affected the rate of photosynthesis and carbon allocation, likely due to glycolate accumulation and subsequent chloroplast dysfunction. These mutants also display early leaf senescence (Dellero et al., 2016). Since GOX converts glycolate and oxygen to glyoxylate and $H_2O_2$, its overexpression could potentially exacerbate oxidative stress under heat stress conditions. However, overexpression of *GOX* in rice has been shown to enhance photosynthetic capacity under high-temperature conditions. While increased $H_2O_2$ and salicylic acid (SA) levels suggest enhanced resistance to photooxidative stress, the underlying mechanism may be more complex than simple antioxidant enzyme activity. These

observed physiological improvements in *GOX*-overexpressing plants might be attributed to the role of $H_2O_2$ and SA as signaling molecules, triggering stress response pathways and promoting plant resilience (Cui et al., 2016).

Growth-deficient phenotypes, notably reduced rosette biomass, were also observed in *Arabidopsis* catalase *cat2* mutants, accompanied by disrupted intracellular redox balance and activated oxidative signaling, particularly under short-day conditions (Queval et al., 2007). In *cat2* lines, photorespiratory ROS, like $H_2O_2$, accumulate due to insufficient catalase activity, intensifying oxidative stress and triggering antioxidant systems (e.g., superoxide dismutase, ascorbate peroxidase, glutathione reductase). Under long-term heat stress, *cat2* mutants exhibit increased $H_2O_2$ accumulation and cell death, while in wild-type plants under the same conditions, *CAT2* levels rise as an adaptive response. Attempts to overexpress *CAT2* in heat-stressed plants, however, revealed minimal differences in mRNA levels between wild-type and overexpression lines, implying that *CAT2* expression is tightly regulated, possibly at the mRNA level, during heat stress (Ono et al., 2021). Another study involved transgenic overexpression of broccoli catalase (BoCAT) in *Arabidopsis*, chosen due to its findings that broccoli with higher catalase activity exhibited improved heat tolerance. This experiment confirmed that transgenic *Arabidopsis* expressing BoCAT showed lower oxidative damage and reduced $H_2O_2$ accumulation under heat stress compared to non-transgenic plants, indicating enhanced cellular protection through catalase activity (Chiang et al., 2014).

Glutamate:glyoxylate aminotransferase (GGAT) catalyzes the transfer of an amino group from glutamate to glyoxylate within the peroxisome, producing glycine and 2-OG. Similarly, serine:glyoxylate aminotransferase (SGAT) transfers an amino group from serine to glyoxylate, forming glycine and hydroxypyruvate. In *Arabidopsis,* mutants for both genes are viable in elevated $CO_2$ conditions; however, *ggat1* mutants display impaired growth and development under ambient conditions, while *sgat1* mutants exhibit lethality (Liepman & Olsen, 2001). *ggat1* mutants also experience a reduction in photosynthetic rates by about 50%, low serine and organic acid levels, and accumulation of glyoxylate, with altered ATP/ADP and NADP/NADPH ratios (Dellero et al., 2016; Wingler et al., 1999). Additionally, *sgat1* mutants accumulate glycine and serine (Wingler et al., 1999). Under heat stress, *GGAT1* gene expression

is known to increase in *Arabidopsis* (Timm et al., 2019). However, studies on SGAT1 activity under elevated temperatures are limited. In lines overexpressing *GGAT1*, levels of serine and glycine were higher than in wild-type plants (Igarashi et al., 2006). Elevated SGAT1 activity has been associated with reduced photosynthetic performance, particularly at higher oxygen concentrations, and changes in amino acid levels. Overexpression lines displayed lower daytime levels of serine and asparagine, suggesting a disruption in nitrogen flow through photorespiration (Modde et al., 2016). No experiments have yet investigated *SGAT1* or *GGAT1* overexpression under heat stress. Given that *SGAT1* overexpression disrupts nitrogen uptake under normal conditions, it is hypothesized that *SGAT1* overexpression may not contribute to heat resilience. Instead, SGAT1 activity appears to require strict regulation to maintain nitrogen balance during stress responses.

Hydroxypyruvate reductase (HPR) functions within the peroxisome, catalyzing the reduction of hydroxypyruvate to glycerate. The peroxisomal enzyme HPR1 is the primary catalyst for this reaction, though it is not essential for photorespiration. *hpr1* mutants exhibit increased hydroxypyruvate levels relative to wild-type plants but display only mild growth impairments under normal conditions, unlike the more severe phenotypes seen in mutants of other core respiratory enzymes. This minimal impact is likely due to the compensatory role of HPR2, a cytosolic enzyme hypothesized to mitigate HPR1 loss, as *hpr2* mutants also accumulate hydroxypyruvate, though to a lesser extent than *hpr1* mutants. The *hpr1-hpr2* double mutant accumulates hydroxypyruvate at extremely high levels (over 106-fold), indicating a severe disruption in photorespiration. This double mutant shows pronounced sensitivity to ambient air and a significant reduction in photosynthetic efficiency. Despite the ability to grow in normal air, the double mutant exhibits notable growth defects compared to *hpr1* single mutants. This finding suggests that the photorespiratory pathway may extend beyond chloroplasts, peroxisomes, and mitochondria to include the cytosol (Timm et al., 2008). *HPR1*, as the primary enzyme, is upregulated in response to heat stress (Timm et al., 2019), while the expression of *HPR2* under these conditions has not been studied. Interestingly, no studies have explored the overexpression of these genes to assess their potential role in enhancing plant resilience under heat stress. Since *HPR1* is naturally upregulated in response to elevated temperature, increased *HPR1* activity could alleviate the accumulation of photorespiratory intermediate

hydroxypyruvate. This process would also contribute to maintaining cellular redox balance within the peroxisome by utilizing NADH as a cofactor in the reduction of hydroxypyruvate.

This section delved into the intricate role of photorespiration in protecting plants during heat stress. Genetic analyses show that mutations in critical photorespiratory enzymes result in conditional lethality or severely compromised growth, indicating that plants are heavily reliant on a functional photorespiratory pathway for survival under normal atmospheric conditions. For example, mutations in *PGLP1* halt photorespiration at its initial step, causing mutant plants to be non-viable outside of $CO_2$-enriched environments, while the disruption of *GLYK* impairs growth at later stages, even with $CO_2$ supplementation. Notably, certain photorespiratory genes respond dynamically to elevated temperatures, with upregulation helping to maintain energy balance and manage intermediate metabolites that could otherwise disrupt cellular redox balance. This adaptive regulation suggests an intrinsic mechanism that allows plants to redirect photorespiratory fluxes under heat stress, enhancing photosynthetic efficiency and reducing ROS production. Moreover, the presence of heightened sensitivity to ambient air and impaired photosynthesis in the double mutant *hpr1-hpr2* reveals that photorespiration relies not only on the peroxisome, mitochondria, and chloroplast, but also involves the cytosol. While experiments have not yet explored the overexpression of some genes, like *HPR1*, to directly enhance resilience under heat, the natural upregulation of such genes suggests a potential strategy for improving tolerance to elevated temperatures (Table 1).

Table 1 | Overview of known photorespiratory genes in *A. thaliana*, detailing their functions, knockout effects, expression changes under heat stress, and the impact of overexpression under heat stress, where available.

| Gene name | Protein product | Protein function | Expression under heat stress | Effect of overexpression in heat stress | Effect of knockout under atmospheric conditions | Species studied |
|---|---|---|---|---|---|---|
| *PGLP1* | PGLP | converts 2-PG to glycolate | ↑ | improved carbon allocation, metabolic stability, increased photosynthesis, primes plants for temperature stress (Timm et al. 2019) | ✕ (Hall et al., 1987; Somerville & Ogren, 1979) | *Arabidopsis thaliana, Hordeum vulgare* |
| *PGLP2* | | | ? | ? | | |
| *GDC** | GDC | converts glycine to serine, releasing $CO_2$ and $NH_3$ | ↔ (Xu & Huang, 2010; Timm et al., 2019) | ? | − (Igamberdiev et al., 2001) | *Hordeum vulgare, Agrostis scabra, Arabidopsis thaliana* |
| *SHMT1* | SHMT | converts glycine and THF into serine and 5,10-$CH_2$-THF | ↔ (Liu et al., 2022; Xu & Huang, 2010; Timm et al., 2019) | ? | ✕ (Voll et al., 2006) | *Arabidopsis thaliana, Solanum lycopersicum, Agrostis scabra* |
| *SHMT2* | | | ? | ? | | |
| *GLYK* | GLYK | phosphorylates glycerate to 3-PGA | ? | ? | ✕ (Boldt et al., 2005) | *Arabidopsis thaliana* |
| *GS** | GS | synthesizes glutamine from glutamate and ammonia using ATP | ↑ (Liu et al., 2016) | ? | ✕ (Wallsgrove et al., 1987) | *Solanum lycopersicum, Hordeum vulgare* |
| *Fd-GOGAT* | GOGAT | converts glutamine and 2-OG to glutamate using ferredoxin | ↑ (Liu et al., 2016) | ? | ✕ (Somerville & Ogren, 1980) | *Solanum lycopersicum* |
| *GOX1* | GOX | oxidizes glycolate to glyoxylate, producing $H_2O_2$ | ? | ? | − (Dellero et al., 2016) | *Arabidopsis thaliana* |
| *GOX2* | | | ? | ? | | |
| *CAT2** | CAT | breaks down $H_2O_2$ into water and oxygen | ↑ | lower oxidative damage and reduced $H_2O_2$ accumulation (Chiang et al., 2014) | − (Queval et al., 2007) | *Arabidopsis thaliana* |
| *GGAT1** | GGAT | catalyzes conversion between glycine and glutamate to serine intermediates | ↑ (Timm et al., 2019) | ? | − (Liepman & Olsen, 2001) | *Arabidopsis thaliana* |

| | | | | | | |
|---|---|---|---|---|---|---|
| *SGAT1** | SGAT | converts glyoxylate and glutamate to glycine and 2-OG | ? | ? | ✕ (Liepman & Olsen, 2001) | *Arabidopsis thaliana* |
| *HPR1* | HPR | reduces hydroxypyruvate to glycerate | ↑ (Timm et al., 2019) | ? | − (Timm et al., 2008) | *Arabidopsis thaliana* |
| *HPR2* | | | ? | ? | | |

Note: An asterisk next to the gene name indicates the presence of isoforms with unknown functions in photorespiration, which are not discussed in this review. (✕) denotes lethality, (−) indicates reduced growth and productivity but viable mutants, and (?) signifies the lack of data. Expression changes are represented as: (↑) for upregulation, (↓) for downregulation, and (↔) for species-specific changes. This table highlights the significant gaps in understanding photorespiratory gene responses to heat stress.

# 4. Discussion

Photorespiration, often considered a wasteful process, has emerged as a critical player in plant response to heat stress. The studies reviewed in this work have shed light on the crucial role of photorespiration in mitigating the deleterious effects of elevated temperatures.

## *Upregulation of specific photorespiratory genes may enhance plant heat stress resilience, though the role of some genes remains unknown*

Studies indicate that overexpressing certain genes can improve plant resilience under heat stress. Genes like *PGLP1, GS/GOGAT, CAT2, GGAT,* and *HPR1* are naturally upregulated in response to heat stress, suggesting a protective role. While overexpression studies on *PGLP1*, *GOX*, and *CAT2* have demonstrated positive effects, upregulating *GS* and *GOGAT* has shown limited benefits, likely due to metabolic imbalances. Further research is needed to assess the effects of overexpressing genes like *GGAT* and *HPR1* under elevated temperatures.

A promising approach is the co-overexpression of *PGLP1* and *GOX*. *PGLP1* is significantly upregulated in wild-type plants under heat stress, enhancing resilience. Combining *GOX* overexpression with *PGLP1* could accelerate the conversion of glycolate to glyoxylate, reducing glycolate buildup that inhibits RuBisCO activity. In maize, *GOX* disruption slows photosynthetic induction, suggesting that GOX activity influences photorespiratory rates and that its bottleneck slows the pathway (Zelitch et al., 2009). Overexpressing *GOX* could alleviate this by boosting photorespiratory flux. During heat stress, excess ATP and NADPH can lead to ROS accumulation and oxidative stress if not utilized efficiently. GOX activity generates $H_2O_2$, whose detoxification via CAT2 consumes energy and prevents ROS buildup. Although $H_2O_2$ can contribute to oxidative stress, its controlled production within the photorespiratory pathway - where *CAT2* is naturally upregulated during heat stress - helps balance ROS levels. If the plant's antioxidative systems can manage the increased ROS, co-overexpression of *PGLP1* and *GOX* could balance energy and ROS levels, improving heat stress resilience. This strategy should be tested in *Arabidopsis* and later in C3 crops like rice, wheat, soybean, and barley. It offers significant potential for agricultural

systems in regions experiencing higher temperatures due to climate change, where such strategies could mitigate declining crop yields.

## *Photorespiration presents several disadvantages, particularly under non-stress conditions, making it a prominent target for re-engineering efforts*

While this review highlights the advantages of photorespiration under heat stress, it is equally important to address its drawbacks. Significant crop losses linked to photorespiration have driven efforts to re-engineer this process. One ambitious approach involves incorporating the more efficient C4 photosynthesis pathway, with its 50% efficiency and higher photosynthetic rates, into rice (Hibberd et al., 2008), though this has yet to yield success. Other strategies aim to reduce oxygenation events or develop more energy-efficient pathways that release photorespiratory $CO_2$ in the chloroplast, increasing $CO_2$ concentrations around RuBisCO (Walker et al., 2016). However, due to the intricate integration of photorespiration with primary metabolic pathways, the majority of these strategies are unlikely to be immediately functional or beneficial for plants (Xin et al., 2015). Additionally, most remain untested under field conditions (Walker et al., 2016). Re-engineering crops based on these concepts requires significant time (Walker et al., 2016), even as climate change accelerates rising atmospheric $CO_2$ levels and temperatures. Shifting research perspectives toward optimizing photorespiration for climate resilience could transform this process from a perceived inefficiency to a key tool for enhancing crop adaptation to environmental challenges.

## *Addressing research gaps in photorespiration to predict plant responses to climate change*

Advancing our understanding of photorespiration and its connections to core metabolic pathways requires addressing several significant knowledge gaps. While recent studies highlight the protective role of photorespiration under heat stress and its potential for improving crop resilience, the specific functions of individual photorespiratory isoforms remain poorly understood. Gene expression profiles of

mutant lines under heat stress conditions are yet to be fully characterized, and overexpression lines for key photorespiratory genes such as *SGAT1*, *GGAT1*, *HPR1*, and *HPR2* are lacking. The effects of overexpressing these genes under heat stress remain unexplored. Similarly, while *GS* and *GOGAT* overexpression has been studied in nitrogen assimilation, their impact on photorespiration and $CO_2$ assimilation under heat stress is unknown. Future research should focus on characterizing all photorespiratory isoforms, testing mutant lines (e.g., *glyk*, *gox*, *sgat*, *hpr2*) under heat stress, and evaluating their effects on plant resilience.

Further challenges include understanding metabolite transport between cellular compartments, as several transporters remain unidentified. Regulatory mechanisms controlling photorespiratory fluxes under fluctuating environmental conditions also require further investigation (Hodges et al., 2016), particularly how these mechanisms vary across plant species and developmental stages. A comprehensive understanding of the interplay between photorespiration and primary metabolic pathways is essential for accurately predicting future $CO_2$ assimilation rates in plants amidst climate change. However, the complexity of these interactions presents significant challenges.

The intricate interconnections between metabolic pathways complicate modeling and interpreting fluxes. Changes in one pathway often affect others through feedback loops, and metabolites such as glycine, serine, and 2-OG participate in multiple pathways, making it difficult to assign their functions to specific processes. Technical limitations also hinder progress. For example, stable isotope methods, which trace labeled substrates through metabolic pathways, are constrained by the limited availability of labeled compounds (De Falco et al., 2022). Isotope tracing offers more specific insights into metabolic fluxes and can help build robust models for predicting plant metabolism under varying environmental conditions. The method allows researchers to track the flow of carbon through metabolic pathways and quantify fluxes in real time, offering insights into the dynamics of photorespiration under different conditions (Hodges et al., 2016). However, improvements in the spatial and temporal resolution, as well as the sensitivity of metabolic flux analysis, particularly at the subcellular level, are still needed (Arunachalam et al., 2023).

Finally, predictive modeling approaches must be refined. Sophisticated models that account for the complexity of metabolic networks are essential for interpreting flux

measurements and predicting metabolic behavior under diverse environmental conditions. Such advancements will enable more accurate assessments of photorespiratory contributions to plant resilience and their broader implications for crop performance.

## *Conclusion*

Considering all the points discussed, photorespiration should not be dismissed as wasteful but recognized as a crucial metabolic pathway that enhances plant resilience, particularly under heat stress. While it does reduce photosynthetic efficiency and consume energy under non-stress conditions, photorespiration transforms into a vital protective mechanism during heat stress, helping plants adapt to increasingly challenging environments. Eliminating this process would be counterproductive, especially as climate change drives rising temperatures and $CO_2$ levels. By shifting the research focus from minimizing photorespiration to optimizing its protective functions, we can unlock its potential to strengthen plant resilience and secure crop production in a warming world.

# Generative AI Statement

I used ChatGPT to assist with paraphrasing certain sentences in this work. However, all ideas, content, and analysis presented are my own, and the paraphrasing was done to improve clarity and readability while maintaining the original meaning. The final work is entirely my own.

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