Shortcutting photorespiration protects potato photosynthesis and tuber yield against heatwave stress.

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Abstract

Over two growing seasons, a chloroplast localized synthetic glycolate metabolic pathway expressed in potato enhanced tuber biomass. We confirmed that this yield benefit did not come at the cost of tuber quality. In 2022 after two early season natural heatwaves, we observed enhanced daily carbon assimilation rates and increased photosynthetic capacity, with transformed plants having up to 23% higher V_{cmax} and 13% higher J_{max} during tuber bulking stages, indicating that transformed plants were better able to withstand growing season heatwaves than untransformed controls. The increases in photosynthetic capacity and potato tuber mass after early season heatwaves was greater than in seasons without heatwaves and presents the AP3 pathway as a promising avenue for yield increases in the face of forecast increased intensity and duration of heatwave events as a result of global warming.

Introduction

Global food insecurity has continued to rise[1] in tandem with plateauing yield increases for key global food crops[2]. The recent coronavirus pandemic further exacerbated global food supply constraints [3] spotlighting the pressing need for improved domestic and subsistence agricultural production of key food crops to ensure local food security for those at greatest risk. Compounding the challenge, climate predictions forecast rising global mean temperatures and increased frequency, duration, and intensity of heatwave events that could be further detrimental to crop yields[4-6]. To sustainably meet food demand, crop yields must increase per unit land area without proportional increase in inputs and greenhouse gas emissions. Improving photosynthesis together with thermal tolerance offers avenues to meet this challenge [7-9].

Photorespiration has been identified as a process that reduces photosynthetic carbon fixation in C3 cereal crops by 20-50%[10] making it a promising target for improving photosynthetic efficiency, heat stress tolerance and crop yield [11-14]. Photosynthetic carbon fixation in plants occurs with the reaction of Ribulose-1,5-bisphosphate (RuBP) and CO_2 catalyzed by the enzyme Rubisco. However, Rubisco can also fix O_2 rather than CO_2 to RuBP, forming phosphoglycolate, which is converted to glycolate initiating the photorespiratory pathway. Photorespiration is energy-expensive and results in a net loss of fixed carbon[15,16]. In dry and hot conditions, RuBP oxygenation occurs more frequently due to Rubisco's higher affinity for O_2 as temperatures increase[17,18]. Thus photorespiratory linked losses in productivity are heat sensitive, and are likely to have greater impact in geographic regions facing climate extremes, which are already among the most food insecure[19].

The introduction of various photorespiratory bypasses has been shown to increase plant biomass in a range of species[11,20-22]. Though most of these have been demonstrated in model plants, the expression of glycolate dehydrogenase in potato by Nölke, et al. [23] and the introduction of cyanobacterial

photorespiratory glycolate catabolism into potato plants by Ahmad, et al. [24], both of which increased plant biomass and photosynthetic performance, demonstrate the potential of this strategy for increasing yield of agronomically important crops. However, their role in conferring thermotolerance has only been explored in model plants[26].

Recently, we engineered an alternative photorespiratory pathway (AP3) that demonstrated enhanced photosynthetic performance and large increases in plant biomass in field experiments in model species *Nicotiana tabacum*[25] and our subsequent field study with elevated temperature treatments, confirmed enhanced thermotolerance in tobacco plants expressing AP3[26]. As such we implemented AP3 described in South, et al. [25] (Fig. 1) into potato (*Solanum tuberosum*). In terms of global production, potato is the most important non-grain crop and the fourth most highly produced crop behind maize, wheat and rice[27] and shares with maize and rice the highest production of calories/land area[28]. It is also likely that species with vegetative storage will show greater potential for yield increase compared with grain crops, given the enhanced carbon sink capacity as well as duration and thus the possibility for increased CO_2 fixation to translate to yield gains. Grain crops are known to suffer a detriment from heatwave events at reproduction[29-31] that may be offset in tuber and storage root crops.

In this study, we hypothesized that field grown potato tuber mass would increase as a result of introduction of AP3, due to reduced photorespiratory carbon losses and increased heatwave thermostability. Over two growing seasons, we tested and carried out single location field trials, which indicated that AP3 increased tuber biomass, in one year driven to the highest yield advantage by thermoprotection to early season heatwave events. The nutritional quality of potato tubers was not impacted.

Results

Production and selection of potato transformants

Solanum tuberosum cv. Desiree was genetically transformed by BTI *Agrobacterium tumefaciens* strain 3C5ZR with kanamycin selection according to Van Eck, et al. [32] at The Boyce Thompson Institute (Cornell University, NY, USA). In this study, we used two versions of alternative pathway constructs (Extended Data Fig. 1). The first, EC36030 (AP3), was transformed to overexpress plant malate synthase (cmMS) and *Chlamydomonas reinhardtii* glycolate dehydrogenase (CrGDH). The introduced genes divert flux from the native pathway to metabolize glycolate in the chloroplast (Fig. 1). The second construct, EC36031 (AP3+RNAi), introduced the same two genes with the addition of an RNA interference (RNAi) module to reduce expression of the glycolate-glycerate transporter (PLGG1) to minimize export of glycolate from the chloroplast into the peroxisome as in the native photorespiratory pathway (Fig. 1). The transformed plantlets were screened with qRT-PCR to confirm expression of AP genes before choosing events for field testing. Three events from each construct with confirmed expression were carried forward for field testing.

Gene Expression confirmed in field-grown plants

From preliminary field trials in 2019 using three events per construct, the best performing event from each construct was selected to enable greater repetition for robust statistical power in subsequent field testing. Best performing events were determined as those with the highest tuber mass per plant (AP3 line 36030-9, and AP3+RNAi line 369031-17) (Extended Data Fig. 2A). Gene expression of MS and GDH was confirmed with qPCR in both events, and reduced PLGG1 expression confirmed in AP3+RNAi event 369031-17 (Extended Data Fig2B-D).

Transgene qRT-PCR expression analysis for plants grown in 2020 and 2022 field trials showed MS and GDH expression for AP3 and AP3+RNAi events (Fig. 2A,B,D&E) and PLGG1 knocked down in the AP3 RNAi events (Fig. 2C&F).

AP3 Pathway increases tuber mass

In the 2019 growing season, three independent AP3 events and three independent AP3+RNAi events were tested against two azygous controls in a randomized complete block (RCB) field experiment where n=3 plots (Extended Data Fig. 3A). AP3 line 36030-9 showed a 23% increase in tuber mass per plant compared with the best-performing control (Cntrl 1) where p=0.04. AP3+RNAi line 36031-17 showed a 7.5% increase (p=0.05) compared with Cntrl 2 (Extended Data Fig. 2A) warranting subsequent field testing of these best-performing lines. In 2020, AP3 event 36030-9 and AP3+RNAi event 36031-17 were grown in an RCB field experiment where n=6 plots (Extended Data Fig. 3B) showing 9.5% increase in tuber mass per plant for AP3 line 36030-9 (p=0.05) compared with best performing control (Cntrl 1) (Fig. 3A). In 2022, the consistently poorest performing control (Cntrl 2) was removed from the experiment allowing an RCB field test where n=8 plots (Extended Data Fig. 4C), showing an increase in tuber mass per plant of 30% (AP3, 36030-9 where p=0.0006) and 14% (AP3+RNAi, 36031-17 where p=0.049) compared with Cntrl 1 (Fig. 3B). In 2020 there were no differences in average mass per tuber (Extended data Fig. 4A) but in 2022 a 14% increase in mass per marketable tuber was apparent in the AP3+RNAi line compared with the control (Extended data Fig. 4B).

Diurnal measurements reveal higher CO_2 assimilation rates in AP3 plants.

Given that no significant increases in photosynthetic capacities, leaf optical or diffusional properties (V_{cmax}, J_{max}, ϕPSII and ϕCO_2, g_m and leaf absorptance) were seen in the 2020 season (Extended Data Fig. 5), and our observation that AP3+RNAi offers thermal protection to modified tobacco plants[26], measurements of diurnal CO_2 assimilation under heat stress became the focus for 2022. We captured two diurnal measurements in two of three observed natural heatwaves, recording higher rates of CO_2 assimilation in the bypass plants over multiple time points (Figs. 4A-B). At 23 DAT (June 14, 2022) during mid-vegetative growth, plants expressing the AP3 pathway showed a 14% greater CO_2 assimilation rate relative to the control at 13:00 and a 20% greater rate at 15:30pm, and AP3+RNAi plants had higher rates ranging from 17-23% between 14:00 and 18:00hrs (Fig. 4A and Extended Data Table 2). Due to

126 variable cloud cover, which violates the requirement for homogeneous light conditions for comparable
127 survey measurements of CO_2 assimilation, no measurements were made in the morning on the first
128 diurnal measurement at 23 DAT (June 14 2022). During diurnal measurements on June 21 at 30 DAT (Fig.
129 4B), the AP3+RNAi line showed 20% higher CO_2 assimilation rates compared with the control at 8:00 and
130 18:00 only (Fig. 4A; Extended data Table 2), with no significant differences observed for the AP3 line.
131 The two measurement days represent not only differences in vegetative development, but also
132 differences in the thermal environment. Calculation of iWUE (intrinsic Water Use Efficiency) as net
133 photosynthetic assimilation/ stomatal conductance (A_n/g_s) for both diurnal measurement days showed
134 no difference between lines at any measured time point (Extended data Fig. 8).

135 The total carbon fixed over the afternoon period (A'), integrated for each diurnal from 13:00-18:00hrs,
136 also reflects the differences between the measurement days, with AP3 lines fixing 18% (AP3) and 19%
137 (AP3+RNAi) more carbon than controls (Fig. 4C), while no differences were observed between any
138 genotypes on June 21 (Fig 4D). These differences are likely driven by temperature, as the relationship
139 between leaf temperature and CO_2 assimilation throughout the afternoon (i.e. 13:00-18:00hrs) is
140 negatively correlated in all lines on June 14 (Extended data Fig. 6A), suggesting that plants were
141 experiencing heat stress. On June 21st there is no relationship during afternoon hours. (Extended data
142 Fig. 6C).

143 **Photosynthetic capacity increases and CO_2 compensation point decreases in AP3 plants during tuber**
144 **bulking**

145 Photosynthetic capacities measured on June 22 (31 DAT) during vegetative growth, showed no change
146 in V_{cmax} or J_{max} (Figs. 5A-B respectively). Later in the season on July 18 (57 DAT) during mid tuber bulking,
147 photosynthetic capacity increased in the transgenic lines with a significant 20% and 23% increase in V_{cmax}
148 in the AP3 and AP3+RNAi lines respectively compared with the control (Fig. 5D). J_{max} increased by 12%

(p=0.05) in the AP3 line and by 13% in AP3+RNAi line (Fig. 5E). While no differences between lines were observed in the CO_2 compensation point (Γ) on June 22 (Fig. 5C), Γ was reduced in both AP3 lines by 8% compared with the control by July 18th (Fig. 5F).

In 2022 no differences were observed in ϕCO_2 or leaf absorptance values between any line (Extended data Fig. 7).

Seasonal temperature and plant growth stages

In 2020, potato plantlets were propagated later than desired due to AHPIS permitting delays and field transplant was delayed until July 1st (Fig. 6A). From transplant through vegetative growth daily temperature ranges remained fairly consistent, with average daily maximum temperatures of 30.6°C ±2°C and minimum temperatures of 19°C ±2.1°C. Through flowering and tuber bulking temperature remained fairly consistent with an average maximum daily temperature of 27.2°C ±3.2°C and minimum 15.9°C ±4°C. During maturation from the onset of leaf senescence through to tuber harvest, temperatures cooled with average highs of 22.7°C ±4.9°C and lows 7.4°C ±4.1°C (Fig 6A). In 2022, potato plantlets were transplanted to the field in late spring on May 23rd (Fig. 6B). From transplant through vegetative growth, plants experienced cooler temperatures at establishment but overall higher daily maximal temperatures during late vegetative growth when compared to vegetative growth in 2020. An unseasonal heatwave event occurred between June 13 through June 16, 2022, with temperatures reaching over 35°C for four consecutive days during vegetative growth, followed by a return to seasonal temperatures for three days before another 35°C day on June 22, 2022 (Fig. 6B). Through flowering and tuber bulking 2022 temperatures were fairly consistent with average highs of 28°C ±2.5°C and lows of 17.8°C ±2.8°C. During maturation in 2022, temperatures cooled with average highs of 26.7°C ±2.9°C and lows 15.5°C ±3.1°C (Fig. 6B), with higher average temperatures during maturation than were experienced in 2020 due to earlier senescence and harvest. In 2020 daily maximum temperatures never

reached above a maximum 33.8°C (July 7th) throughout the entire growing season, yet three early season heatwaves with maximum daily temperature above 35°C were observed in 2022 (Fig. 6C).

No penalty observed in tuber nutritional composition in bypass events with biomass increases

In 2020, while no differences between transgenic and control tubers were observed for starch, protein, amino acids, free sugars, calcium and potassium (Extended Data Table 3), an 8% increase in total dietary fiber (TDF) was apparent in the AP3 line (Fig. 7B), decreased Vitamin C (27%) in the AP3+RNAi line (Fig. 7B), and increased iron in both the AP3 (44%) and AP3+RNAi line (52%) (Fig. 7C). All percentage change values are compared with the best performing Control, Cntrl1. In 2022, no changes in nutritional composition were observed for any assay (Fig. 7D-F).

AP3 alters leaf photorespiratory metabolite pools.

In a growth chamber experiment where potato plants experienced simulated heatwaves mimicking the 2022 early field season growth patterns, mass spectrometric analysis showed increased accumulation of glycolate, glycine, serine, pyruvate, malate (Fig. 8A-E respectively), in leaves of AP3 lines compared with the control, showing altered photorespiratory metabolism. Glyoxylate levels were undectable in any line at analysis resolution.

Discussion

In this study, we have shown that an alternative photorespiratory pathway (AP3) increased tuber mass in a globally important agricultural food crop over two growing seasons. We built on previous work that showed AP3 improved growth and performance of field-grown model crop tobacco under seasonal conditions[25] and in field conditions under elevated heat stress[26]. We engineered the AP3 pathway into potato cv. Desiree, hypothesizing that the introduction of genes to metabolize glycolate in the chloroplast would cause increased leaf photosynthetic rates by reducing the export of glycolate into the

costly native photorespiratory pathway and increase thermotolerance under heat stress, ultimately resulting in potato tuber yield increases.

We demonstrated 9-30% increases in tuber biomass in replicated single location field experiments across two years, and confirmed that this yield benefit does not come at the expense of tuber quality (Fig 7). In 2020 we saw a 9.5% tuber mass per plant increase in AP3 compared with controls (Fig. 3A), but increases in photosynthetic capacity were not detectable (Extended Data Fig. 5). In 2022, we observed a 30% and 14.2% increase in tuber mass per plant in AP3 and AP3 RNAi lines respectively compared with controls (Fig. 3B), and observed temporal increases in both photosynthetic rates (Fig. 4) and photosynthetic capacity (Fig. 5) after early season heatwaves (Fig. 6), which highlights the interplay between photosynthetic metabolism, development, and acclimation to heatwaves with daily max temperature greater than 35°C. The reduction in CO_2 compensation point in AP3 lines during tuber bulking (Fig. 5F) and the altered pool sizes of photorespiratory metabolites in AP3 compared with controls (Fig. 8) indicate the photosynthetic increases and tuber biomass gains are a result of a functioning AP3 pathway diverting flux from native photorespiration, accelerated by heat stress during early growth.

AP3 function increases photosynthetic rates and capacity

The AP3 pathways are predicted to enhance photorespiratory CO_2 refixation in the chloroplast, via the oxidation of glycolate to glyoxylate, which is converted to malate via MS, and subsequently decarboxylated, resulting in the release of two molecules of CO_2 in the chloroplast (South, et al. [25]; Fig 1)[33,34]. CrGDH is a mitochondrial localized enzyme in *Chlamydomonas* where it uses ubiquinone as an electron acceptor. In plant chloroplasts, CrGDH localizes to the thylakoid membrane [25] potentially allowing it access to plastoquinone as an electron receptor, which would capture a significant amount of redox energy contributing to the energetic advantage over the native pathway where this redox energy

is released as heat when peroxisomal glycolate oxidase reduces oxygen, thus, theoretical photosynthetic performance would be improved. Indeed, previous studies have shown AP3 tobacco plants to have altered photorespiratory metabolite profiles and improved photosynthetic performance compared with controls[25,26], including enhanced carboxylation capacity (V_{cmax}), quantum efficiency (ϕCO_2), and ϕPSII under photorespiratory stress. Similarly, in these potato studies, CO_2 compensation points have been shown to reduce in AP3 lines compared with controls due to increased release of CO_2 in the chloroplast. However, the photosynthetic benefit was not consistent in all environments. In the preliminary study of AP3-transformed tobacco from South, et al. [25] maximum carboxylation rates of Rubisco (V_{cmax}) were shown to increase and CO_2 compensation point reduced as hypothesized due to the increased availability of CO_2 as a substrate for Rubisco at the site of carboxylation[25]. However, Cavanagh, et al. [26], while observing lower CO_2 compensation points at leaf temperatures over 40°, observed no increases in $V_{c,max}$ in AP3+RNAi lines, neither in ambient temperature or under imposed heat stress (ranging from 15-45°C)[26] using tobacco plants with the same AP3+RNAi construct design used in South, et al., 2019. Instead, net CO_2 assimilation measured over the diurnal period was shown to increase in transgenic lines compared to control plants in both studies, and this benefit is further enhanced under heat stress[26], indicating that it is likely that cumulative diurnal increases rather than increased V_{cmax}, drove the observed increases in AP3-tobacco biomass compared with controls.

In 2022 we observed enhancements in photosynthetic capacity and reduced CO_2 compensation points in transformed plants during tuber bulking stages (Fig. 5D-F), but no differences in photosynthetic capacity were observed during vegetative growth early in the season (Fig 5A-C). The reduction of CO_2 compensation point in AP3 lines corresponds with the increases in photosynthetic capacity during tuber bulking, supporting the notion that AP3 plants are better able to assimilate more carbon at low CO_2 concentrations after heat stress. A similar heat stress protection is seen by Timm, et al. [35] in *Arabidopsis thaliana* photorespiratory mutants with 2-PG overexpressed, where faster photorespiratory metabolism

reduces Γ under heat stress. The lack of variation between lines in iWUE (Extended Fig. 8), indicates that AP3 benefits are indeed a result of altered photorespiratory pathways rather than physiological changes to stomatal opening.

Further, given that limited tuber sink is known to reduce photosynthetic efficiency via carbohydrate accumulation in the leaves of potato plants[36] the developing tuber likely represents a strong photosynthate sink, which explains the temporal observations of increased V_{cmax} and J_{max} during tuber bulking (Fig 5D-E). These results highlight the importance of increasing crop sink capacity alongside improved crop photosynthetic performance to ensure increased source capacity translates to greater crop yields, particularly in grain crops[37,38]. AP3 pathways may offer a greater advantage to tuber crops over grain crops considering that previous work in grain crops have concluded the yield loss driven by heat stress is a combination of the inhibition of photosynthesis and effects of high temperature directly on reproductive processes[29-31]. Because potato tuber yield does not depend on temperature sensitive reproduction[39] it is likely that the observed tuber mass increases in our study are due almost entirely to AP3 mitigation of impacts of temperature on photosynthetic physiology.

While the observance of increased metabolite pools in AP3 leaves under heat stress suggests that photorespiratory flux is altered by AP3 genes, targeted mechanistic assessment of metabolomic fluxes under different temperature regimes and growth stages as in Timm, et al. [40] would offer greater insight into AP3 function, given that single point in time metabolite analyses do not provide information about metabolic flux[41,42].

AP3 improves temperature acclimation and thermotolerance

Given the delay of the field transplant in 2020, plantlets experienced very different early growth conditions between the two years. Vegetative potato plant growth thrives in cool conditions best supported in the air temperature range of 20–25 °C[43,44], while the optimal range for tuberization is even

lower between 15–20°C[45,46]. High temperatures (28°C) during vegetative growth and early tuber formation have been shown to reduce stolon number and potential tuber sites in cv. Desiree over 5 fold compared to optimal 18°C [47], and reduce tuber mass 10 fold compared with control plants[48]. In our 2020 field testing, plantlet establishment occurred in temperatures above the ideal 20-25°C and temperatures remained consistently above ideal for tuber development and maturation through the mid tuber bulking phase (Fig 6A). This, along with late field transplant, is likely contributed to the overall lower tuber yields in both AP3 lines and controls in 2020 compared with 2022 (Fig. 3).

In 2022 an earlier field transplant facilitated optimal early season cooler temperatures during establishment and early vegetative growth, allowing optimal stolon formation and tuber initiation. In addition, plants experienced significant heat shock three weeks after transplant from June 13-16 (Fig. 6 B-C). Given past observations of increased thermotolerance resulting from AP3 pathways, this heat shock likely enabled the significantly higher diurnal CO_2 assimilation observed in AP3 and AP3+RNAi plants compared with the control measured on June 14th (Daily high of >37°C; Fig. 4 A& C). However, when measured 7 days later, no significant differences in the rate of carbon gain compared to controls were detectable (Fig. 4D). While the second round of diurnal measurements of CO_2 assimilation in 2022 was also carried out under unseasonably high temperatures on June 21 (daily high of 35°C), the leaves measured may have acclimated to a higher thermal environment. On June 21, measurements were made on leaves that had developed under a mean daily high temperature regime of 27°C (i.e. between Jun 3rd-12th), as opposed to the leaves measured on June 14 which developed under a mean daily high temperature regime of 32.2°C (i.e. between June 11th-20th). This distinction likely underpins the differential response, as pre-existing leaves have less capacity to adjust or acclimate to a new growth temperature[49,50]. In potato, temperature acclimation involves a shift of the thermal optimum[51], and indeed potato cultivars acclimated to high growth temperature maintain rates of net photosynthesis similar to those grown at the thermal optimum[52]. Overall, our observations are consistent with the lack

of an observed benefit to A' in AP3+RNAi compared to WT controls in tobacco grown under cool temperatures[26], supporting the hypothesis that modifications to photorespiration will offer photosynthetic thermal protection in high temperature conditions that promote high photorespiratory flux. It is likely that thermotolerance is increased in AP3 lines if introduced CrGDH maintains lower amounts of glycolate in the chloroplast of the AP3 plants. In this case, when glycolate production is high at high temperatures, AP3 is able to keep glycolate below the threshold that causes inhibition of the photosynthetic carbon reduction cycle.

While AP3 confers thermal protection at temperatures over 35°C in tobacco[26], and our study suggests that a glycolate metabolic pathway enhances tuber mass in potato when heatwaves above 35°C occur during vegetative growth (Fig. 6C), more species specific investigation is required into the critical temperature optimum for bypass benefits in potato. Further, while our results suggest that mid/late vegetative growth high temperature stress is mitigated by AP3 pathways, more work is required to determine at which critical seasonal time-points AP3 may have the best advantage.

Tuber nutritional content is unchanged by AP3

To determine if potato tubers from plants with altered photorespiratory metabolism displayed key differences in overall protein content or nutritional quality, in 2020 we examined a suite of tuber nutrients (Extended Data Table 3) observing a large increase in tuber iron content in transformed plants and small differences in vitamin C and total dietary fiber (Fig. 7A-C). A repeat of these three assays in 2022 showed no differences in nutritional content (Fig. 7D-F).

Tuber protein content did not vary between either of the transgenic lines and controls (Extended Data Table 3), though potatoes are low in protein in general. However, potatoes are an important dietary source of vitamin C and vitamin B6, and can also supply micronutrients and dietary fiber, with global potato consumption reaching ~ 35 kg/capita/yr in 2013[53]. Thus, any attempt to increase tuber yield via

biotechnology must also consider the potential impact on nutritional content. AP3 genes caused no decline in tuber nutrient content in 2020 or 2022 (Fig. 7 and Extended data Table 3), yet in 2020 the increase in tuber yield observed in the AP3 event (Fig. 3A) was accompanied by a 48% increase in tuber iron content (Fig. 7C). Given that the large increases in iron content in both AP3 and AP3+RNAi events (48% and 52% respectively) in 2020 (Fig. 7C) were not seen in 2022 (Fig. 7F), the increases may have been due to a nutrient deficit or triggered due to insufficient time for iron accumulation due to a shifted growing season. Studies have shown that yield increases from elevated CO_2 in rice[54], wheat[55] and tobacco[56] were limited in correlation with limited nutrient supply, yet elevated CO_2 improved plant iron content in tomato roots and shoots under iron limited conditions via increased ferric chelate reductase activity, increased root proton secretion for increased iron solubility and the expression of *FER*, *FRO1*, and *IRT,* genes involved in iron uptake[57]. It follows that the hypothesized increased chloroplastic CO_2 availability stimulated by the AP3 pathway, in conjunction with potentially limited iron supply, may have caused a similar alleviation of iron-deficiency induced in potato tubers. Increased CO_2 assimilation has also been shown to increase sucrose accumulation[58-60], in turn stimulating auxin signaled iron deficiency responses via FIT-mediated transcriptional regulation of iron uptake genes in Arabidopsis[61]. Given the demonstrated increase in CO_2 assimilation and photosynthetic capacity in AP3 potatoes in this study, sucrose accumulation in AP3 potato plants may have increased potentially triggering a similar mechanism under iron deficiency, supported by increased tuber sucrose contents observed in transgenic lines compared with controls in 2020 (ANOVA P=0.1) and 2022 (ANOVA P=0.3) (Extended Data Table 3). In our study, soil cores were not taken during 2020 field testing, but soil analysis of 2022 cores showed no iron deficiency (Extended Data Table 4).

Though focused experiments aimed at understanding the relationship between iron uptake and photorespiratory mechanisms fall outside the scope of this study, investigation of the role of AP3 biochemistry in nutrient deficit soils may be important to explore expansion of potato growth into semi-

arid calcareous soils and to support potato iron bio fortification research to address anemia in developing countries[62,63].

Conclusions

The increases in photosynthetic capacity and tuber mass in AP3 potato after early season heatwaves in this study presents the AP3 pathway as a promising avenue for yield increases in the face of a warming planet. Considering the maintenance of tuber quality, our work anticipates that an alternative glycolate photorespiratory bypass may future proof crops, and root storage crops in particular, against not only increased global mean temperatures but also the likely increased intensity and duration of heatwave events forecast to result from global warming.

METHODS

Construct design and genetic transformation of plants.

Solanum tuberosum cv. Desiree was genetically transformed to contain the AP3 photorespiratory pathway outlined in South, et al. [25] with *Chlamydomonas reinhardtii* glycolate dehydrogenase (CrGDH), *Cucurbita maxima* malate synthase (CmMS), and RNAi targeting the glycolate-glycerate transporter *PLGG1* (Fig. 1). Control plants had been through the transformation protocol, but did not contain the transgenic construct. Transformation was performed by BTI *Agrobacterium tumefaciens* strain 3C5ZR with kanamycin selection according to Van Eck, et al. [32]. Plasmids schematics are depicted in Extended Data Fig. 1.

The transformation generated 6 AP3 and 9 AP3+RNAi lines, verified by qPCR to show the presence of the introduced genes and qRT-PCR to determine levels of gene expression. Three independent transformation events of each construct were taken forward for preliminary field testing in 2019.

Plant growth

2019

Three AP3 lines (36030-6, 36030-9, 36030-14), three AP3+RNAi lines (36031-5, 36031-7, 36031-17) and two independent untransformed controls were selected for preliminary field trials testing. For field transplant, plants were propagated from tubers. On May 8th 2019, tubers were quartered, chitted, transplanted to 1 gallon pots in potting soil and kept in growth chamber conditions on a 22°C/16hr light 15°C /8hr dark cycle, 60% RH for 3 weeks. After 3 weeks plants were moved to greenhouse conditions (natural light 22°C day/15°C night) for seven days, then hand transplanted to the field site on June 4th 2019 in a randomized block design using three replicate blocks (Extended Fig. 3A). Each plot contained 10 plants per line per row with 10" spacing between each plant, 36" between rows and 20" between lines in row.

2020

In 2020, the highest yielding AP3 (36030-9) and AP3+RNAi (36031-17) transformation events from 2019 and two controls (Cntrl1 and Cntrl2), were selected for field analysis. Due to uneven plant establishment in 2019, in 2020 plants were propagated from tissue culture plantlets grown *in vitro* rather than from tubers to ensure homogenous establishment. For propagation, plantlets were divided into nodal cuttings sectioned from a single shoot according to Roca, et al. [64] and placed in MS growth media[65]. Cuttings were kept in a walk-in growth chamber at 20°C/16h day, 18°C/8hr night at 50% relative humidity and daytime light intensity of 700 µmol m^{-2} s^{-1}.

At eight weeks after tissue culture division, plantlets were transplanted into peat pots in potting soil, and kept in growth chamber (Conviron) conditions 22°C/14hr day, 18°C/10 hr night at 700 µmol m^{-2} s^{-1} daytime light intensity and 50% RH for 12 days. Five days prior to field planting, plantlets were moved

into the greenhouse for acclimation to diurnal solar conditions (26°C/14hr day, 20°C/10 hr night) (Extended Fig. 9) before being transplanted to the field.

Plants were transplanted to the field on July 1st in a complete randomized block design with 6 replicate plots of each event (Extended Data Fig. 3B). Plants were sown 12 inches apart in a single row of 10 plants per plot with 20 inch spacing between lines in a single row and 36 inches between rows. Throughout growth, plants were hilled (soil mounded to cover lower leaves about 20cm from ground level) to promote tuber growth four times at 10, 25, 40 and 55 days after transplant to field (DAT) (Pictured in Extended Data Fig. 10). Plots were watered as needed between 8:00-09:00hrs through drip irrigation installed either side of each hill (pictured in Extended data Fig. 11).

2021

In 2021, the same plant tissue culture propagation and establishment protocol was followed as in 2020, but the consistently poorer performing control (Cntrl 2) was dropped from the experiment to allow increased repetition, leaving only Cntrl 1. Plantlets were transplanted to the field on June 18th 2021 in a complete randomized block design with 8 replicate plots of each event, AP3 (36030-9), AP3+RNAi (36031-17) and Cntrl 1 (Extended Data Fig. 3C). Each plot was spaced in the same dimensions 2020. At 8 days after field transplant, the experiment was destroyed by a flooding event.

2022

The 2021 protocol and field plan (Extended Data Fig. 3C) was followed to repeat the flooded-out experiment, with plants transplanted to the field on May 23rd, 2022. Mounding took place at 10, 25 and 40 Days after transplant (as in Extended Data Fig. 10). Plots were watered as needed between 8:00-09:00hrs through drip irrigation on both sides of each hill in each plot (pictured in Extended data Fig. 11).

All field trials were carried out at University of Illinois Energy Farm Facility in Urbana, Illinois (40°03'46.4"N 88°12'25.4"W, 215 m above sea level).

Gene expression analysis

In 2019, 100 mg of leaf material was harvested from three plants per plot, flash frozen in liquid nitrogen and stored at −80 °C. RNA was extracted using the Nucleospin RNA kit (Macherey-Nagal GmbH& Co.KG, Düren, Germany). cDNA was generated using the Qiagen Quantinova reverse transcription kit. Gene expression analysis was performed on a Bio-Rad CFX Real-Time PCR system. Relative changes in gene expression were determined using reference genes *L25 and Eif1a* with three technical replicates per biological sample. Amplification was performed using the Bio-Rad SSO advanced SYBR green master mix, and relative levels were determined using the ΔΔCT method.

In 2020 and 2022, at 21 days after transplant, leaf tissue was collected from the last fully expanded leaf of each plant for total RNA extraction. A single hole punch from each plant in a plot, totaling 10 leaf discs, which was approximately 100 mg. Tissue was collected from every plot in the field experiment. The tissue was immediately flash frozen in liquid nitrogen and stored at −80 °C. Total RNA was extracted with the RNeasy Plant Mini Kit (74904: Qiagen) and treated with DNase I (79254: Qiagen) to remove gDNA. Qubit RNA IQ Assay Kit was used, (Q33221) to ensure RNA had sufficient integrity and quality, with an IQ score greater than 9/10 before cDNA synthesis. The RNA was reverse-transcribed using the SuperScript™ III First-Strand Synthesis System (18080051; Invitrogen). An additional non-reverse transcriptase (NRT) control was made for each RNA sample by following the same protocol but replacing the SSIII enzyme with water. The cDNA was then diluted to 5 ng/µL. A qPCR run was conducted to ensure there was minimal genomic DNA contamination by comparing Cq values of synthesized cDNA and the NRT control. Each sample was determined to have a difference in Cq values of at least 5 between its corresponding NRT control and cDNA.

Real time quantitative PCR (RT-qPCR) assays were conducted on the CFX Connect™ Real-Time System (Bio-Rad) using SsoAdvanced Universal SYBR® Green Supermix (1725270: Bio-Rad) to detect two reference genes, cyclophilin (cyc) and tubulin *(tub)* and three genes of interest (CrGDH, CmMS and PLGG1). The software qbase+ (Biogazelle) was used to calculate relative gene expression from the Cq values by employing a generalized delta-delta-Ct model according to Hellemans, et al. [66]. Normalized relative quantities (NRQ) were calculated for each sample using the average Cq value for its technical replicates, normalized factors that were calculated based on the reference genes, as well as amplification efficiencies calculated for each primer set.

All primers used in this work are listed in Extended data Table 1.

Photosynthetic Measurements

Diurnal CO_2 assimilation

Diurnal CO_2 assimilation was measured in 2022 only. On June 14th, 2022 and June 21st, 2022 diurnal measurements of photosynthesis began at 8:00hrs until around 18:30hrs, with measurement sets throughout the day commencing at 8:00, 10:00, 11:30, 13:00, 14:00, 15:30, 16:45 and 18:00hrs. On June 14th only data after 13:00hrs is presented due to intermittent morning cloud cover. Snapshot measurements were made on the last fully expanded terminal leaflet on the main stem on three plants per plot (n=8 with 3 subsamples per plot), with Li-6800 (LI-COR, Lincoln NE). Light levels inside the measuring cuvette were set to match ambient using the PPFD sensor on the Li6800. Reference CO_2 was set to match ambient at 420 ppm for all measurements. Between the June 14th and June 21st a new leaflet had emerged and measurements were made on a new last fully expanded leaf on June 21st.

A/Ci response curves

The response of assimilation (*A)* to intercellular CO_2 partial pressure (C_i) was measured on the last fully expanded terminal leaflet on the main stem with Li-6800 in both 2020 and 2022 field experiments. In

both years, curves were made at a saturating light intensity of 1,800 µmol mol^{-2} s^{-1}. Measurements of A began at ambient 400 µmol mol^{-1} CO_2 to create a response curve at the following CO_2 concentrations: 400, 300, 200, 150, 100, 75, 50, 25, 400, 400, 700, 1000, 1200, 1500, 1800, 2000 µmol mol^{-1}. V_{cmax} and J_{max} were calculated using the C_3 photosynthesis biochemical model of Farquhar, et al. [67] using the curve fitting utility from Sharkey, et al. [68] In 2020, curves were made in a single campaign over two days Aug 5-6th (35-36 DAT, early tuber bulking) at 25°C between 8:00-14:00hrs. In 2022 curves were made in two separate campaigns, the first on June 22nd during vegetative growth measured at 28°C and the second on July 18th during mid tuber bulking at 30°C, both between 8:00-14:00hrs. In 2020, curves were measured in 3 plants per plot where n=6 plots (18 subsamples). In 2022 two plants per plot were measured where n=8 plots with 2 samples per plot (16 subsamples).

Γ (CO_2 compensation point) was calculated from the initial slope of A/Ci response curves at Ci values below 200 µmol mol^{-2} s^{-1} as the x intercept. [69]

A/Q response curves

The response of photosynthesis to light (A/Q) was measured in 2020 and 2022. In 2020 A/Q curves were made during the gas exchange campaign Aug 5-6th, after the completion of an A/Ci curve on a given plant, leaves were stabilized at 400 µmol mol^{-1} CO_2 and saturating light intensity 1,800 µmol m^{-2} s^{-1}. The response of A to Q was then measured at the following light levels: 1800, 1500, 1000, 400, 200, 150, 100, 75, 50, 30, 20, 10, 0 µmol m^{-2} s^{-1}. Temperature exchange point was set to 25°C for curve duration.

In 2022 measurements were made in a single campaign over two days, June 28 and 29th (36-37 DAT). A/Q curves were made independently and did not follow A/Ci curves on a given leaf. The last fully expanded terminal leaflet on the main stem was stabilized at 420 µmol mol^{-1} CO_2 and saturating light intensity 1,800 µmol m^{-2} s^{-1} inside the measuring cuvette. A/Q was then measured at the following light levels: 1800, 1500, 1000, 700, 400, 200, 150, 100, 75, 50, 30, 20, 0 µmol m^{-2} s^{-1} with temperature

exchange point was set to 28°C for curve duration. For all A/Q curves in both years, the minimum wait time between logging at a new light level was 180secs. For all curves a saturating pulse was made at each data log to capture chlorophyll fluorescence dynamics.

From A/Q curves the quantum yield of CO_2 fixation (ϕCO_2) was calculated from the slope of the relationship between A/Q below 100 μmol m^{-2} s^{-1} absorbed irradiance. Quantum yield of PSII (ϕPSII) was calculated from the slope of the relationship between ϕPSII/Q below 100 μmol m^{-2} s^{-1} absorbed irradiance. In both years, leaf absorption was calculated on the last fully expanded terminal leaflet on 3 plants per plot, as the average of three measurements per leaf, where measured absorption = 1-(transmittance+reflectance) with a Jaz spectrometer (Ocean Optics).

Intrinsic Water Use Efficicency (iWUE)

iWUE was calculated at the leaf level from diurnal photosynthetic gas exchange measurements as the ratio of net photosynthetic assimilation (A_n) to stomatal conductance (g_s) according to Leakey, et al. [70].

Isotopic gas exchange and mesophyll conductance

Fully-expanded potato leaves, exposed to full light were cut between 05:00-05:30 hrs from 3-13 August 2020 (34-43DAT). Cut leaves were immediately placed in a bucket of water before being re-cut underwater and taken to the lab were they remained in the dark until photosynthesis was measured. Leaves were placed in a growth cabinet (Gen 1000, Conviron) with an irradiance of ~700 μmol m^{-2} s^{-1}, set to 25°C for approximately five minutes before the terminal leaflet was placed in the multiphase flash fluorometer chamber of the LI-6800 (LI-6800 Biosciences, Lincoln, NE, USA) located in the growth cabinet. Leaf temperature was set to 25°C, irradiance 1800 μmol m^{-2} s^{-1}, CO_2 sample 400 μmol mol^{-1}, chamber relative humidity 60% and an [O_2] of either 20.9 kPa or 1.99 kPa. Leaves acclimated in the chamber for between 20 and 35 minutes before photosynthesis stabilized. Photosynthesis and

mesophyll conductance were estimated under steady-state conditions for approximately 30 minutes before the [O_2] was changed and the leaf was allowed to acclimate for 10 to 15 minutes to the other [O_2] before photosynthesis and mesophyll conductance were measured for approximately 30 minutes. The order of the [O_2] was random. The LI-6800 was coupled to a tunable diode laser absorption spectroscope (TDL—model TGA 200A, Campbell Scientific) that measures $^{12}CO_2$ and $^{13}CO_2$[71]. The TDL setup and calibration are previously described in Tazoe et al. 2009,[72]. The TDL measured each site for 20 s. The LI-6800 was set to auto log every 180 s, once per TDL measurement cycle. The TDL was connected to the LI-6800 reference and sample using the reference and sample ports on the back of the LI-6800 head, respectively. Photosynthetic discrimination ($\Delta^{13}C_{obs}$) and mesophyll conductance (g_m) including the ternary effect[73] were calculated according to Evans et al.[74] and Evans and von Caemmerer[75].

Tuber Harvest

All tuber harvests were conducted by hand in a single day. Above ground biomass was removed, tubers were dug up and separated from roots, cleaned, counted and weighed on a per plot basis (Extended Fig. 12). In 2019, harvest took place on Oct 9th 2019. In 2020, harvest took place on October 15th. In 2019 and 2020 tubers <2g were not included in tuber biomass totals. In 2022 harvest took place on September 14th and tubers below <5g were counted and weighed and subtracted from the total per plot to give total marketable tuber mass per plot.

Tuber Nutritional content

In 2020, 3 subsamples from each plot were analyzed for nutrient content, n= 6 with 3 subsamples per repetition per genotype. Immediately after tuber harvest, 500g of tubers between 55-65g each were selected for each subsample (1.5kg per plot). Unpeeled tubers were washed, dried and ground. Unpeeled tuber dry matter (DM) and organic matter (OM)/ash were determined according to AOAC official methods 934.01, (2006) and 942.05 (2006) respectively. Total starch was determined according to AOAC Official Method 979.10 (2006) and total dietary fiber (TDF) following AOAC Official Method

985.29 (2006). Free Sugars were quantified according to Campbell, et al. [76]. Total crude protein (CP), Minerals and Amino acids were analyzed at Agricultural Experiment Station Chemical Lab (University of Missouri-Columbia) all according to 2006 AOAC Official Methods as follows: AOAC method 990.03 for Crude Protein by combustion, 985.01 (a,b,d) for minerals Calcium and Potassium and Iron using inductively coupled plasma (ICP) atomic emission spectroscopy, 982.30 E(a,b,c) for amino acids and 988.15 for Tryptophan by alkaline hydrolysis. Vitamin C and B6 were analyzed at Eurofins according to AOAC Official Method 967.22, 2006 for Vitamin C and J. AOAC, 88:30-37, 2005 for Vitamin B6.

From 2020 analysis, significant differences were observed between transformed plants and controls for TDF, Iron and Vitamin C. As such these three assays were repeated from 2022 grown tubers, from 2 subsamples per plot (n=8, 16 subsamples per line), following the same protocol used in 2020.

Soil composition Analysis

Soil analysis was completed fee for service by KSI Laboratories (Shelbyville, IL, USA), on samples taken on June 23, 2022, at 32 DAT. Four soil cores were extracted from various positions in every plot and combined prior to analysis. pH tests were performed with electrode readings in 10g of soil and 10ml of distilled water and buffer pH tested using the Sikora buffer method[77]. Phosphorus, potassium, calcium, magnesium, sulfur, boron, zinc, copper, iron, manganese, sodium were determined using ICP spectroscopy, using Mehlich 3 extraction[78]. Organic Matter was determined colormetrically[79].

Leaf Photorespiratory Metabolite Analysis

To assess the response of leaf photorespiratory metabolite pool sizes, plants from AP3 line 36030-9, AP3+RNAi line 36031-17, and control 1 were grown in growth chamber conditions where n=8 plants per line. Chamber conditions were set to simulate field temperature and humidity conditions observed during 2022 field growing season, including two early season heat waves (Chamber settings in Extended

Data Table 5). In heat wave 1, plants were exposed to daily highs of 37°C over four days, and in heat wave 2, to daily highs of 37°C over three days.

Mass spectrometry

~100 mg of fresh leaf tissue was harvested from the last fully expanded terminal leaflet from each plant in mid-afternoon (between 15:00—16:00hrs) on day four of simulated heat wave 1. Tissue was immediately flash frozen in liquid nitrogen. Metabolite analysis was performed by the Carver Metabolomics Core, University of Illinois at Urbana-Champaign Roy J. Carver Biotechnology Center. Metabolites were analyzed using a GC-MS system (Agilent) consisting of an Agilent 7890 gas chromatograph, an Agilent 5975 mass selective detector, and a HP 7683B autosampler. Gas chromatography was performed on a ZB-5MS capillary column (60-m × 0.32-mm i.d. and 0.25-µm film thickness; Phenomenex). The inlet and MS interface temperatures were 250°C, and the ion source temperature was adjusted to 230°C. An aliquot of 1 µL was injected with the split ratio of 10:1. The helium carrier gas was kept at a constant flow rate of 2 mL/min. The temperature program was a 5-min isothermal heating at 70°C, followed by an oven temperature increase of 5°C min−1 to 310°C and a final 10 min at 310°C. The mass spectrometer was operated in positive electron impact mode at 69.9 eV ionization energy at m/z 30 to 800 scan range. To allow comparison among samples, all data were normalized to the internal standard in each chromatogram and the sample wet weight. The spectra of all chromatogram peaks were evaluated using the AMDIS 2.71 program (NIST).

Statistical Analysis

Field experiments were analyzed from a complete randomized block design with a linear model ANOVA where $y = \mu + Block + Line + \varepsilon$ with α=0.05. All data were separated by year. Line was considered as a fixed effect and Block was analyzed as a random effect.

For qPCR in n=6 in 2020 , n=8 in 2022 with 3 technical reps per qPCR run using two tailed T-Tests against the Control (AP3), to compare gene expression of each transgenic against the control independently. For photosynthetic A/Ci and A/Q curves, in 2020, n= 6 with 3 subsamples per plot. In 2022 n=8 with 2 subsamples per plot. For diurnal photosynthetic measurements n=3 with 3 subsamples per plot and significance was determined with the ANOVA on the linear model and post hoc Dunnett's test against the control. Harvested tubers were analyzed according to the RCB linear model with ANOVA and post hoc Duncan test. For tuber nutrient analysis in 2020 n=6 with 3 subsamples per plot and in 2022 n=8 with 2 subsamples per plot and significance determined by linear model with ANOVA and post hoc Duncan tests. No block effects were observed in any measurements.

Leaf photorespiratory metabolites were analyzed from a growth chamber experiment where n=8 plants per line and statistical significance determined with a one-way ANOVA and post-hoc Dunnett's test where α=0.05.Statistical analysis was performed in R (version 4.2.1 https://www.R-project.org/).

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Author Contributions

K.M-H. and D.R.O wrote the manuscript. D.R.O supervised the research. P.F.S. designed the AP3 constructs. A.P.C. and P.F.S. carried out molecular characterization of transformed material and designed and analyzed 2019 experiments. A.P.C. led and carried out 2019 field work, analyzed 2019 harvest data, and assisted with manuscript preparation and analysis of Γ. N.F. helped optimize, generate and analyze 2019 qPCR data. K. M-H designed all 2020, 2021 and 2022 experiments and carried out and analyzed all field work, aided by J.F., P.S. and J.L.

R.B supervised 2020 field work and carried out and analyzed gas exchange data, with the help of M.L. and S.S. S.S. collected and analyzed isotopic gas exchange and mesophyll conductance measurements. S.B, J.F and P.S. carried out all gene expression lab work including qPCR optimization and data synthesis, with assistance from J.L.. P.S. assisted with statistical analysis. J.J carried out all protein characterization with P.S.. R.N.D carried out tuber nutritional analysis. All authors were responsible for data analysis of their respective contributions. All authors contributed to final manuscript editing.

References

1 WHO, W. H. O. *The state of food security and nutrition in the world 2020: transforming food systems for affordable healthy diets*. Vol. 2020 (Food & Agriculture Org., 2020).

2 Ray, D. K., Mueller, N. D., West, P. C. & Foley, J. A. Yield trends are insufficient to double global crop production by 2050. *PloS one* **8**, e66428 (2013).

3 Panghal, A. *et al.* Global food security post COVID-19: Dearth or dwell in the developing world? *Agronomy journal* **114**, 878-884 (2022).

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

5 Perkins, S., Alexander, L. & Nairn, J. Increasing frequency, intensity and duration of observed global heatwaves and warm spells. *Geophysical Research Letters* **39** (2012).

6 Breshears, D. D. *et al.* Underappreciated plant vulnerabilities to heat waves. *New Phytologist* **231**, 32-39 (2021).

7 Simkin, A. J., López-Calcagno, P. E. & Raines, C. A. Feeding the world: improving photosynthetic efficiency for sustainable crop production. *Journal of Experimental Botany* **70**, 1119-1140 (2019).

8 Ort, D. R. *et al.* Redesigning photosynthesis to sustainably meet global food and bioenergy demand. *Proceedings of the National Academy of Sciences* **112**, 8529-8536 (2015).

9 Long, S. P., Marshall-Colon, A. & Zhu, X.-G. Meeting the global food demand of the future by engineering crop photosynthesis and yield potential. *Cell* **161**, 56-66 (2015).

10 Walker, B. J., VanLoocke, A., Bernacchi, C. J. & Ort, D. R. The costs of photorespiration to food production now and in the future. *Annual Review of Plant Biology* **67**, 107-129 (2016).

11 Peterhansel, C. *et al.* Engineering photorespiration: current state and future possibilities. *Plant Biology* **15**, 754-758 (2013).

12 Betti, M. *et al.* Manipulating photorespiration to increase plant productivity: recent advances and perspectives for crop improvement. *Journal of Experimental Botany* **67**, 2977-2988 (2016).

13 Hagemann, M. & Bauwe, H. Photorespiration and the potential to improve photosynthesis. *Current Opinion in Chemical Biology* **35**, 109-116 (2016).

14 South, P. F., Cavanagh, A. P., Lopez-Calcagno, P. E., Raines, C. A. & Ort, D. R. Optimizing photorespiration for improved crop productivity. *Journal of Integrative Plant Biology* **60**, 1217-1230 (2018).

15 Ogren, W. L. Photorespiration: pathways, regulation, and modification. *Annual Review of Plant Physiology* **35**, 415-442 (1984).

16 Bauwe, H., Hagemann, M. & Fernie, A. R. Photorespiration: players, partners and origin. *Trends in Plant Science* **15**, 330-336 (2010).

17 Jordan, D. B. & Ogren, W. L. The CO_2/O_2 specificity of ribulose 1, 5-bisphosphate carboxylase/oxygenase. *Planta* **161**, 308-313 (1984).

18 Badger, M. & Andrews, T. Effects of CO_2, O_2 and temperature on a high-affinity form of ribulose diphosphate carboxylase-oxygenase from spinach. *Biochemical and Biophysical Research Communications* **60**, 204-210 (1974).
19 Cavanagh, A. P. & Ort, D. R. Transgenic strategies to improve the thermotolerance of photosynthesis. *Photosynthesis research*, 1-12 (2023).
20 Peterhansel, C. & Maurino, V. G. Photorespiration redesigned. *Plant Physiology* **155**, 49-55 (2011).
21 Kebeish, R. *et al.* Chloroplastic photorespiratory bypass increases photosynthesis and biomass production in Arabidopsis thaliana. *Nature Biotechnology* **25**, 593-599 (2007).
22 Maier, A. *et al.* Transgenic introduction of a glycolate oxidative cycle into A. thaliana chloroplasts leads to growth improvement. *Frontiers in Plant Science* **3**, 38 (2012).
23 Nölke, G., Houdelet, M., Kreuzaler, F., Peterhänsel, C. & Schillberg, S. The expression of a recombinant glycolate dehydrogenase polyprotein in potato (S olanum tuberosum) plastids strongly enhances photosynthesis and tuber yield. *Plant Biotechnology Journal* **12**, 734-742 (2014).
24 Ahmad, R. *et al.* Improvement of biomass accumulation of potato plants by transformation of cyanobacterial photorespiratory glycolate catabolism pathway genes. *Plant Biotechnology Reports* **10**, 269-276 (2016).
25 South, P. F., Cavanagh, A. P., Liu, H. W. & Ort, D. R. Synthetic glycolate metabolism pathways stimulate crop growth and productivity in the field. *Science* **363**, eaat9077, doi:10.1126/science.aat9077 (2019).
26 Cavanagh, A. P., South, P. F., Bernacchi, C. J. & Ort, D. R. Alternative pathway to photorespiration protects growth and productivity at elevated temperatures in a model crop. *Plant Biotechnology Journal* **20**, 711-721 (2022).
27 FAO. (2021).
28 Poore, J. & Nemecek, T. Reducing food's environmental impacts through producers and consumers. *Science* **360**, 987-992 (2018).
29 Siebers, M. H. *et al.* Simulated heat waves during maize reproductive stages alter reproductive growth but have no lasting effect when applied during vegetative stages. *Agriculture, Ecosystems & Environment* **240**, 162-170 (2017).
30 Templ, B. & Calanca, P. Critical increase in the occurrence of heat stress during reproductive growth in Russian wheat beyond 1.5 C global warming. *Weather and Climate Extremes* **30**, 100281, doi:https://doi.org/10.1016/j.wace.2020.100281 (2020).
31 Ferguson, J. N., Tidy, A. C., Murchie, E. H. & Wilson, Z. A. The potential of resilient carbon dynamics for stabilizing crop reproductive development and productivity during heat stress. *Plant, Cell & Environment* **44**, 2066-2089 (2021).
32 Van Eck, J. *et al.* Enhancing beta-carotene content in potato by RNAi-mediated silencing of the beta-carotene hydroxylase gene. *American Journal of Potato Research* **84**, 331-342 (2007).
33 Beezley, B. B., Gruber, P. J. & Frederick, S. E. Cytochemical localization of glycolate dehydrogenase in mitochondria of Chlamydomonas. *Plant Physiology* **58**, 315-319 (1976).
34 Aboelmy, M. H. & Peterhansel, C. Enzymatic characterization of Chlamydomonas reinhardtii glycolate dehydrogenase and its nearest proteobacterial homologue. *Plant Physiology and Biochemistry* **79**, 25-30 (2014).
35 Timm, S., Woitschach, F., Heise, C., Hagemann, M. & Bauwe, H. Faster removal of 2-phosphoglycolate through photorespiration improves abiotic stress tolerance of Arabidopsis. *Plants* **8**, 563 (2019).
36 Basu, P., Sharma, A., Garg, I. & Sukumaran, N. Tuber sink modifies photosynthetic response in potato under water stress. *Environmental and Experimental Botany* **42**, 25-39 (1999).

37 Sonnewald, U. & Fernie, A. R. Next-generation strategies for understanding and influencing source–sink relations in crop plants. *Current Opinion in Plant Biology* **43**, 63-70 (2018).

38 Paul, M. J., Watson, A. & Griffiths, C. A. Linking fundamental science to crop improvement through understanding source and sink traits and their integration for yield enhancement. *Journal of Experimental Botany* **71**, 2270-2280 (2020).

39 Ordoñez, B., Orrillo, M. & Bonierbale, M. W. Technical manual potato reproductive and cytological biology. (2017).

40 Timm, S. *et al.* Metabolite profiling in Arabidopsis thaliana with moderately impaired photorespiration reveals novel metabolic links and compensatory mechanisms of photorespiration. *Metabolites* **11**, 391 (2021).

41 Allen, D. K. Assessing compartmentalized flux in lipid metabolism with isotopes. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids* **1861**, 1226-1242 (2016).

42 Koley, S., Jyoti, P., Lingwan, M. & Allen, D. K. Isotopically nonstationary metabolic flux analysis of plants: recent progress and future opportunities. *New Phytologist* **242**, 1911-1918 (2024).

43 Kirk, W. & Marshall, B. The influence of temperature on leaf development and growth in potatoes in controlled environments. *Annals of Applied Biology* **120**, 511-525 (1992).

44 Fleisher, D. H., Shillito, R. M., Timlin, D. J., Kim, S. H. & Reddy, V. R. Approaches to modeling potato leaf appearance rate. *Agronomy Journal* **98**, 522-528 (2006).

45 Van Dam, J., Kooman, P. & Struik, P. Effects of temperature and photoperiod on early growth and final number of tubers in potato (Solanum tuberosum L.). *Potato Research* **39**, 51-62 (1996).

46 Rykaczewska, K. The effect of high temperature occurring in subsequent stages of plant development on potato yield and tuber physiological defects. *American Journal of Potato Research* **92**, 339-349 (2015).

47 Struik, P. C., Geertsema, J. & Custers, C. H. Effects of shoot, root and stolon temperature on the development of the potato (Solanum tuberosum L.) plant. III. Development of tubers. *Potato Research* **32**, 151-158 (1989).

48 Tang, R. *et al.* Physiological and growth responses of potato cultivars to heat stress. *Botany* **96**, 897-912 (2018).

49 Campbell, C. *et al.* Acclimation of photosynthesis and respiration is asynchronous in response to changes in temperature regardless of plant functional group. *New Phytologist* **176**, 375-389 (2007).

50 Way, D. A. & Yamori, W. Thermal acclimation of photosynthesis: on the importance of adjusting our definitions and accounting for thermal acclimation of respiration. *Photosynthesis Research* **119**, 89-100 (2014).

51 Yamori, W., Noguchi, K., Hikosaka, K. & Terashima, I. Phenotypic plasticity in photosynthetic temperature acclimation among crop species with different cold tolerances. *Plant Physiology* **152**, 388-399 (2010).

52 Wolf, S., Olesinski, A., Rudich, J. & Marani, A. Effect of high temperature on photosynthesis in potatoes. *Annals of Botany* **65**, 179-185 (1990).

53 Wijesinha-Bettoni, R. & Mouillé, B. The contribution of potatoes to global food security, nutrition and healthy diets. *American Journal of Potato Research* **96**, 139-149 (2019).

54 Ziska, L., Weerakoon, W., Namuco, O. & Pamplona, R. The influence of nitrogen on the elevated CO_2 response in field-grown rice. *Functional Plant Biology* **23**, 45-52 (1996).

55 Rogers, G., Milham, P., Gillings, M. & Conroy, J. Sink strength may be the key to growth and nitrogen responses in N-deficient wheat at elevated CO_2. *Functional Plant Biology* **23**, 253-264 (1996).

56 Geiger, M., Haake, V., Ludewig, F., Sonnewald, U. & Stitt, M. Influence of nitrate and ammonium nitrate supply on the response of photosynthesis, carbon and nitrogen metabolism, and growth to elevated carbon dioxide in tobacco. *Plant, Cell and Environment* **22**, 1117-1199 (1999).

57 Jin, C. W. *et al.* Elevated Carbon Dioxide Improves Plant Iron Nutrition through Enhancing the Iron-Deficiency-Induced Responses under Iron-Limited Conditions in Tomato. *Plant Physiology* **150**, 272-280, doi:10.1104/pp.109.136721 (2009).

58 Stitt, M., Wilke, I., Feil, R. & Heldt, H. W. Coarse control of sucrose-phosphate synthase in leaves: alterations of the kinetic properties in response to the rate of photosynthesis and the accumulation of sucrose. *Planta* **174**, 217-230 (1988).

59 Bishop, K. A. *et al.* Similar photosynthetic response to elevated carbon dioxide concentration in species with different phloem loading strategies. *Photosynthesis Research* **137**, 453-464 (2018).

60 Leakey, A. D. B. *et al.* Elevated CO2 effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. *Journal of Experimental Botany* **60**, 2859-2876, doi:10.1093/jxb/erp096 (2009).

61 Lin, X. Y., Ye, Y. Q., Fan, S. K., Jin, C. W. & Zheng, S. J. Increased sucrose accumulation regulates iron-deficiency responses by promoting auxin signaling in Arabidopsis plants. *Plant Physiology* **170**, 907-920 (2016).

62 Singh, B. *et al.* Biofortification strategies to improve iron concentrations in potato tubers: lessons and future opportunities. *Potato Research* **65**, 51-64 (2022).

63 Connorton, J. M. & Balk, J. Iron biofortification of staple crops: lessons and challenges in plant genetics. *Plant and Cell Physiology* **60**, 1447-1456 (2019).

64 Roca, W. M., Espinoza, N., Roca, M. & Bryan, J. A tissue culture method for the rapid propagation of potatoes. *American Potato Journal* **55**, 691-701 (1978).

65 Murashige, T. & Skoog, F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia plantarum* **15**, 473-497 (1962).

66 Hellemans, J., Mortier, G., De Paepe, A., Speleman, F. & Vandesompele, J. qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. *Genome Biology* **8**, 1-14 (2007).

67 Farquhar, G. D., Caemmerer, S. & Berry, J. A. A biochemical model of photosynthetic CO2 assimilation in leaves of C3 species. *Planta* **149**, 78-90, doi:10.1007/bf00386231 (1980).

68 Sharkey, T. D., Bernacchi, C. J., Farquhar, G. D. & Singsaas, E. L. Fitting photosynthetic carbon dioxide response curves for C3 leaves. *Plant, Cell & Environment* **30**, 1035-1040, doi:10.1111/j.1365-3040.2007.01710.x (2007).

69 von Caemmerer, S. *Biochemical Models of Leaf Photosynthesis*. (CSIRO Publishing, 2000).

70 Leakey, A. D. *et al.* Water use efficiency as a constraint and target for improving the resilience and productivity of C3 and C4 crops. *Annual Review of Plant Biology* **70**, 781-808 (2019).

71 Bowling, D. R., Sargent, S. D., Tanner, B. D. & Ehleringer, J. R. Tunable diode laser absorption spectroscopy for stable isotope studies of ecosystem–atmosphere CO_2 exchange. *Agricultural and Forest Meteorology* **118**, 1-19 (2003).

72 Tazoe, Y., Von Caemmerer, S., Estavillo, G. M. & Evans, J. R. Using tunable diode laser spectroscopy to measure carbon isotope discrimination and mesophyll conductance to CO_2 diffusion dynamically at different CO_2 concentrations. *Plant, Cell & Environment* **34**, 580-591 (2011).

73 Farquhar, G. D. & Cernusak, L. A. Ternary effects on the gas exchange of isotopologues of carbon dioxide. *Plant, Cell & Environment* **35**, 1221-1231 (2012).

74 Evans, J., Sharkey, T., Berry, J. & Farquhar, G. Carbon isotope discrimination measured concurrently with gas exchange to investigate CO_2 diffusion in leaves of higher plants. *Functional Plant Biology* **13**, 281-292 (1986).

75 Evans, J. R. & Von Caemmerer, S. Temperature response of carbon isotope discrimination and mesophyll conductance in tobacco. *Plant, Cell & Environment* **36**, 745-756, doi:10.1111/j.1365-3040.2012.02591.x (2013).
76 Campbell, J. M. *et al.* Selected fructooligosaccharide (1-kestose, nystose, and 1F-β-fructofuranosylnystose) composition of foods and feeds. *Journal of Agricultural and Food Chemistry* **45**, 3076-3082 (1997).
77 Sikora, F. A buffer that mimics the SMP buffer for determining lime requirement of soil. *Soil Science Society of America Journal* **70**, 474-486 (2006).
78 Mehlich, A. New extractant for soil test evaluation of phosphorus, potassium, magnesium, calcium, sodium, manganese and zinc. *Communications in Soil Science and Plant Analysis* **9**, 477-492 (1978).
79 Walkley, A. & Black, I. A. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science* **37**, 29-38 (1934).