Enhancing biomass production and yield by maintaining enhanced capacity for CO_2 uptake in response to elevated CO_2

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Dahal, K., Weraduwage, S. M., Kane, K., Rauf, S. A., Leonardos, E. D., Gadapati, W., Savitch, L., Singh, J., Marillia, E.-F., Taylor, D. C., Micallef, M. C., Knowles, V., Plaxton, W., Barron, J., Sarhan, F., Hüner, N., Grodzinski, B. and Micallef, B. J. 2014. **Enhancing biomass production and yield by maintaining enhanced capacity for CO_2 uptake in response to elevated CO_2**. Can. J. Plant Sci. **94**: 1075–1083. Using four model plants, two members of the Gramineae, rye and wheat, and two Brassicaceae, *Brassica napus* and *Arabidopsis thaliana*, two fundamental approaches were exploited to determine how regulating source-sink development would alter photosynthesis, productivity and yield during long-term acclimation to elevated CO_2. In one approach we exploited the cold acclimation response of winter wheat, rye and *B. napus*. In the other approach we modified the dark respiration in *A. thaliana* to alter availability of respiratory substrates required for anabolic processes, such as fatty acid metabolism, thus reducing sink limitations on canopy photosynthesis at elevated CO_2. Taken together, the data show the importance of maintaining strong demand from active sinks when the above-ground canopy is being exposed to elevated levels of the primary substrate of photosynthesis, CO_2.

Key words: Carbon dioxide enrichment, productivity, photosynthesis, respiration, cereals, Brassicaceae

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Mots clés: Hausse du dioxyde de carbone, productivité, photosynthèse, respiration, céréales, Brassicaceae

Abbreviations: **CA**, cold acclimated; **mtPDH**, mitochondrial pyruvate dehydrogenase; **mtPDHK**, mitochondrial pyruvate dehydrogenase kinase; dehydrogenase; **NA**, non-acclimated; **NPQ**, nonphotochemical dissipation; **plPDH**, chloroplastic isozyme of PDH; **TCA**, tricarboxylic acid

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The primary goal of our group within the Green Crop Network is described in the overview article by Smith and Zhou (2014; this issue). The overall goal of Theme 3 was to provide a genetic and metabolic blueprint for enhanced plant carbon storage and biomass production through an enhanced capacity for CO_2 uptake in response to elevated CO_2 that is associated with global warming. To address this goal, two fundamentally different experimental approaches with different plant material were exploited to assess how source-sink interactions during CO_2 enrichment may lead to enhanced plant productivity. The first approach exploited the known enhancement in CO_2 assimilation and biomass production induced by cold acclimation of winter cereals, such as wheat and rye as well as *Brassica napus* under ambient CO_2 conditions (Hurry and Hüner 1991; Boese and Hüner 1992; Öquist et al. 2003; Hurry et al. 1994, 1995; Hüner et al. 1998; Savitch et al. 2002; Rapacz et al. 2008). The second approach exploited the enhancement of seed oil yield previously found at ambient CO_2 in genetic variants of *Arabidopsis thaliana* altered in respiratory metabolism (Zou et al. 1999; Marilla et al. 2003). We hypothesized that this genetic modification would reduce sink limitations on photosynthesis and growth at elevated CO_2 through greater availability of respiratory substrates required for anabolic processes such as fatty acid synthesis in seeds.

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THE EFFECTS OF COLD ACCLIMATION ON PHOTOSYNTHESIS AND PRODUCTIVITY AT HIGH CO_2

During cold acclimation of cold-tolerant cultivars and species, increased photosynthetic capacity is, in part, the result of the enhanced activities of key regulatory enzymes of primary carbon metabolism such as Rubisco, cFBPase, and SPS (Hurry et al. 1995, 2000; Stitt and Hurry 2002) and the concomitant export to sinks during cold acclimation (Leonardos et al. 2003). Although spring cereals can grow and develop at low temperatures, they exhibit decreased photosynthetic capacity estimated as light-saturated rates of carbon assimilation, A_{sat}, in response to low temperatures in contrast to winter cultivars (Hurry and Huner 1991). Because spring cultivars exhibit a limited sink demand coupled with a retardation of carbon export from photosynthetically active source tissue to plant sink tissue (Hurry et al. 1995), this results in inhibition of cytosolic sucrose biosynthesis, which leads to Pi limitations within the chloroplast which, in turn, decreases the utilization of stromal phosphorylated intermediates of the Calvin–Benson cycle. This results in an inhibition of CO_2 assimilation due to feedback-limited photosynthesis (Hurry et. al. 1995; Savitch et al. 2002). Therefore, spring cultivars exhibit decreased plasticity to low growth temperatures and exhibit reduced photosynthetic capacity during cold acclimation (Hurry et al. 1995; Savitch et al. 2002). Winter cultivars overcome the potential for low-temperature-induced feedback inhibition of photosynthesis by the maintenance of high sink capacity, which maintains a high flux of carbon export from the source.

Is the enhanced photosynthetic capacity exhibited by cold-acclimated winter cultivars under ambient CO_2 conditions related to an enhanced efficiency for light energy conversion into biomass and seed production?

A major focus of photosynthesis research continues to be the enhancement of crop yield coupled with minimal increases in energy inputs. Classical breeding has been used in attempts to maximize photosynthetic efficiency through alteration of canopy structure to minimize light saturation effects on individual leaves (Zhu et al. 2010). Under light saturated conditions, photosynthetic efficiency is minimal, and plants protect the photosynthetic apparatus from the potential damaging effects of excess light by dissipating the excess absorbed energy as heat through induction of the xanthophyll cycle (Ort 2001). The evolution of the xanthophyll cycle to regulate nonphotochemical dissipation (NPQ) of excess absorbed light is considered essential for the survival of sun plants to fluctuations in irradiance (Demmig-Adams and Adams 1992). Enhanced rates of photosynthesis should decrease the propensity for energetically wasteful dissipation of absorbed light has heat. Dahal et al. (2012c, 2013) reported that cold acclimation of winter wheat and rye increased the quantum requirement to close PSII reaction centers with a concomitant increase in the quantum requirement to induce NPQ relative to nonacclimated plants. This means that it is more difficult to close PSII reaction centers in cold acclimated versus nonacclimated plants. This is consistent with the increased flux of electrons through photosynthetic electron transport, the enhanced rates of CO_2 assimilation and the decreased light sensitivity of NPQ in cold acclimated versus nonacclimated plants (Dahal et al. 2012c, 2013). This is not only coupled to an increase in the content of major photosynthetic components (e.g., Rubisco, psaB, psaA, cFBPase) per unit chlorophyll, but also a decrease in the low temperature sensitivities of CO_2 assimilation as well as photosynthetic electron transport (Dahal et al. 2012a,b, 2013). Thus, cold acclimation increases energy conversion efficiency at the expense of NPQ such that more absorbed energy is trapped as fixed carbon and subsequently converted into biomass. Interestingly, cold acclimation of spring wheat and spring rye decreases energy conversion efficiency, which is coupled to increased dissipation of absorbed light as NPQ (Dahal et al. 2012a, 2013). Furthermore, this differential capacity to modulate energy conversion efficiency is translated into a 40% increase in grain yield (grams of seeds/plant) in winter wheat than spring wheat (Dahal et al. 2013).

Can cold acclimated plants maintain this enhanced capacity for CO_2 assimilation and biomass production during long-term growth under elevated CO_2 conditions?

Although an immediate increase in the rates of net CO_2 assimilation has been observed following a short-term shift of C_3 plants from ambient to elevated CO_2 due to enhanced carboxylation velocity (Cheng et al. 1998; Long et al. 2004; Ainsworth and Rogers 2007), long-term growth and development of C_3 plants at high CO_2 concentration may lead to an end product inhibition of photosynthetic capacity due to accumulation of non-structural carbohydrates in the cytosol (Stitt and Quick 1989; Foyer 1990). At elevated CO_2, photosynthesis is usually limited either by the capacity of photosynthetic electron transport to supply ATP and NADPH to regenerate RuBP, or by the capacity of starch and sucrose synthesis to utilize triose phosphates and consequently regenerate P_i.

The P_i regeneration-limited photosynthesis is governed by the balance between the source leaves to assimilate carbon and the sink strength to utilize photoassimilates (Arp 1991; Drake et al. 1997). It has been suggested that the increased carbon uptake resulting from initial stimulation of photosynthesis alters the balance between supply and demand due to limited sink capacity to utilize carbohydrates and concomitant retardation of carbon export to the sinks (Kramer 1981; Arp 1991; Drake et al. 1997). This results in the accumulation of sucrose in the source leaves followed by inhibition of sucrose synthesis and a short-term decrease in utilization of phosphorylated intermediates and depletion in stromal P_i. Low availability of stromal P_i triggers inhibition of ATP synthesis and thereby a decrease in the rate at which PGA is converted to triose phosphate, which results in feedback inhibition of CO_2 assimilation (Stitt and Quick 1989; Sharkey and Vaderveer 1989). In the long-term, the feedback inhibition of photosynthesis may lead to the downregulation of the expression of key regulatory enzymes of photosynthetic carbon metabolism (Drake et al. 1997; Moore et al. 1999).

We hypothesized that the cold acclimation-induced increase in photosynthetic performance of winter wheat and winter rye at ambient CO_2 is maintained under long-term growth and development at elevated CO_2. Consistent with our hypothesis, the low-temperature-induced increase in photosynthetic capacity of winter cultivars, Norstar and Musketeer, at ambient CO_2, was maintained under growth and development of plants at elevated CO_2 (Dahal et al. 2012). In fact, the cold acclimated (CA) winter cultivars exhibited about 1.4-fold higher A_{sat} than non-acclimated (NA) controls when grown at elevated CO_2. This 1.4-fold enhancement of photosynthetic capacity in CA versus NA winter cultivars at elevated CO_2 was associated with increased SLW, and, as a consequence, an increase in total leaf protein content and subsequent levels of major photosynthetic enzymes such as Rubisco (rbcL), cFBPase and components of photosynthetic electron transport, Lhcb1, PsbA and PsaA on a leaf area basis upon cold acclimation (Dahal et al., in preparation). These were consistent with increased quantum requirements to close PSII reaction centers as well as to induce energy dissipation by NPQ coupled with a lower C_i requirement to open PSII reaction centers and a lower propensity to dissipate absorbed energy through NPQ under CO_2 saturated conditions in CA versus NA winter cultivars irrespective of growth CO_2. This indicates that compared with NA winter cultivars, CA winter cultivars grown at elevated CO_2 maintain an enhanced efficiency to utilize absorbed light energy and convert it to biomass with a concomitant decrease in dissipation of absorbed energy through NPQ. Furthermore, the enhanced energy conversion efficiency is translated into a greater increase in grain yield (grams of seeds/plant) in winter wheat than spring wheat, even during long-term growth at elevated CO_2 (Dahal et al., in preparation).

Can the target genes that govern the cold acclimation-induced enhancement in photosynthetic capacity and biomass production be identified?

We hypothesized, first, that the cold acclimation-induced increase in photosynthetic performance of *B. napus* at ambient CO_2 is maintained under long-term growth and development at elevated CO_2 similar to that observed for CA winter cereals (Dahal et al. 2012a,b). Second, based on our previous results (Savitch et al. 2005), we hypothesized that the *BnCBF17* over-expressor grown at 20°C should respond similarly to growth and development under elevated CO_2 as does cold acclimated WT *B. napus*. The recent results of Dahal et al. (2012c) indicate that the over-expression of *BnCBF17* mimics the effects of cold acclimation of *B. napus* with respect to photosynthetic performance, the efficiency of energy conversion and WUE, which is still maintained even after long-term growth and development under elevated CO_2 conditions. Neither cold acclimated Brassica nor the *BNCBF17* over-expressor exhibited feedback inhibition of photosynthesis during long-term growth and development at elevated CO_2. Thus, we suggest that the transcription factor, *BnCBF17*, may be a central component which governs the regulation of photosynthetic capacity and energy conversion efficiency of crop plants at ambient as well as at elevated CO_2 conditions.

Can this information be used to genetically modify plants to induce enhanced photosynthetic capacity and biomass production without the requirement for cold acclimation?

We suggest that our results for over-expression of *BNCBF17* in *B. napus* (Dahal et al. 2012c) provide important new insights into potential molecular and genetic approaches focussed on the maintenance or even the enhancement of plant productivity under suboptimal growth

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conditions associated with climate change independent of the requirement for cold acclimation. Thus, we suggest that CBFs/DREBs are critical transcription factors that govern plant phenotypic plasticity associated with cold acclimation from the level of gene expression and freezing tolerance to whole plant architecture, WUE as well as photosynthetic energy conversion efficiency into biomass and, ultimately, seed production. Based on our data, over-expression of CBFs circumvents the need for cold acclimation to induce enhanced CO_2 assimilation rates, energy conversion efficiency and increased biomass production in important crop plants such as wheat, rye and *B. napus*.

THE EFFECT OF RESPIRATORY METABOLISM ON SOURCE-SINK BALANCE AT ELEVATED CO_2: LESSONS LEARNED FROM GENETIC VARIANTS OF *ARABIDOPSIS THALIANA* ALTERED IN MITOCHONDRIAL PYRUVATE DEHYDROGENASE

At elevated atmospheric CO_2 as predicted for this century, plant productivity is likely to be limited by the development and metabolism of sink-tissues (sink-strength), causing feedback effects on photosynthesis (source-strength) (Grodzinski 1992; Morgan et al. 2005; Ainsworth and Rogers 2007). Studies on plants with large sinks, and experimental manipulations of sinks, have implicated sink strength as an important factor in maintaining high photosynthetic rates in plants under CO_2-enrichment (Ainsworth et al. 2004). Thus far, studies examining the influence of metabolism on sink strength at elevated CO_2 have focused on anabolic processes such as starch synthesis and nitrogen assimilation (Smidansky et al. 2002; Stitt and Krapp 1999). Dark respiration has rarely been considered a promising target for increasing sink strength and productivity (Long et al. 2006), in part since dark respiration is associated with catabolism (Fig. 1). Studies at ambient CO_2 have demonstrated correlations between decreased respiratory CO_2 release and enhanced productivity (Winzeler et al. 1989).

However, an up-regulation of mitochondrial numbers and mitochondrial genes and proteins can occur in source tissues under high CO_2 (Robertson et al. 1995; Leakey et al. 2009). We believe that this respiratory adaptation to elevated CO_2 reflects the crucial role of dark respiration in anabolic processes, such as fatty acid and protein synthesis (Fig. 1) (Weraduwage et al. 2011). Stated another way, we believe that an increased demand for intermediary substrates derived from dark respiration like acetyl-CoA occurs at elevated CO_2 that support enhanced plant growth (sink strength) and that need to be supplied to prevent feedback effects on photosynthesis (source strength).

To examine the relationship between dark respiration and source-sink balance in plants at elevated CO_2, we utilized genetic variants of *Arabidopsis thaliana* affected in mitochondrial pyruvate dehydrogenase (mtPDH) activity (Zou et al. 1999; Marilla et al. 2003). Mitochondrial pyruvate dehydrogenase catalyzes the oxidative decarboxylation of pyruvate to acetyl-CoA, thus linking glycolysis with the tricarboxylic acid (TCA) cycle (Fig. 1) (Budde and Randall 1990; Marillia et al. 2003). Acetyl-CoA is required for the synthesis of fatty acids, a number of plant growth regulators, and secondary metabolites including terpenoids (Fig. 1) (Gemel and Randall 1992; Luethy et al. 1994; Marillia et al. 2003; Weraduwage et al. 2011). Up-regulation of mtPDH protein does occur in wheat at elevated CO_2 (Robertson et al. 1995).

To modify mtPDH activity in *Arabidopsis thaliana* we used antisense technology to reduce the activity of mitochondrial pyruvate dehydrogenase kinase (mtPDHK) (Zou et al. 1999; Marillia et al. 2003). The protein phosphorylation activity of mtPDHK is believed to deactivate mtPDH (Yeaman et al. 1978; Thelen et al. 2000; Tovar-Mėndez et al. 2003). A chloroplastic isozyme of PDH (plPDH) also exists that is not regulated by phosphorylation (Patel and Korotchkina 2003). *Arabidopsis thaliana* transgenic lines showing antisense suppression of mtPDHK do exhibit increased mtPDH activities in leaves, and an increased 100-seed oil content and 100-seed specific weight (i.e., enhanced sink activity in the seed), particularly in lines showing seed-specific expression of the antisense construct, when grown at 22–25°C and ambient CO_2 (Zou et al. 1999; Marillia et al. 2003). However, under these growing conditions overall plant biomass and yield in the transgenic lines is either not enhanced or it decreases (Zou et al. 1999; Marillia et al. 2003). We believe this occurs because source activity at ambient CO_2 cannot adapt sufficiently to the enhanced respiratory activity in the mtPDHK transgenics. Expression of mtPDHK in these transgenic lines has not been examined at elevated CO_2.

Using four transgenic lines each of *Arabidopsis thaliana* that express an antisense construct for mtPDHK in either a constitutive or seed-specific manner (eight transgenic lines total) and three control lines [an untransformed control (Wt) and a plasmid-only control corresponding to either the constitutive or seed-specific lines], we tested the following hypothesis: antisense suppression of mtPDHK in *Arabidopsis thaliana* will enhance photosynthesis and overall plant productivity at elevated CO_2 due to complementary increases in source and sink strength particularly for seed-specific lines.

Objective 1 – What is the effect of atmospheric CO_2 levels on mtPDHK expression and the enzyme activities of mtPDH and mtPDHK in leaves and seeds of Arabidopsis?

Leaf mtPDHK transcript levels were lower in both control and constitutive transgenic lines at elevated CO_2 relative to ambient CO_2, and mtPDHK transcript levels in leaves were lower in constitutive transgenic lines relative to control lines at both ambient and elevated CO_2 (Weraduwage 2013). To quantify mtPDH and mtPDHK enzyme activities in leaves and seeds, two high-throughput in vitro assays using crude extracts were

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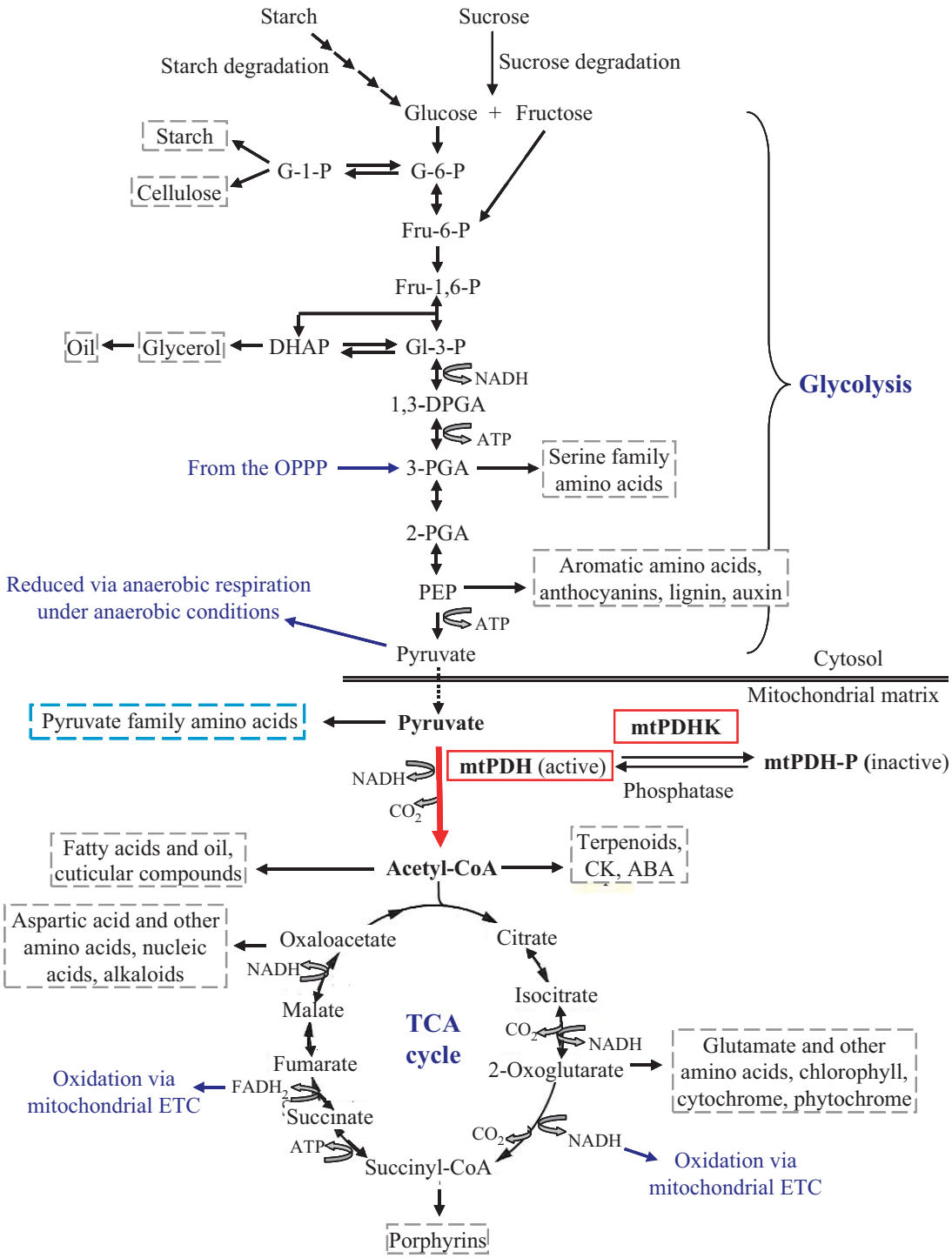

Fig. 1. The anabolic and catabolic roles for dark respiration in plants. The pyruvate-synthesizing glycolytic pathway and pyruvate-oxidizing TCA cycle are shown. The mtPDH complex, which links glycolysis and the TCA cycle, is marked by a red arrow. The metabolic junctions where other respiratory pathways such as the OPPP, aerobic respiratory pathway, or mitochondrial ETC and the anaerobic respiratory pathways interconnect with glycolysis and the TCA cycle through metabolites are denoted with blue arrows. The numerous anabolic pathways that depend on intermediary substrates arising from the respiratory pathways are given in boxes outlined in grey. Reversible reactions (↕) and irreversible reactions (↓) are noted. Abbreviations: ABA, abscisic acid; CK, cytokinin; ETC, electron transport chain; mtPDH, mitochondrial pyruvate dehydrogenase; mtPDHK, mtPDH kinase; mtPDH-P, phosphorylated mtPDH; OPPP, oxidative pentose phosphate pathway; TCA, tricarboxylic acid.

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developed that favor the mtPDH isozyme over plPDH based both on precipitation of plPDH and optimization of substrates and co-factors for mtPDH (Weraduwage 2013). The mtPDHK activity assay examines the inactivation of mtPDH over time in the presence of 5 mM ATP, and it allows the determination of contaminating plPDH activity in crude extracts since plPDH is not phosphorylated like mtPDH (Weraduwage 2013). These assays show that mtPDH activities are enhanced and mtPDHK activities are reduced in leaves at elevated CO_2 for transgenic lines relative to controls, which is consistent with changes in leaf mtPDHK transcript levels (Weraduwage 2013). Seed mtPDH activities are also enhanced in constitutive and seed-specific transgenic lines compared with controls at both ambient and elevated CO_2 (Weraduwage 2013). Collectively, the data support both an up-regulation of mtPDH activity in leaves and seeds at elevated CO_2 relative to ambient CO_2, and an enhancement of mtPDH activity at elevated CO_2 in both constitutive and seed-specific transgenic lines showing antisense repression of mtPDHK relative to control lines.

Interestingly, at ambient CO_2 maximal mtPDH activity was exhibited by lines showing intermediate repression of mtPDHK (Weraduwage 2013), which challenges the notion that mtPDHK is strictly a negative regulator of mtPDH activity (Yeaman et al. 1978; Thelen et al. 2000; Tovar-Mėndez et al. 2003). Thus, at ambient CO_2 either a high or a low level of phosphorylation appears to decrease mtPDH activity, although this requires confirmation using phospho-specific antibodies directed to mtPDH (Weraduwage 2013). We hypothesize that this parabolic relationship between mtPDHK and mtPDH activities at ambient CO_2 reflects a role for mtPDHK in balancing photosynthetic and respiratory processes in plants based on the energy status (e.g., ATP levels) of the plant, since ATP is a substrate for mtPDHK.

Objective 2 – Will sink activity be enhanced by a repression of mtPDHK?

To examine the impact of mtPDHK expression on sink activity at ambient and elevated CO_2, we quantified a number of vegetative and reproductive growth parameters including total seed and oil production (Weraduwage 2013). A number of growth and seed oil parameters were improved in transgenic lines, particularly at elevated CO_2; many of these parameters showed a significant linear or quadratic correlation with mtPDHK transcript levels and mtPDH activity in the leaf and seed (Weraduwage 2013). We consistently found enhanced 100-seed oil content and 100-seed specific weight for all transgenic lines compared with control lines as found in previous studies (Zou et al. 1999; Marillia et al. 2003). The greatest enhancement in total seed and oil productivity was found for two constitutive lines at elevated CO_2 (up to 2.8 times) that also exhibit a significant increase in inflorescence size, the lowest rate of mtPDH complex inactivation by ATP in leaves, and an intermediary enhancement of mtPDH complex activity in seeds (Weraduwage 2013). Increases in productivity at elevated CO_2 for these two constitutive lines also correlate with altered developmental parameters, including enhanced seed number per silique, enhanced harvest index, and a larger inflorescence canopy (Weraduwage 2013). Three-dimensional laser scanning imaging also show that these two constitutive lines have larger canopy areas, larger stem volumes, and a faster growth rate (Zhao 2009; Yang 2010). Interestingly, the enhanced productivity found for these two transgenic lines at elevated CO_2 was greatest under low humidity and high plant density (Weraduwage 2013), suggesting that mtPDHK may be involved in stress adaptation in plants. These data show that sink activity at elevated CO_2 can be altered through dosage-dependent expression of mtPDHK.

Objective 3 – Is photosynthesis and respiratory CO_2 release altered at elevated CO_2 for transgenic Arabidopsis showing reduced expression of mtPDHK?

We used both leaf-level and whole-plant gas exchange techniques to examine the effects of altered mtPDHK expression on net carbon exchange rates in the light and dark, to assess the impact of mtPDHK on source activity and to establish if respiratory CO_2 release is increased or decreased in *Arabidopsis* grown at elevated CO_2 (Rauf 2012; Weraduwage 2013). Gas exchange measurements of individual rosette leaves demonstrated enhanced photosynthesis for constitutive and seed-specific transgenic lines at both ambient and elevated CO_2, providing evidence for reduced feedback effects on photosynthesis early in plant development before a significant inflorescence is established (Weraduwage 2013). An altered inflorescence canopy architecture described in Objective 2 above for constitutive transgenic lines is significant, since we have also demonstrated clearly that the inflorescence in Arabidopsis contributes up to 90% of whole plant C gain through photosynthesis at later stages of development (Rauf 2012). Thus, enhanced inflorescence development later in plant development significantly impacts whole-plant photosynthesis and carbon gain during reproductive development.

Measurements of net C exchange for both single leaves and the whole plant consistently showed that both photosynthesis and respiratory CO_2 release are enhanced at elevated CO_2 for Arabidopsis (Rauf, 2012). The enhancement of respiratory CO_2 release at elevated CO_2 provides evidence for enhanced sink activity, and these data help to resolve the controversy on whether plants grown at elevated CO_2 show reduced or enhanced respiration (Grodzinski 1992; Drake et al. 1999; Leaky et al. 2009). These data are consistent with recent work by Leakey et al. (2009) showing an up-regulation of dark respiratory genes in source tissues under high CO_2. The effect of CO_2 was greatest on photosynthesis and (growth and maintenance) respiration at stages of development when we have very active sink development, first during the

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leaf expansion phase and then at the silique-to-seed filling stage (Rauf 2012). Possibly the effects on canopy architecture and stress response discussed above occur due to alterations in synthesis of terpenoid-based plant growth regulators in transgenic plants showing altered mtPDH activity and thus altered capacity for acetyl-CoA synthesis.

Objective 4 – Is mtPDH is a viable target site for improving crop productivity at elevated CO_2?

Our study is the first attempt at increasing dark respiration at elevated CO_2 to enhance sink activity in plants, thus challenging the belief that decreased respiratory flux will enhance crop productivity (Weraduwage et al. 2011). Our definition of respiratory flux is the rate at which carbon enters the TCA cycle through mtPDH. Once carbon enters the TCA cycle, it can either be oxidized to CO_2 or shunted into anabolic pathways (Fig. 1). Interestingly, we did not find differences in the rate of respiratory CO_2 release on a leaf area or dry matter basis for transgenic lines compared with controls, although mtPDH activity was enhanced in transgenic lines (Rauf 2012; Weraduwage 2013). We have shown previously by feeding ^{14}C-pyruvate to developing seeds that the flux through mtPDH is increased in transgenic lines showing repression of mtPDHK as evidenced by enhanced ^{14}C-label in fatty acids (Marilla et al. 2003). It is possible that PEP carboxylase has been enhanced in our transgenic lines owing to its anapleurotic role in replenishing TCA cycle intermediates (Weraduwage et al. 2011), thus reducing CO_2 respiratory release in the transgenic lines.

By using transgenic *Arabidopsis* having repressed mtPDHK expression, we have shown that dark respiratory processes play an important role in regulating source-sink balance in plants subjected to elevated CO_2. Contrary to our original hypothesis, transgenic lines showing constitutive and not seed-specific expression of the mtPDHK antisense construct showed the greatest improvement in productivity at elevated CO_2 (Weraduwage 2013). This finding is consistent with our observations that these yield increases for constitutive lines correlated with a number of morphological parameters, including size of the inflorescence canopy, and thus a number of tissues did respond to altered mtPDHK expression in the constitutive lines. We have discovered a method to greatly enhance both plant and oil productivity at elevated CO_2 in *Arabidopsis* by directly increasing dark respiratory flux to harness its anabolic properties in a dosage-responsive manner. We believe this approach will inspire future studies to exploit the anabolic aspects of dark respiration to its fullest potential so that future agricultural systems can reap maximal benefits of elevated CO_2 under both field and controlled environments, including the production of plant oils that are important for the future bioeconomy.

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