REVIEW

Transgenic strategies to improve the thermotolerance of photosynthesis

Amanda P. Cavanagh[1,2] · Donald R. Ort[2,3]

Abstract

Warming driven by the accumulation of greenhouse gases in the atmosphere is irreversible over at least the next century, unless practical technologies are rapidly developed and deployed at scale to remove and sequester carbon dioxide from the atmosphere. Accepting this reality highlights the central importance for crop agriculture to develop adaptation strategies for a warmer future. While nearly all processes in plants are impacted by above optimum temperatures, the impact of heat stress on photosynthetic processes stand out for their centrality. Here, we review transgenic strategies that show promise in improving the high-temperature tolerance of specific subprocesses of photosynthesis and in some cases have already been shown in proof of concept in field experiments to protect yield from high temperature-induced losses. We also highlight other manipulations to photosynthetic processes for which full proof of concept is still lacking but we contend warrant further attention. Warming that has already occurred over the past several decades has had detrimental impacts on crop production in many parts of the world. Declining productivity presages a rapidly developing global crisis in food security particularly in low income countries. Transgenic manipulation of photosynthesis to engineer greater high-temperature resilience holds encouraging promise to help meet this challenge.

Keywords Temperature stress · Rubisco · Photorespiration · Rca · Calvin–Benson cycle · Climate warming

Introduction

Agricultural crop production is threatened by numerous global climate change-associated abiotic stresses, the most pervasive of which is progressive growing season warming. Global mean temperatures have risen by approximately 1 °C since the Industrial Revolution and will increase an additional 1.5 °C over the 2001 average global temperature by mid-century (IPCC 2018). Warming temperatures have already contributed to global yield losses in wheat and maize (Asseng et al. 2015; Lobell and Gourdji 2012; Lobell et al. 2011), and future temperature increases are predicted to further negatively affect global yields of these as well as other key crops, including rice and soybean (Zhao et al. 2017).

These temperature increases have been and will continue to be spatially heterogeneous, with some areas of the world warming more rapidly than others (Ciais et al. 2013; IPCC 2018; Teixeira et al. 2013) and terrestrial temperatures increasing at a faster rate than those over water (IPCC 2018). Areas of greater warming in the Northern Hemisphere will most likely occur between 40 and 60°N, which overlap with major agricultural regions (Bita & Gerats 2013; Teixeira et al. 2013). However, a notable exception is the Great Plains of the central USA—the US corn belt. The midwestern US corn belt is one of the few regions globally that has experienced negligible increase in average annual temperature during the last century. The warming that has occurred has been during the cool portion of the year whereas the summer season has not shown a warming trend, in fact there may be a slight cooling trend (Arritt 2016). The intensive agricultural land use in this region is likely the explanation of this "warming hole" (Alter et al. 2018). The amount of corn harvested annually from this region has increased 400%

✉ Donald R. Ort
   d-ort@illinois.edu

1   School of Life Sciences, University of Essex, Colchester CO4 3SQ, UK

2   Carl R. Woese Institute for Genomic Biology, University of Illinois, Urbana, IL 61801, USA

3   Departments of Plant Biology and Crop Sciences, University of Illinois, Urbana, IL 61801, USA

over the past sixty years (National Agricultural Statistics Service 2016) due to increased acreage under cultivation, along with increased planting densities with more vigorous plants. The increase in photosynthesis that this regional intensification represents is accompanied by a proportional increase in water evaporation from the crop canopy (i.e., transpiration) causing surface cooling. The subsequent condensation of this water vapor at the top of the troposphere pumps heat away from surface of the earth where crops are and is consistent with the increase in growing season precipitation that region has experienced. Eastern China, another area of extensive intensification of agriculture, also has not experienced the summer warming characteristic of nearly all other terrestrial regions of similar latitude. This mitigation of warming in the US corn belt and eastern China may not last if intensification of these crops plateaus and greenhouse gas emissions do not abate.

Heat stress will likely have its most detrimental outcomes in lower income countries (Deryng et al. 2014). The African continent warrants special attention as many of its countries are already food insecure. Additionally, with > 75% of its population below the age of 35, the demographics portend that by mid-century 40% of all the children born globally will be African (UN Department of Economic and Social Affairs Population Facts Dec 2018 No. 2019/6) demanding greater output from the African food and agricultural system. The year 2021 was the third warmest year on record for Africa, which has been warming faster than the global average, at a rate of 0.3 °C per decade for the last three decades. In 2021, the temperature across North Africa was 1.2 °C and across West Africa 0.9 °C above the 1981–2010 average (State of the Climate of Africa 2021). In addition to the rising average annual temperatures, Africa is also experiencing a rise in the frequency of extremely hot days (State of the Climate of Africa 2021) the hallmark of increasing frequency, intensity, and duration of heat waves (Coumou & Robinson 2013; Meehl & Tebaldi 2004; Wang et al. 2015). For the decade beginning in 1980, there were 24 days in which the mean temperature was in the warmest 1% of record range, whereas in the last ten years there were 109 days in the warmest 1% (State of the Climate of Africa 2021). Increased temperature is a leading cause of the 34% decrease in agricultural productivity growth in Africa since 1961. Whereas, the growth in productivity has stagnated globally this decrease in Africa is greater than any other region and is expected to decline further in future. It is projected that additional warming of 1.5 °C would drive nearly a 10% decrease in maize productivity in West Africa and a 20–60% in the wheat harvest in Southern and North Africa. Perhaps no region in the world has been affected as much

as the African Sahel1 region, [1] which is experiencing rapid 2.8% per year population growth in an environment with depleted natural resources and where temperature increases are projected to be 1.5 times higher than in the rest of the world (State of the Climate in Africa 2019). Agriculture is critical to Africa's economy, accounting for the majority of lifework across the continent where IPCC projections suggest that warming scenarios portend debilitating effects on crop production and food security. For countries across the Sahel, modeled shifts to mid-century temperatures represent novel climates not currently observed for any crop on the continent, limiting adaptation strategies (Burke et al. 2009). Whereas, millet and sorghum have greater resilience to heat stress conditions than other African crops, 5–8% yield losses are projected even in these crops by mid-century (State of the Climate in Africa 2019).

The recent and ongoing rise in global average temperature near Earth's surface is caused primarily by the accumulation of greenhouse gases in the atmosphere. In 2021 anthropogenic activities released an estimated 10.9 Gt C into the atmosphere, surpassing terrestrial and marine sinks (net uptake of ~ 6.4 Gt C yr^{-1}) and resulting in an estimated increase of 5.2 Gt C in the form of higher CO_2 (i.e., in increase in atmospheric CO_2 concentration by ~ 2.5 ppm; Friedlingstein et al. 2022). This accumulation of CO_2 in the atmosphere drives radiative forcing by absorbing a greater fraction of outgoing radiation such that incoming radiation exceeds outgoing radiation, resulting in tropospheric warming. Apart from CO_2, methane and nitrous oxide are even more potent greenhouse gases than CO_2 (> 30 and 300 times greater radiative forcing potential, respectively). However, the increase in atmospheric concentration is relatively short, 12 years for methane and 114 years for nitrous oxide compared to 300–1,000 years for CO_2 (Forster et al. 2008). The scope of the threat to agriculture is revealed by the fact that atmospheric temperature increase caused by increasing atmospheric CO_2 will not decrease appreciably even if zero carbon emissions is achieved (Solomon et al. 2009). Warming caused by atmospheric CO_2 accumulation is persistent and will remain irreversible on a millennium time scale unless countered by still to be developed negative carbon technologies.

---

[1] The Sahel is a transitional region between the wooded Sudanian savanna to the south and the Sahara to the north. It spans ~6000 km from the Atlantic Ocean on the west coast of Africa to the Red Sea in the east. The Sahel belt from west to east includes parts of northern Senegal, southern Mauritania, central Mali, northern Burkina Faso, the extreme south of Algeria, Niger, the extreme north of Nigeria, Cameroon and Central African Republic, central Chad, central and southern Sudan, the extreme north of South Sudan, Eritrea and Ethiopia. (https://en.wikipedia.org/wiki/Sahel#cite_note-2)

Unless practical technologies can be rapidly developed and deployed at scale to remove and sequester carbon dioxide from the atmosphere, anthropogenic atmospheric warming is irreversible over at least the next 10 generations. Accepting this reality highlights the paramount importance for crop agriculture to develop adaptation strategies for a warmer future. For many crops, high-temperature stress during their reproductive phase results in yield losses more detrimental than those incurred during periods of vegetative heat stress (Hedhly et al. 2009; Hatfield et al. 2011; Jagadish 2020). However, the magnitude of these losses will depend on the timing of heat stress, which may vary on an annual basis. Temperature-sensitive vegetative processes, like photosynthesis, will be impacted by warming throughout the growing season, thus strategies to enhance their resilience could provide broad scope for improvement. There have been numerous authoritative reviews synthesizing the effect of rising temperature on plant carbon metabolism (Dusenge et al. 2019; Ferguson et al. 2021) and crop photosynthetic performance (Slattery and Ort 2019; Moore et al. 2021; Ainsworth and Ort 2010). Concurrently, there is a renewed focus surrounding the potential to engineer photosynthesis for increased yield (recently reviewed in: Long et al. 2015; Bailey-Serres et al. 2019; Burgess et al. 2023; Garcia et al. 2023). Here, we expand these to focus this review on transgenic adaptation strategies for the effects of a warming climate on photosynthesis and photorespiration.

Net photosynthetic carbon assimilation (A_n) in C3 plants is well explained by the proportion of Rubisco in the active state and ribulose bisphosphate (RuBP) regeneration capacity (Farquhar et al. 1980). Under current atmospheric $[CO_2]$ and saturating light, the temperature dependence of photosynthesis is well described by Rubisco biochemistry, although a decline in electron transport rates at high temperatures can also limit CO_2 fixation (Sage and Kubien 2007; Schrader et al. 2004; Busch and Sage 2017). Rubisco is regulated by a heat-labile chaperone protein Rubisco activase (Rca), which is essential for removing inhibitory sugar phosphate inhibitors from the catalytic sites of Rubisco and maintaining photosynthetic carboxylation capacity (Carmo-Silva et al. 2015; Qu et al. 2022). Rubisco is a dual-function enzyme and its substrate specificity declines with increased temperature, which increases the oxygenation, rather than carboxylation of RuBP and promotes CO_2 release via photorespiration (Badger and Andrews 1974; Ogren 1984). Photorespiration salvages one molecule of 3-phosphoglcerate (3-PGA) from two molecules of the oxygenation product 2-phosphoglycolate (2-PG) with the release of one molecule of CO_2 via a series of enzymatic conversions and transport steps spanning the chloroplast, peroxisome, and mitochondria (Peterhansel et al. 2010). The photorespiratory pathway is energetically costly, requiring 3.5 ATP and 2 NADPH equivalents to recover RuBP from 2-PG and 3-PGA and
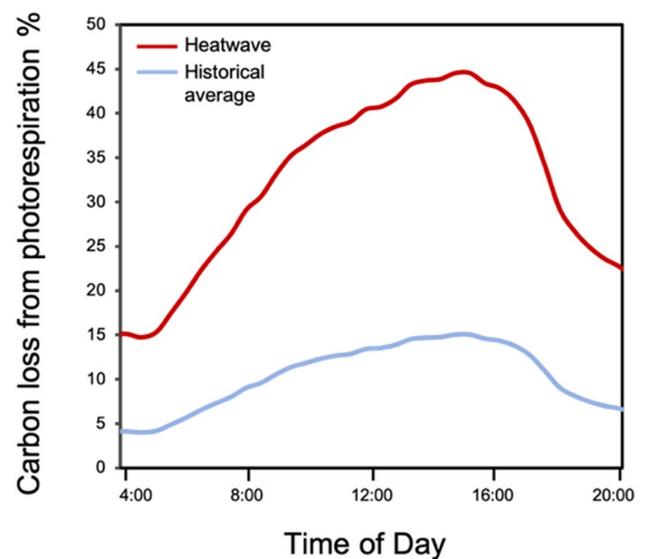
Fig. 1 Modeled losses in daily canopy carbon gain attributed to photorespiration associated with the UK 2022 July heat wave using weather data recorded at NIAB Cambridge on July 19, 2022 (red) compared to the historical average July temperatures (1990–2020; blue) assuming CO_2 concentrations at 400 ppm. Model parameters and assumptions follow Walker et al. 2016

releases NH_3 and 25% of the previously fixed CO_2 in themitochondria. This can result in a dramatic yield drag in C3 crops, with modeled wheat yield losses of 20% across the USA annually and yield reductions of 50% or more observed in warmer growing regions (Walker et al. 2016). Although photorespiratory losses are less pronounced in cool growing regions, modeling the impact of heat waves similar to those experienced in the UK in July 2022 reveals daily carbon losses associated with photorespiration of > 40% for a typical C3 crop (Fig. 1). Models of photorespiratory losses in wheat and soybean under future climate scenarios suggest that yield penalties of 8–20% would persist at atmospheric CO_2 concentrations of 1000 ppm when accompanied by a 3.7 °C temperature increase across the USA (Walker et al. 2016). As a result of the direct impacts of temperature on Rubisco biochemistry, transgenic strategies aimed at improving Rubisco carboxylation or lowering the cost of photorespiration have been targets for improvement of photosynthetic thermal tolerance.

Improving Rubisco carboxylation above the thermal optima

Improving Rubisco substrate specificity, particularly at higher temperatures, is a long-standing aspiration of photosynthesis research. Direct replacement of a crop Rubisco

with a higher specificity version has been proposed to achieve this (McGrath and Long 2014), but this is complicated by inter-specific assembly incompatibility due to the specialized assembly requirements of Rubisco (Aigner et al. 2017). Despite this, bioengineering efforts have replaced tobacco and potato Rubisco with faster but less CO_2-specific versions of Rubisco from the proteobacterium *Rhodospirillum rubrum* (Whitney and Andrews 2003; Manning et al. 2023) and *Halothiobacillus neapolitanus* (Chen et al. 2022), the cyanobacterium *Synechococcus elongatus* (Lin et al. 2014; Occhialini et al. 2016), the purple bacteria *Rhodobacter sphaeroides* (Gunn et al. 2020), as well as plant Rubiscos from closely related species (Martin-Avila et al. 2020). However, attempts have yet to overcome the growth impairment that results from reduced Rubisco specificity and content compared to the untransformed controls.

Improving Rubisco to enhance photosynthetic carbon assimilation above the thermal optima can also be achieved through means other than direct engineering of its catalytic properties. The nuclear-encoded Rubisco small subunit (*rbcS*) enhances Rubisco CO_2 specificity (Schulz et al. 2022) and has been demonstrated to vary in response to growth temperature in *Arabidopsis* (Cavanagh et al. 2023), highlighting the potential for future engineering efforts to identify a more efficient Rubisco isoform (Mao et al. 2022). Rubisco activation is mediated by Rca, which removes inhibitory sugar phosphates from inactive and inhibited Rubisco. Transgenic rice plants overexpressing a rice isoform of both Rca and *rbcS* maintain higher rates of A_n at elevated (i.e., 36 °C) temperatures, likely due to their increased proportion of active Rubisco (Suganami et al. 2020). Similarly, rice overexpressing *rbcS* and a maize Rca maintain higher rates of A_n at ambient and elevated temperatures showing increases in maximum carboxylation rate (V_{cmax}) at 40 °C, relating to their increased proportion of activated Rubisco (Qu et al. 2021). This increase in photosynthesis at elevated temperatures resulted in a 26% increase in dry weight biomass relative to WT under growth in controlled conditions at 40 °C (Qu et al. 2021). Although these transgenic targets remain to be tested in field settings, rice overexpressing Rubisco was reported to have 16–28% increased yields with improved nitrogen use efficiency in sufficiently fertilized (i.e., > 10 g N m^{-2}) paddy conditions (Yoon et al. 2020). Over the four years of field plantings, yields were highest in seasons with relatively high vegetative growth phase temperatures (Yoon et al 2020), suggesting that co-expressing Rca with Rubisco could drive further improvements under climate warming scenarios.

Improving thermal stability of Rca

Rubisco itself remains active at temperatures above 50 °C, although most isoforms of Rca inactivate around 40 °C (Crafts-Brandner and Salvucci 2000). Thus, the observed in vivo deactivation of Rubisco at higher temperatures can be attributed to the heat sensitivity of Rubisco activase, which in turn leads to photosynthetic inhibition and subsequent yield loss (Carmo-Silva et al. 2015; Qu et al. 2022). *Arabidopsis* plants expressing a thermostable Rca isoform exhibit higher photosynthesis and recovery after a short-term high-temperature exposure (Kumar et al. 2009) and increased biomass and seed yield compared to WT plants (Kumar et al. 2009) or those expressing unaltered isoforms (Kurek et al. 2007) when grown at elevated temperature. Although overexpression of Rca alone can result in lower Rubisco content and impaired growth at ambient temperatures (Fukayama et al. 2012, 2018), overexpressing a thermotolerant isoform from a rice wild relative improved both photosynthesis and yield above a threshold leaf temperature in the range of 38–42 °C (Scafaro et al. 2016, 2018). Given the additive benefit of co-overexpressing Rubisco and Rca discussed above, combining this strategy with a thermostable superior Rca may drive a growth benefit under optimal as well as heat stress conditions.

Many species have multiple isoforms of Rca with differing heat sensitivity (Crafts-Brandner and Salvucci 2000; Crafts-Brandner et al. 1997; Law and Crafts-Brandner 2001; Carmo-Silva et al. 2015; Scafaro et al. 2019; Kim et al. 2021). In wheat, increased temperatures induce the expression of a more thermostable, but less efficient Rca (Scafaro et al. 2019; Degen et al. 2020). This trade-off can be overcome via mutagenesis and is the result of a single amino acid substitution (Degen et al. 2020). Further, the trade-off between high-temperature stability and activity is not present in the highly active CAM isoform found to be 10 °C more thermostable than both rice and Arabidopsis Rca (Shivhare and Mueller-Cajar 2017), indicating that engineering Rca for enhanced performance and thermostability is possible.

Because in C4 plants CO_2 is concentrated around Rubisco in bundle sheath chloroplasts suppressing the oxygenation reaction, the stimulation of photorespiration by elevated temperatures is minimal or absent. As a consequence, photosynthesis of C4 plants generally has a higher temperature optimum than in C3 plants (Sage and Kubien 2007). PEP carboxylase (PEPC) is the primary carboxylase in C4 plants, which fixes bicarbonate rather than CO_2. Thus C4 photosynthesis at low CO_2 levels is co-limited by CO_2 diffusion as well as by the activities of carbonic anhydrase and PEPC. In high CO_2 environments, Rubisco activity and RuBP regeneration become important regulators of C4 photosynthesis

(von Caemmerer & Furbank 2016). At high temperature, electron transport rate and RuBP regeneration have been reported as limitations to C4 photosynthesis (Dwyer et al. 2007; Kubien et al. 2003; Pittermann & Sage 2001; Sage 2002). Using RNAi transgenics to reduce Rca content in the C4 dicot *Flaveria bidentis* confirmed the requirement for Rca, but demonstrated that losses in Rubisco activation state and activity above the thermal optimum are not related to Rca content, indicating that other factors regulate Rubisco activation at high temperatures in C4 species (Hendrickson et al. 2008). Yet another report concluded that heat sensitivity of Rca function is linked with decreased Rubisco activation in C4 grasses (Perdomo et al. 2017). C4 grass species, such as sorghum, have separate α and β genes as do many

other species but in these grass species the Rca-α form is expressed only under heat stress (> 42 °C). Kim et al. (2021) found that the induction profile of Rca-α protein paralleled the recovery profile of both A_n and Rubisco activation from a 42-°C heat treatment. This correlation between Rca-α isoform protein accumulation and the maintenance of Rubisco activation suggests that manipulating the regulation of Rca-α isoform expression could be a promising strategy to increase the temperature tolerance of this group of C4 grasses.
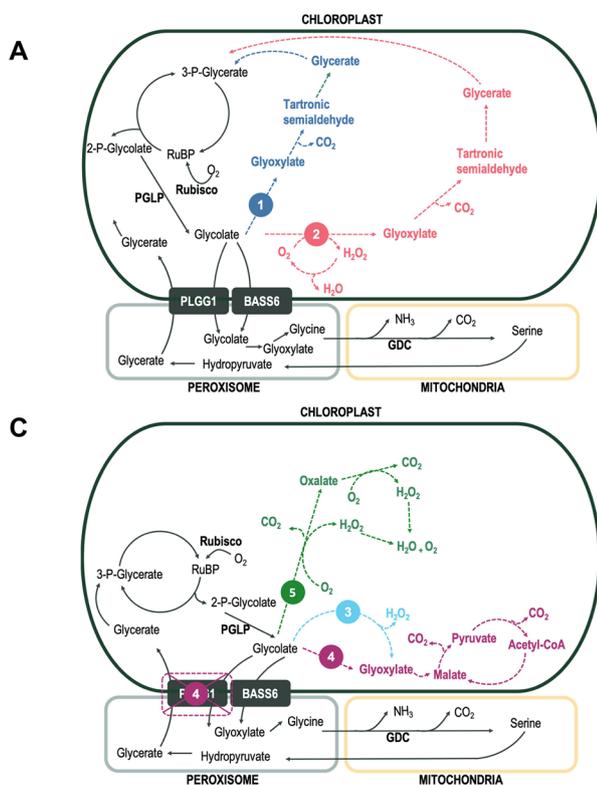
Fig. 2 Chloroplastic photorespiratory bypass pathways tested in plants. **a** Pathway 1 (dark blue) converts glycolate to glycerate using five genes from the *E. coli* glycolate pathway encoding the enzymes glycolate dehydrogenase, glyoxylate carboligase, and tartronic semialdehyde reductase. Pathway 2 (pink) achieves this using *Oryza sativa* glycolate oxidase and *E. coli* catalase (to remove the hydrogen peroxide generated by glycolate oxidase), glyoxylate carboligase, and tartronic semialdehyde reductase. Major native photorespiratory flux through the chloroplast, peroxisome, and mitochondria (gray, solid lines) are also shown, including steps catalyzed by phosphoglycolate phosphatase (PGLP) and the glycine decarboxylase cleavage (GDC) system. **b** Differences in above-ground biomass (solid) and grain yield (dashed) for various species expressing pathway 1 (dark blue) or pathway 2 (pink) relative to unmodified controls. Values greater than 1 indicate an increase relative to WT controls. **c** Glycolate oxidation
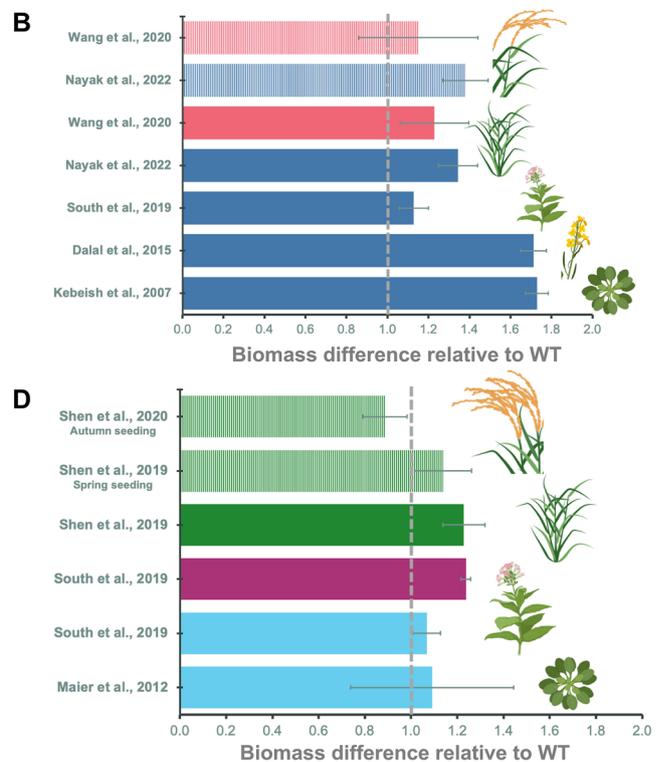
pathways release CO_2 in the chloroplast. Pathway 3 (light blue) introduces genes encoding glycolate oxidase, malate synthase, and catalase. Pathway 4 (purple; AP-3) uses *Chlamydomonas reinhardtii* glycolate dehydrogenase (eliminating the need for catalase) and malate synthase and downregulates the native glycolate-glycerate exchange transporter PLGG1 via RNAi suppression to increase metabolic flux through the alternative metabolic pathway. Pathway 5 (green) uses glycolate oxidase, oxalate oxidase, and catalase for the consecutive decarboxylation of glycolate with production of CO_2. **d** Differences in above-ground biomass (solid) and grain yield (dashed) for various species expressing pathway 3 (light blue), pathway 4 (purple, AP-3), or pathway 5 (green) relative to unmodified controls. Values greater than 1 indicate an increase relative to WT controls. Values reported are aggregate means from original study, and error is the coefficient of variation. Created with BioRender.com

Manipulating photorespiration to improve thermotolerance

To lower the cost of photorespiration, synthetic pathways have been designed to metabolize glycolate and release CO_2 in the chloroplast stroma, where it can be immediately re-fixed by Rubisco (Fig. 2; recently reviewed in: Eisenhut et al. 2019). These "photorespiratory bypass" approaches have primarily relied on transgenic technologies to introduce metabolic pathways in plant chloroplasts to either convert glycolate to glycerate (Fig. 2A-B; Kebeish et al. 2007; Dalal et al. 2015; Wang et al. 2020; Nayak et al. 2022) or fully decarboxylate glycolate to release CO_2 in the chloroplast (Fig. 2C-D; Shen et al. 2019; Maier et al. 2012; South et al. 2019). Expression of glycolate oxidase in the chloroplast intended to fully decarboxylate glycolate releasing CO_2 within the chloroplast has demonstrated a growth and yield advantage in field-grown model and agronomic crops (South et al. 2019; Shen et al. 2019; Wang et al. 2020). South et al. (2019) modified the original glycolate oxidation pathway proposed by Maier et al. (2012) to overexpress *Chlamydomonas reinhardtii* glycolate dehydrogenase to remove the need for catalase and repressed glycolate transport from the chloroplast through suppression of the glycolate/glycerate antiporter PLGG1 in tobacco. This pathway (named AP-3) led to 5–8% increases in the daily integral of photosynthetic carbon assimilation (*A*') and drove 19–37% increases in biomass in field-grown tobacco relative to a WT control (South et al. 2019). Field-grown rice plants overexpressing an alternative full decarboxylation strategy combining chloroplast-localized glycolate oxidase, oxalate oxidase, and catalase (the GOC pathway) again demonstrate a photosynthetic and biomass advantage over WT controls in agriculturally relevant settings (Shen et al. 2019). When the reported diurnal measurements of photosynthesis are converted to *A*' (following: BernacchiI et al. 2006), the transgenic rice plants fixed 15–25% more daily carbon, driving a 14–35% increase in biomass over multiple growing seasons, although grain yield increases were unstable over growing seasons (Shen et al. 2019). Further modification of this pathway in rice to return inputs to the Calvin–Benson cycle displays stable yield enhancement (Wang 2020).

In rice and tobacco, the overexpression of the glycolate oxidation pathways in chloroplasts appear to benefit photosynthesis through increased chloroplast CO_2 concentration, suggesting that synthetic glycolate metabolism could mitigate the impact of warming on photosynthetic carbon fixation and growth at elevated temperatures. This hypothesis was confirmed by comparing the cost of growth at elevated temperature between AP-3 and WT tobacco plants under canopy warming conditions in the field (Cavanagh et al. 2022). Following a short-term shift to elevated temperature, plants with engineered photorespiration maintain higher rates of net assimilation compared to WT controls. Measured over two field plantings, transgenic plants maintained ~ 16% greater *A*' relative to WT plants under heated conditions (i.e., canopy warmed 5 °C relative to ambient). Transgenic plants sustained 19% less biomass loss under heated conditions compared to control plants, having produced 26% more total biomass than WT plants under heated conditions, compared to 11% under ambient conditions (Cavanagh et al. 2022). Overall, this argues against the suggestion that alternative pathways could be maladaptive under adverse conditions (Burgess et al. 2023) and provides strong proof of concept for targeting photorespiration to improve the high-temperature resilience of photosynthesis.

Increasing RuBP regeneration

As temperatures increase and exceed the optimum for photosynthesis, it is predicted that the rate of RuBP regeneration will become limiting (Sage and Kubien 2007). Rising $[CO_2]$ will also shift control away from Rubisco toward RuBP regeneration in C3 crops (Bernacchi et al. 2005; Sage and Kubien 2007). Therefore, transgenic strategies enhancing RuBP regeneration, such as the overexpression of the Calvin Cycle enzyme sedoheptulose-1,7-bisphosphatase (SBPase) (Raines 2003), should confer an advantage to C3 photosynthesis at elevated temperatures, particularly when combined with increased $[CO_2]$ treatments. Transgenic rice overexpressing SBPase maintain higher rates of A_n (measured at 25 °C) than WT and untransformed control plants following a 2-h temperature stress at all temperatures above 35 °C (Feng et al. 2007). Transgenic plants also maintained a higher growth rate over the three days following heat exposure (between 35 and 45 °C) (Feng et al. 2007). A progressive decline in the activation state of both Rubisco and phosphoribulokinase (PRK) was associated with temperature treatments, but SBPase overexpressing lines maintained higher rates of activation of both enzymes at all temperatures above 35 °C. High temperatures also caused Rca to form a greater association with the thylakoid membranes in WT but not transgenic plants (Feng et al. 2007), suggesting an unresolved link between SBPase activity, RuBP regeneration, and Rubisco activation state at elevated temperatures. While these results indicate that overexpression of SBPase could be a transgenic target to improve photosynthetic thermal tolerance, they have not demonstrated a benefit over sustained heat treatment. To explore the full benefit of this manipulation, field-grown transgenic soybean overexpressing the cyanobacterial bifunctional FBP/SBPase was simultaneously exposed to elevated $[CO_2]$ and elevated

temperature (+ 2.7 °C) (Köhler et al. 2016). Transgenic plants maintained higher photosynthetic carbon assimilation, maximal rates of carboxylation, and RuBP regeneration than controls across all treatments. Under ambient [CO_2] (400 ppm) conditions, canopy heating resulted in yield declines across all genotypes. However, under elevated [CO_2] (600 ppm), overexpression of the bifunctional FBP/SBPase was thermoprotective and heated plants maintained the same yield as unheated plants, while WT showed yield reductions of 11–22% (Köhler et al. 2016). Thus, there is strong and continually accumulating evidence that accelerating the rate of RuBP regeneration is a viable strategy for simultaneously adapting C3 photosynthesis to two components of climate change: increasing atmospheric [CO_2] and increasing growing season temperatures. That it appears this adaptation could be achieved by the upregulation of single native genes (e.g., SBPase) indicates the proof of concept achieved by transgenic manipulation might be possible by screening for genetic variation in a natural, wild, or mutagenized populations.

Other Promising targets not yet directly tested

Transgenic overexpression of photorespiration enzymes

Theory suggests that C3 plants overinvest in photorespiration enzymes for optimal growth in current and future atmospheres (Zhu et al. 2007). However, antisense knockdowns of the enzymes involved in the mitochondrial glycine decarboxylase cleavage (GDC) system, which plays a central role in regulating photorespiratory flux (Hagemann and Bauwe 2016; Timm and Bauwe 2013; Fu et al. 2023), result in poor photosynthesis and growth rates in potato (Heineke et al. 2001) and rice (Xu et al. 2009). Conversely, overexpression the GDC-H and GDC-L proteins separately improve photosynthesis and plant growth in Arabidopsis (Timm et al. 2012, 2015; Simkin et al. 2017) and tobacco (López-Calcagno et al. 2019). This effect is perhaps realized through minimizing the inhibitory accumulation of 2-PG in the chloroplast while also boosting RuBP regeneration by increasing the rate of return of 3-PGA to the C3 cycle (Betti et al. 2016; Peterhansel et al. 2013; Flügel et al. 2017).

Boosting the capacity of the photorespiratory pathway will likely drive a benefit under heat stress, when the rate of Rubisco oxygenation increases. *Arabidopsis* overexpressing the stromal protein phosphoglycolate phosphatase (PGLP) maintain higher photosynthetic rates after short- and long-term exposure to a 10-°C temperature increase than do control plants (Timm et al. 2019), supporting the suggestion that a faster photorespiratory metabolism improves thermotolerance. However, no growth impacts are reported for the plants overexpressing phosphoglycolate phosphatase under heat stress. Further, no other single photorespiratory enzyme overexpressing line has been directly tested under heat stress conditions, though field-grown tobacco GDC-H overexpression lines have a greater growth benefit than greenhouse-grown plants (26–47% compared to 13–38%; López-Calcagno et al. 2019), suggesting that this modification can confer resilience under higher photorespiratory conditions in the field (high light and temperatures). Given the increases in thermotolerance conferred by alternative photorespiration (Cavanagh et al. 2022), future experiments are required to know if enhancing photorespiratory capacity can directly boost photosynthetic thermotolerance.

Other photorespiration transgenic targets remain to be explored. For example, catalase mutants have an increased stoichiometry of CO_2 release from photorespiration, due to increased non-enzymatic decarboxylation of pyruvate in the peroxisome (Bao et al. 2021). Although the full contribution of non-enzymatic decarboxylation on photorespiratory CO_2 release remains to be resolved, Bao et al. (2021) do note that the activity of glycolate oxidase increases more rapidly with temperature than that of catalase in *Beta vulgaris*, which could potentially drive non-enzymatic CO_2 degradation at elevated temperatures (Grodzinski and Butt 1976). These findings support preliminary work on variation in photorespiration (Zelitch 1989). Therefore, the transgenic overexpression of peroxisomal catalase, or of a more thermotolerant version of the enzyme, might improve the efficiency of photorespiration under elevated temperatures.

Increasing canopy albedo by reducing leaf chlorophyll level

Enhanced reflectance of incident solar radiation by leaves (i.e., increased albedo) can result in reduced canopy temperature and transpiration, which would be expected to be adaptive by mitigating the increase in temperature, drought, and vapor pressure deficit that global change forecasts. Field experiments with a pale green soybean mutant showed that short wavelength radiative forcing was lower by 4.1 ± 0.6 W m^{-2} during the growing season and by 1 ± 0.1 W m^{-2} across the full year (Genesio et al. 2021). Moreover, modeling by Singarayer et al. (2009) predicted that an increase in canopy albedo of 0.04 could have a regionally and seasonally specific cooling as much as ~1 °C. Thus, designing higher albedo canopies is considered a plausible mitigation strategy for global warming (Genesio et al. 2021).

Because reduced absorption of solar radiation that accompanies increased reflectance (i.e., increased albedo) also lowers leaf temperature, higher albedo canopies should increase net CO_2 assimilation (A_n) by mitigating negative heat stress effects (Ainsworth and Ort 2010) and in turn also improve water use efficiency (WUE). Cooler soybean canopies lower vapor pressure deficit, resulting in higher WUE, which was reported for alfalfa with reduced chlorophyll content compared to the full green control (Estill et al. 1991). Albedo is measured on a scale from zero for a perfectly black surface to 1 for a perfect reflecting white surface. Considerable natural genetic variation in albedo exists within and between important crop species such as wheat, barley, corn, oats, rye, soybean, and sunflower with values varying from 0.02 to 0.1 (Breuer et al. 2003). Differences in albedo can be driven by differing thickness and types of leaf waxes (Uddin and Marshall, 1988), leaf hairiness as well as the arrangement of the leaves in the canopy (Hatfield and Carlson 1979), but differences in chlorophyll content may be the most predictable and least pleiotropic way to engineer albedo (Slattery and Ort 2021). For example, it was shown that under field conditions, a soybean mutant containing half of the chlorophyll of the near isogenic wild type had lower leaf temperatures throughout the growing season (Slattery et al. 2017). It is evident that increasing crop canopy albedo has the potential to be thermoprotective to photosynthesis and productivity at the crop level as well as decrease radiative forcing on a regional scale if higher albedo crops are adopted at large scale. While there is the legitimate worry that reducing absorption of a crop canopy will reduce canopy carbon gain and therefore yield, it has been well documented that this does not happen for select low chlorophyll mutants. Because so much of the light absorbed at the top of dense crop canopies exceeds the capacity of photosynthesis to utilize, leaf chlorophyll can be reduced as much as 70% with no or little penalty on carbon gain or yield (Slattery et al. 2017; Walker et al. 2018). But the direct experimental validation of the hypothesis that lower leaf temperature of low chlorophyll content crops has not yet been accomplished.

Concluding thoughts

Almost every biological process in a plant from phenology to reproduction to water relations is affected by high-temperature stress. As mentioned above, high-temperature stress during the reproductive phase of many crops has the largest impact on season long yield (Hatfield et al. 2011). Integrating the impact of temperature on both carbon metabolism and reproduction is crucial to reveal potential cross-talk between pathways (Ferguson et al. 2021). For example, Siebers et al. (2015) measured reductions in mid-day photosynthesis ranging between 15 and 58% in field-grown soybean

exposed to a 6-°C heat wave when temperatures warmed to above the thermal optimum, regardless of the developmental stage of the crop. However, a 10% reduction in end of season yield was only observed when heat waves were applied during early pod developmental stages. Heat waves imposed during vegetative growth had no lasting impacts on yield, indicating that the yield loss was not only due to direct impacts of the heat waves on photosynthesis but that effects on reproductive process also played a major role. Thomey et al. (2019) extended this work to investigate interactions of high temperature with elevated [CO_2] showing that the stimulation of yield by [CO_2] fertilization was reduced when the temperature optimum of soybean was surpassed, further implicating temperature sensitivity of reproduction in heat-induced yield loss. Thus, it is evident that in order to get the full benefit of improving the resilience of photosynthesis to warming temperatures a commensurate improvement in the resilience of reproduction processes will be needed.

There can be no doubt that adapting crops to higher temperatures is nowhere more important or more urgent than in Africa. Our focus in this review has been on transgenic strategies to improve thermotolerance in crop photosynthesis and this may seem a particularly difficult route toward adaptation in Africa. While that may be true, the development and the recently approved release of Bt-containing Pod Borer-Resistant (PBR) Cowpea for commercial production in Nigeria is a path setting demonstration that transgenic adaptation in Africa may be possible. Moreover, transgenic technologies can also be valuable in providing proof-of-concept research that an adaptation concept is worthwhile before investing in searching for useful genetic variation in a natural or mutagenized populations and then introducing the trait into target food crops by introgressive hybridization.

Declarations

References

Ainsworth EA, Ort DR (2010) How do we improve crop production in a warming world? Plant Physiol 154(2):526–530. https://doi.org/10.1104/pp.110.161349

Aigner H, Wilson RH, Bracher A, Calisse L, Bhat JY, Hartl FU, Hayer-Hartl M (2017) Plant RuBisCo assembly in *E. coli* with five chloroplast chaperones including BSD2. Sci 358(6368):1272–1278. https://doi.org/10.1126/science.aap9221

Alter RE, Douglas HC, Winter JM, Eltahir EAB (2018) Twentieth century regional climate change during summer in the central United States attributed to agricultural intensification. Geophys Res Lett 45:1586–1594

Arritt R (2016) Climate change in the corn belt. CSCAP-0193-2016. Ames, IA: Cropping Systems Coordinated Agricultural Project (CAP): Climate Change, Mitigation, and Adaptation in Corn-based Cropping Systems. https://store.extension.iastate.edu/Product/Climate-Change-in-the-Corn-Belt

Asseng S, Ewert F, Martre P, Rötter RP, Lobell DB, Cammarano D, Kimball BA, Ottman MJ, Wall GW, White JW, Reynolds MP, Alderman PD, Prasad PVV, Aggarwal PK, Anothai J, Basso B, Biernath C, Challinor AJ, De Sanctis G, Doltra J, Fereres E, Garcia-Vila M, Gayler S, Hoogenboom G, Hunt LA, Izaurralde RC, Jabloun M, Jones CD, Kersebaum KC, Koehler AK, Müller C, Naresh Kumar S, Nendel C, O'Leary G, Olesen JE, Palosuo T, Priesack E, Eyshi Rezaei E, Ruane AC, Semenov MA, Shcherbak I, Stöckle C, Stratonovitch P, Streck T, Supit I, Tao F, Thorburn PJ, Waha K, Wang E, Wallach D, Wolf J, Zhao Z, Zhu Y (2015) Rising temperatures reduce global wheat production. Nat Clim Chang 5(2):143–147. https://doi.org/10.1038/nclimate2470

Badger MR, Andrews TJ (1974) Effects of CO_2, O_2 and temperature on a high-affinity form of ribulose diphosphate carboxylase-oxygenase from spinach. Biochem Biophys Res Commun 60(1):204–210. https://doi.org/10.1016/0006-291X(74)90192-2

Bailey-Serres J, Parker JE, Ainsworth EA, Oldroyd GED, Schroeder JI (2019) Genetic strategies for improving crop yields. Nature 575(7781):109–118. https://doi.org/10.1038/s41586-019-1679-0

Bao H, Morency M, Rianti W, Saeheng S, Roje S, Weber APM, Walker BJ (2021) Catalase protects against nonenzymatic decarboxylations during photorespiration in *Arabidopsis thaliana*. Plant Direct 5(12):e366. https://doi.org/10.1002/pld3.366

Bernacchi CJ, Leakey ADB, Heady LE, Morgan PB, Dohleman FG, McGrath JM, Gillespie KM, Wittig VE, Rogers A, Long SP, Ort DR (2006) Hourly and seasonal variation in photosynthesis and stomatal conductance of soybean grown at future CO_2 and ozone concentrations for 3 years under fully open-air field conditions. Plant Cell Environ 29(11):2077–2090. https://doi.org/10.1111/j.1365-3040.2006.01581.x

Bernacchi CJ, Morgan PB, Ort DR, Long SP (2005) The growth of soybean under free air [CO_2] enrichment (FACE) stimulates photosynthesis while decreasing in vivo Rubisco capacity. Planta 220(3):434–446. https://doi.org/10.1007/s00425-004-1320-8

Betti M, Bauwe H, Busch FA, Fernie AR, Keech O, Levey M, Ort DR, Parry MAJ, Sage R, Timm S, Walker B, Weber APM (2016) Manipulating photorespiration to increase plant productivity: recent advances and perspectives for crop improvement. J Exp Bot 67(10):2977–2988. https://doi.org/10.1093/jxb/erw076

Bita CE, Gerats T (2013) Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. Front Plant Sci 4:1–18

Breuer L, Eckhardt K, Frede HG (2003) Plant parameter values for models in temperate climates. Ecol Model 169:237–293

Burgess AJ, Masclaux-Daubresse C, Strittmatter G, Weber APM, Taylor SH, Harbinson J, Yin X, Long S, Paul MJ, Westhoff P, Loreto F, Ceriotti A, Saltenis VLR, Pribil M, Nacry P, Scharff LB, Jensen PE, Muller B, Cohan J-P, Foulkes J, Rogowsky P, Debaeke P, Meyer C, Nelissen H, Inzé D, Klein Lankhorst R, Parry MAJ, Murchie EH, Baekelandt A (2023) Improving crop yield potential: underlying biological processes and future

prospects. Food Energy Secur 12(1):e435. https://doi.org/10.1002/fes3.435

Burke MB, Lobell DB, Guarino L (2009) Shifts in African crop climates by 2050, and the implications for crop improvement and genetic resources conservation. Glob Environ Chang 19(3):317–325. https://doi.org/10.1016/j.gloenvcha.2009.04.003

Busch FA, Sage RF (2017) The sensitivity of photosynthesis to O_2 and CO_2 concentration identifies strong Rubisco control above the thermal optimum. New Phytol 213(3):1036–1051. https://doi.org/10.1111/nph.14258

Carmo-Silva E, Scales JC, Madgwick PJ, Parry MAJ (2015) Optimizing Rubisco and its regulation for greater resource use efficiency. Plant Cell Environ 38(9):1817–1832. https://doi.org/10.1111/pce.12425

Cavanagh AP, Slattery R, Kubien DS (2023) Temperature-induced changes in Arabidopsis Rubisco activity and isoform expression. J Exp Bot 74(2):651–663. https://doi.org/10.1093/jxb/erac379

Cavanagh AP, South PF, Bernacchi CJ, Ort DR (2022) Alternative pathway to photorespiration protects growth and productivity at elevated temperatures in a model crop. Plant Biotechnol J 20(4):711–721. https://doi.org/10.1111/pbi.13750

Ciais P, Sabine C, Bala G, Bopp L, Brovkin V, Canadell J, … Thornton P. 2013. Carbon and other biogeochemical cycles. In *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. (eds T.F. Stocker, D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, … P.M. Midgley), pp. 465–570. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Chen T, Riaz S, Davey P, Zhao Z, Sun Y, Dykes GF, Zhou F, Hartwell J, Lawson T, Nixon PJ, Lin Y, Liu L-N (2022) Producing fast and active Rubisco in tobacco to enhance photosynthesis. Plant Cell 35(2):795–807. https://doi.org/10.1093/plcell/koac348

Coumou D, Robinson A (2013) Historic and future increase in the global land area affected by monthly heat extremes. Environ Res Lett 8:034018

Crafts-Brandner SJ, Salvucci ME (2000) Rubisco activase constrains the photosynthetic potential of leaves at high temperature and CO_2. Proc Natl Acad Sci USA 97(24):13430–13435. https://doi.org/10.1073/pnas.230451497

Crafts-Brandner SJ, van de Loo FJ, Salvucci ME (1997) The two forms of Ribulose-1,5-bisphosphate carboxylase/oxygenase activase differ in sensitivity to elevated temperature. Plant Physiol 114(2):439–444. https://doi.org/10.1104/pp.114.2.439

Dalal J, Lopez H, Vasani NB, Hu Z, Swift JE, Yalamanchili R, Dvora M, Lin X, Xie D, Qu R, Sederoff HW (2015) A photorespiratory bypass increases plant growth and seed yield in biofuel crop *Camelina sativa*. Biotechnol Biofuels 8(1):175. https://doi.org/10.1186/s13068-015-0357-1

Degen GE, Worrall D, Carmo-Silva E (2020) An isoleucine residue acts as a thermal and regulatory switch in wheat Rubisco activase. Plant J 103(2):742–751. https://doi.org/10.1111/tpj.14766

Deryng D, Conway D, Ramankutty N, Price J, Warren R (2014) Global crop yield response to extreme heat stress under multiple climate change futures. Environ Res Lett 9:034011

Dusenge ME, Duarte AG, Way DA (2019) Plant carbon metabolism and climate change: elevated CO_2 and temperature impacts on photosynthesis, photorespiration and respiration. New Phytol 221(1):32–49. https://doi.org/10.1111/nph.15283

Dwyer SA, Ghannoum O, Nicotra A, von Caemmerer S (2007) High temperature acclimation of C4 photosynthesis is linked to changes in photosynthetic biochemistry. Plant, Cell Environ 30:53–66

Eisenhut M, Roell M-S, Weber APM (2019) Mechanistic understanding of photorespiration paves the way to a new green revolution.

New Phytol 223(4):1762–1769. https://doi.org/10.1111/nph.15872

Estill K, Delaney RH, Smith WK, Ditterline RL (1991) Water relations and productivity of alfalfa leaf chlorophyll variants. Crop Sci 31:1229–1233

Farquhar GD, von Caemmerer S, Berry JA (1980) A biochemical model of photosynthetic CO_2 assimilation in leaves of C3 species. Planta 149:78–90

Feng L, Wang K, Li Y, Tan Y, Kong J, Li H, Li Y, Zhu Y (2007) Overexpression of SBPase enhances photosynthesis against high temperature stress in transgenic rice plants. Plant Cell Rep 26(9):1635–1646. https://doi.org/10.1007/s00299-006-0299-y

Ferguson JN, Tidy AC, Murchie EH, Wilson ZA (2021) The potential of resilient carbon dynamics for stabilizing crop reproductive development and productivity during heat stress. Plant Cell Environ 44(7):2066–2089. https://doi.org/10.1111/pce.14015

Flügel F, Timm S, Arrivault S, Florian A, Stitt M, Fernie AR, Bauwe H (2017) The Photorespiratory metabolite 2-phosphoglycolate regulates photosynthesis and starch accumulation in Arabidopsis. Plant Cell 29(10):2537–2551. https://doi.org/10.1105/tpc.17.00256

Forster P, Ramaswamy V, Artaxo P, Berntsen T, Betts R, Fahey D, Haywood J, Lean J, Lowe D, Myhre G, Nganga J, Prinn R, Raga G, Schulz M, Van Dorland R (2008) Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the IPCC, S. Solomon et al. (eds.), Cambridge University Press, Cambridge, UK, Chapter 2

Friedlingstein P, O'Sullivan M, Jones MW, Andrew RM, Gregor L, Hauck J, Le Quéré C, Luijkx IT, Olsen A, Peters GP, Peters W, Pongratz J, Schwingshackl C, Sitch S, Canadell JG, Ciais P, Jackson RB, Alin SR, Alkama R, Arneth A, Arora VK, Bates NR, Becker M, Bellouin N, Bittig HC, Bopp L, Chevallier F, Chini LP, Cronin M, Evans W, Falk S, Feely RA, Gasser T, Gehlen M, Gkritzalis T, Gloege L, Grassi G, Gruber N, Gürses Ö, Harris I, Hefner M, Houghton RA, Hurtt GC, Iida Y, Ilyina T, Jain AK, Jersild A, Kadono K, Kato E, Kennedy D, Klein Goldewijk K, Knauer J, Korsbakken JI, Landschützer P, Lefèvre N, Lindsay K, Liu J, Liu Z, Marland G, Mayot N, McGrath MJ, Metzl N, Monacci NM, Munro DR, Nakaoka SI, Niwa Y, O'Brien K, Ono T, Palmer PI, Pan N, Pierrot D, Pocock K, Poulter B, Resplandy L, Robertson E, Rödenbeck C, Rodriguez C, Rosan TM, Schwinger J, Séférian R, Shutler JD, Skjelvan I, Steinhoff T, Sun Q, Sutton AJ, Sweeney C, Takao S, Tanhua T, Tans PP, Tian X, Tian H, Tilbrook B, Tsujino H, Tubiello F, van der Werf GR, Walker AP, Wanninkhof R, Whitehead C, Willstrand Wranne A, Wright R, Yuan W, Yue C, Yue X, Zaehle S, Zeng J, Zheng B (2022) Global Carbon Budget 2022. Earth Syst Sci Data 14(11):4811–4900. https://doi.org/10.5194/essd-14-4811-2022

Fu X, Gregory LM, Weise SE, Walker BJ (2023) Integrated flux and pool size analysis in plant central metabolism reveals unique roles of glycine and serine during photorespiration. Nat Plants 9(1):169–178. https://doi.org/10.1038/s41477-022-01294-9

Fukayama H, Mizumoto A, Ueguchi C, Katsunuma J, Morita R, Sasayama D, Hatanaka T, Azuma T (2018) Expression level of Rubisco activase negatively correlates with Rubisco content in transgenic rice. Photosynth Res 137(3):465–474. https://doi.org/10.1007/s11120-018-0525-9

Fukayama H, Ueguchi C, Nishikawa K, Katoh N, Ishikawa C, Masumoto C, Hatanaka T, Misoo S (2012) Overexpression of Rubisco Activase decreases the photosynthetic CO_2 assimilation rate by reducing Rubisco content in rice leaves. Plant Cell Physiol 53(6):976–986

Garcia A, Gaju O, Bowerman AF, Buck SA, Evans JR, Furbank RT, Gilliham M, Millar AH, Pogson BJ, Reynolds MP, Ruan Y-L, Taylor NL, Tyerman SD, Atkin OK (2023) Enhancing crop yields through improvements in the efficiency of photosynthesis

and respiration. New Phytol 237(1):60–77. https://doi.org/10.1111/nph.18545

Genesio L, Bassi R, Migiletta F (2021) Plants with less chlorophyll. A global change perspective. Glob Chang Biol 27:959–967

Grodzinski B, Butt VS (1976) Hydrogen peroxide production and the release of carbon dioxide during glycolate oxidation in leaf peroxisomes. Planta 128(3):225–231. https://doi.org/10.1007/BF00393233

Gunn LH, Martin Avila E, Birch R, Whitney SM (2020) The dependency of red Rubisco on its cognate activase for enhancing plant photosynthesis and growth. Proc Natl Acad Sci USA 117(41):25890–25896. https://doi.org/10.1073/pnas.2011641117

Hagemann M, Bauwe H (2016) Photorespiration and the potential to improve photosynthesis. Curr Opin Chem Biol 35:109–116. https://doi.org/10.1016/j.cbpa.2016.09.014

Hatfield JL, Boote KJ, Kimball BA, Ziska L, Izaurralde RC, Ort DR, Thomson AM, Wolfe D (2011) Climate impacts on agriculture: implications for crop production. Agron J 103:351–370

Hatfield J, Carlson RE (1979) Light quality distributions and spectral albedo of three maize canopies. Agric Meteorol 20:215–226

Hedhly A, Hormaza JI, Herrero M (2009) Global warming and sexual plant reproduction. Trends Plant Sci 14(1):30–36

Heineke D, Bykova N, Gardeström P, Bauwe H (2001) Metabolic response of potato plants to an antisense reduction of the P-protein of glycine decarboxylase. Planta 212(5):880–887. https://doi.org/10.1007/s004250000460

Hendrickson L, Sharwood R, Ludwig M, Whitney SM, Badger MR, von Caemmerer S (2008) The effects of Rubisco activase on C4 photosynthesis and metabolism at high temperature. J Exp Bot 59:1789–1798

IPCC (2018) Summary for policymakers. In: Global warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change,. (eds V. Masson-Delmotte, P. Zhai, H.-O. Pörtner, D. Roberts, J. Skea, P.R. Shukla, … T. Waterfield), World Meteorological Organization, Geneva, Switzerland, 32 pp.

Jagadish SVK (2020) Heat stress during flowering in cereals—effects and adaptation strategies. New Phytol 226:1567–1572

Kebeish R, Niessen M, Thiruveedhi K, Bari R, Hirsch HJ, Rosenkranz R, Stäbler N, Schönfeld B, Kreuzaler F, Peterhänsel C (2007) Chloroplastic photorespiratory bypass increases photosynthesis and biomass production in *Arabidopsis thaliana*. Nat Biotechnol 25(5):593–599. https://doi.org/10.1038/nbt1299

Kim SY, Slattery RA, Ort DR (2021) A role for differential Rubisco activase isoform expression in C4 bioenergy grasses at high temperature. GCB Bioenergy 13(1):211–223. https://doi.org/10.1111/gcbb.12768

Köhler IH, Ruiz-Vera UM, VanLoocke A, Thomey ML, Clemente T, Long SP, Ort DR, Bernacchi CJ (2016) Expression of cyanobacterial FBP/SBPase in soybean prevents yield depression under future climate conditions. J Exp Bot 68(3):715–726. https://doi.org/10.1093/jxb/erw435

Kubien DS, von Caemmerer S, Furbank RT, Sage RF (2003) C4 photosynthesis at low temperature. A study using transgenic plants with reduced amounts of Rubisco. Plant Physiol 132:1577–1585

Kumar A, Li C, Portis AR (2009) *Arabidopsis thaliana* expressing a thermostable chimeric Rubisco activase exhibits enhanced growth and higher rates of photosynthesis at moderately high temperatures. Photosynth Res 100(3):143–153. https://doi.org/10.1007/s11120-009-9438-y

Kurek I, Chang TK, Bertain SM, Madrigal A, Liu L, Lassner MW, Zhu G (2007) Enhanced thermostability of Arabidopsis Rubisco Activase improves photosynthesis and growth rates under moderate

heat stress. Plant Cell 19(10):3230–3241. https://doi.org/10.1105/tpc.107.054171

Law RD, Crafts-Brandner SJ (2001) High temperature stress increases the expression of wheat leaf Ribulose-1,5-Bisphosphate Carboxylase/oxygenase Activase Protein. Arch Biochem Biophys 386(2):261–267. https://doi.org/10.1006/abbi.2000.2225

Lin MT, Occhialini A, Andralojc PJ, Parry MAJ, Hanson MR (2014) A faster Rubisco with potential to increase photosynthesis in crops. Nature 513(7519):547–550. https://doi.org/10.1038/nature13776

Lobell DB, Gourdji SM (2012) The influence of climate change on global crop productivity. Plant Physiol 160:1686–1697

Lobell DB, Schlenker W, Costa-Roberts J (2011) Climate trends and global crop production since 1980. Science 333:616–62

Long SP, Marshall-Colon A, Zhu X-G (2015) Meeting the global food demand of the future by engineering crop photosynthesis and yield potential. Cell 161(1):56–66. https://doi.org/10.1016/j.cell.2015.03.019

López-Calcagno PE, Fisk S, Brown KL, Bull SE, South PF, Raines CA (2019) Overexpressing the H-protein of the glycine cleavage system increases biomass yield in glasshouse and field-grown transgenic tobacco plants. Plant Biotechnol J 17(1):141–151. https://doi.org/10.1111/pbi.12953

Maier A, Fahnenstich H, von Caemmerer S, Engqvist M, Weber A, Flügge U-I, Maurino V (2012) Transgenic introduction of a glycolate oxidative cycle into *A. thaliana* chloroplasts leads to growth improvement. Front Plant Sci. https://doi.org/10.3389/fpls.2012.00038

Manning T, Birch R, Stevenson T, Nugent G, Whitney S (2023) Bacterial Form II Rubisco can support wild-type growth and productivity in *Solanum tuberosum* cv. Desiree (potato) under elevated CO2. PNAS Nexus. https://doi.org/10.1093/pnasnexus/pgac305

Mao Y, Catherall E, Díaz-Ramos A, Greiff GRL, Azinas S, Gunn L, McCormick AJ (2022) The small subunit of Rubisco and its potential as an engineering target. J Exp Bot 74(2):543–561. https://doi.org/10.1093/jxb/erac309

Martin-Avila E, Lim Y-L, Birch R, Dirk LMA, Buck S, Rhodes T, Sharwood RE, Kapralov MV, Whitney SM (2020) Modifying plant photosynthesis and growth via simultaneous chloroplast transformation of Rubisco large and small subunits. Plant Cell 32(9):2898–2916. https://doi.org/10.1105/tpc.20.00288

McGrath JM, Long SP (2014) Can the cyanobacterial carbon-concentrating mechanism increase photosynthesis in crop species? A theoretical analysis. Plant Physiol 164(4):2247–2261. https://doi.org/10.1104/pp.113.232611

Meehl GA, Tebaldi C (2004) More intense, more frequent, and longer lasting heat waves in the 21st century. Science 305:994–997

Moore CE, Meacham-Hensold K, Lemonnier P, Slattery RA, Benjamin C, Bernacchi CJ, Lawson T, Cavanagh AP (2021) The effect of increasing temperature on crop photosynthesis: from enzymes to ecosystems. J Exp Bot 72(8):2822–2844. https://doi.org/10.1093/jxb/erab090

Nayak L, Panda D, Dash GK, Lal MK, Swain P, Baig MJ, Kumar A (2022) A chloroplast glycolate catabolic pathway bypassing the endogenous photorespiratory cycle enhances photosynthesis, biomass and yield in rice (*Oryza sativa* L.). Plant Sci 314:103. https://doi.org/10.1016/j.plantsci.2021.111103

National Agricultural Statistics Service (2016) United States Department of Agriculture (USDA), "Quick Stats 2.0". Retrieved from https://quickstats.nass.usda.gov/

Occhialini A, Lin MT, Andralojc PJ, Hanson MR, Parry MAJ (2016) Transgenic tobacco plants with improved cyanobacterial Rubisco expression but no extra assembly factors grow at near wild-type rates if provided with elevated CO2. Plant J 85(1):148–160. https://doi.org/10.1111/tpj.13098

Ogren WL (1984) Photorespiration: pathways, regulation, and modification. Annu Rev Plant Physiol 35(1):415–442. https://doi.org/10.1146/annurev.pp.35.060184.002215

Peterhansel C, Horst I, Niessen M, Blume C, Kebeish R, Kürkcüoglu S, Kreuzaler F 2010. Photorespiration. The Arabidopsis Book 2010 (8)

Peterhansel C, Krause K, Braun H-P, Espie GS, Fernie AR, Hanson DT, Keech O, Maurino VG, Mielewczik M, Sage RF (2013) Engineering photorespiration: current state and future possibilities. Plant Biol 15(4):754–758. https://doi.org/10.1111/j.1438-8677.2012.00681.x

Perdomo JA, Capo-Bauca S, Carmo-Silva E, Galmes J (2017) Rubisco and Rubisco activase play an important role in the biochemical limitations of photosynthesis in rice, wheat, and maize under high temperature and water deficit. Front Plant Sci 8:490

Pittermann J, Sage RF (2001) The response of the high altitude C4 grass *Muhlenbergia montana* (Nutt.) to long- and short-term chilling. J Exp Bot 52:829–838

Population facts (2019) UN Department of Economic and Social Affairs Population Facts Dec 2018 No. 2019/6. Retrieved from: www.un.org › pdf › popfacts

Qu Y, Mueller-Cajar O, Yamori W (2022) Improving plant heat tolerance through modification of Rubisco activase in C3 plants to secure crop yield and food security in a future warming world. J Exp Bot 74(2):591–599. https://doi.org/10.1093/jxb/erac340

Qu Y, Sakoda K, Fukayama H, Kondo E, Suzuki Y, Makino A, Terashima I, Yamori W (2021) Overexpression of both Rubisco and Rubisco activase rescues rice photosynthesis and biomass under heat stress. Plant Cell Environ 44(7):2308–2320. https://doi.org/10.1111/pce.14051

Raines CA (2003) The Calvin cycle revisited. Photosynth Res 75(1):1–10. https://doi.org/10.1023/A:1022421515027

Sage RF (2002) Variation in the k(cat) of Rubisco in C3 and C4 plants and some implications for photosynthetic performance at high and low temperature. J Exp Bot 53(369):609–620

Sage RF, Kubien DS (2007) The temperature response of C3 and C4 photosynthesis. Plant Cell Environ 30(9):1086–1106. https://doi.org/10.1111/j.1365-3040.2007.01682.x

Scafaro AP, Atwell BJ, Muylaert S, Reusel BV, Ruiz GA, Rie JV, Gallé A (2018) A thermotolerant variant of Rubisco Activase From a wild relative improves growth and seed yield in rice under heat stress. Front Plant Sci. https://doi.org/10.3389/fpls.2018.01663

Scafaro AP, Bautsoens N, den Boer B, Van Rie J, Gallé A (2019) A conserved sequence from heat-adapted species improves Rubisco Activase thermostability in wheat. Plant Physiol 181(1):43–54. https://doi.org/10.1104/pp.19.00425

Scafaro AP, Gallé A, Van Rie J, Carmo-Silva E, Salvucci ME, Atwell BJ (2016) Heat tolerance in a wild *Oryza* species is attributed to maintenance of Rubisco activation by a thermally stable Rubisco activase ortholog. New Phytol 211(3):899–911. https://doi.org/10.1111/nph.13963

Schrader SM, Wise RR, Wacholtz WF, Ort DR, Sharkey TD (2004) Thylakoid membrane responses to moderately high leaf temperature in Pima cotton. Plant Cell Environ 27(6):725–735. https://doi.org/10.1111/j.1365-3040.2004.01172.x

Schulz L, Guo Z, Zarzycki J, Steinchen W, Schuller JM, Heimerl T, Prinz S, Mueller-Cajar O, Erb TJ, Hochberg GKA (2022) Evolution of increased complexity and specificity at the dawn of form I Rubiscos. Science 378(6616):155–160. https://doi.org/10.1126/science.abq1416

Shen B-R, Wang L-M, Lin X-L, Yao Z, Xu H-W, Zhu C-H, Teng H-Y, Cui L-L, Liu EE, Zhang J-J, He Z-H, Peng X-X (2019) Engineering a new chloroplastic photorespiratory bypass to Increase photosynthetic efficiency and productivity in rice. Mol Plant 12(2):199–214. https://doi.org/10.1016/j.molp.2018.11.013

Shivhare D, Mueller-Cajar O (2017) In vitro characterization of thermostable CAM Rubisco activase reveals a Rubisco interacting surface loop. Plant Physiol 174(3):1505–1516. https://doi.org/10.1104/pp.17.00554

Siebers MH, Yendrek CR, Drag D, Locke AM, Rios Acosta L, Leakey AD, Ainsworth EA, Bernacchi CJ, Ort DR (2015) Heat waves imposed during early pod development in soybean (*Glycine max*) cause significant yield loss despite a rapid recovery from oxidative stress. Glob Chang Biol 21:3114–3125

Simkin AJ, Lopez-Calcagno PE, Davey PA, Headland LR, Lawson T, Timm S, Bauwe H, Raines CA (2017) Simultaneous stimulation of sedoheptulose 1,7-bisphosphatase, fructose 1,6-bisphophate aldolase and the photorespiratory glycine decarboxylase-H protein increases CO_2 assimilation, vegetative biomass and seed yield in Arabidopsis. Plant Biotechnol J 15(7):805–816. https://doi.org/10.1111/pbi.12676

Singarayer JS, Ridgwell A, Irvine P (2009) Assessing the benefits of crop albedo bio-geoengineering. Environ Res Lett 4(4):045110

Slattery RA, Ort DR (2019) Carbon assimilation in crops at high temperatures. Plant Cell Environ 42(10):2750–2758. https://doi.org/10.1111/pce.13572

Slattery RA, Ort DR (2021) Perspectives on improving light energy distribution and light use efficiency in crop canopies. Plant Physiol 185:34–48

Slattery RA, Vanloocke A, Bernacchi CJ, Zhu X-G, Ort DR (2017) Photosynthesis, light use efficiency, and yield of reduced chlorophyll soybean mutants in field conditions. Front Plant Sci 8:549

Solomon S, Plattner GK, Knutti R, Friedlingstein P (2009) Irreversible climate change due to carbon dioxide emissions. Proc Natl Acad Sci USA 106:1704–1709

South PF, Cavanagh AP, Liu HW, Ort DR (2019) Synthetic glycolate metabolism pathways stimulate crop growth and productivity in the field. Science 363(6422):eaat9077. https://doi.org/10.1126/science.aat9077

State of the Climate in Africa (2019) (WMO-No. 1253) Retrieved from: https://library.wmo.int/index.php?lvl=notice_display&id=21778#.Y_PCPrTMLUK

State of the Climate in Africa (2021) (WMO-No.1300) Retrieved from: https://public.wmo.int/en/resources/library/state-of-climate-africa-2021-0

Suganami M, Suzuki Y, Tazoe Y, Yamori W, Makino A (2020) Co-overproducing Rubisco and Rubisco activase enhances photosynthesis in the optimal temperature range in rice. Plant Physiol 185(1):108–119. https://doi.org/10.1093/plphys/kiaa026

Teixeira EI, Fischer G, van Velthuizen H, Walter C, Ewert F (2013) Global hot-spots of heat stress on agricultural crops due to climate change. Agric for Meteorol 170:206–215

Thomey ML, Slattery RA, Köhler I, Bernacchi CJ, Ort DR (2019) Yield response of field-grown soybean exposed to heat waves under current and elevated [CO_2]. Glob Chang Biol 25:4352–4368

Timm S, Bauwe H (2013) The variety of photorespiratory phenotypes—employing the current status for future research directions on photorespiration. Plant Biol 15(4):737–747. https://doi.org/10.1111/j.1438-8677.2012.00691.x

Timm S, Florian A, Arrivault S, Stitt M, Fernie AR, Bauwe H (2012) Glycine decarboxylase controls photosynthesis and plant growth. FEBS Lett 586(20):3692–3697. https://doi.org/10.1016/j.febslet.2012.08.027

Timm S, Wittmiß M, Gamlien S, Ewald R, Florian A, Frank M, Wirtz M, Hell R, Fernie AR, Bauwe H (2015) Mitochondrial dihydrolipoyl dehydrogenase activity shapes photosynthesis and photorespiration of *Arabidopsis thaliana*. Plant Cell 27(7):1968–1984. https://doi.org/10.1105/tpc.15.00105

Timm S, Woitschach F, Heise C, Hagemann M, Bauwe H (2019) Faster removal of 2-phosphoglycolate through photorespiration improves abiotic stress tolerance of Arabidopsis. Plants 8(12):563

Uddin MN, Marshal DR (1988) Variation in epicuticular wax content in wheat. Euphytica 38:3–9

von Caemmerer S, Furbank RT (2016) Strategies for improving C4 photosynthesis. Curr Opin Plant Biol 31:125–134

Walker BJ, Drewry DT, Slattery RA, Vanloocke A, Cho YB, Ort DR (2018) Chlorophyll can be reduced in crop canopies with little penalty to photosynthesis. Plant Physiol 176:1215–1232

Walker BJ, VanLoocke A, Bernacchi CJ, Ort DR (2016) The costs of photorespiration to food production now and in the future. Annu Rev Plant Biol 67(1):107–129. https://doi.org/10.1146/annurev-arplant-043015-111709

Wang L, Huang J, Luo Y, Yao Y, Zhao Z (2015) Changes in extremely hot summers over the global land area under various warming targets. PLoS ONE 10:1–11

Wang L-M, Shen B-R, Li B-D, Zhang C-L, Lin M, Tong P-P, Cui L-L, Zhang Z-S, Peng X-X (2020) A synthetic photorespiratory shortcut enhances photosynthesis to boost biomass and grain yield in rice. Mol Plant 13(12):1802–1815. https://doi.org/10.1016/j.molp.2020.10.007

Whitney SM, Andrews TJ (2003) Photosynthesis and growth of tobacco with a substituted bacterial Rubisco mirror the properties of the introduced Enzyme. Plant Physiol 133(1):287–294. https://doi.org/10.1104/pp.103.026146

Xu H, Zhang J, Zeng J, Jiang L, Liu E, Peng C, He Z, Peng X (2009) Inducible antisense suppression of glycolate oxidase reveals its strong regulation over photosynthesis in rice. J Exp Bot 60(6):1799–1809. https://doi.org/10.1093/jxb/erp056

Yoon D-K, Ishiyama K, Suganami M, Tazoe Y, Watanabe M, Imaruoka S, Ogura M, Ishida H, Suzuki Y, Obara M, Mae T, Makino A (2020) Transgenic rice overproducing Rubisco exhibits increased yields with improved nitrogen-use efficiency in an experimental paddy field. Nature Food 1(2):134–139. https://doi.org/10.1038/s43016-020-0033-x

Zelitch I (1989) Selection and characterization of tobacco plants with novel O2-resistant photosynthesis. Plant Physiol 90(4):1457–1464. https://doi.org/10.1104/pp.90.4.1457

Zhu X-G, de Sturler E, Long SP (2007) Optimizing the distribution of resources between enzymes of carbon metabolism can dramatically increase photosynthetic rate: a numerical simulation using an evolutionary algorithm. Plant Physiol 145(2):513–526. https://doi.org/10.1104/pp.107.103713

Zhao C, Liu B, Piao S, Wang X, Lobell DB, Huang Y, Huang M, Yao Y, Bassu S, Ciais P, Durand J-L, Elliott J, Ewert F, Janssens IA, Li T, Lin E, Liu Q, Martre P, Müller C, Peng S, Peñuelas J, Ruane AC, Wallach D, Wang T, Wu D, Liu Z, Zhu Y, Zhu Z, Asseng S (2017) Temperature increase reduces global yields of major crops in four independent estimates. Proc Nat Acad Sci 114 (35):9326–9331. https://doi.org/10.1073/pnas.1701762114