Elevated CO₂ induces age-dependent restoration of growth and metabolism in gibberellin-deficient plants

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Abstract

Main conclusion The effect of elevated [CO₂] on the growth of tomato plants with reduced GA content is influenced by developmental stage.

The increase of carbon dioxide (CO₂) in the atmosphere during the last decades has aroused interest in the function of this gas in the growth and development of plants. Despite the known association between elevated CO₂ concentration ([CO₂]) and plant growth, its effects in association with gibberellin (GA), plant hormone that regulates de major aspects of plant growth, are still poorly understood. Therefore, we evaluated the effect of elevated [CO₂] on growth and primary metabolism in tomato plants with drastic reduction in GA content (*gib-1*) at two different growth stages (21 and 35 days after germination, dag). Disruption on growth, photosynthetic parameters and primary

metabolism were restored when *gib-1* plants were transferred to elevated [CO₂] at 21 dag. Elevated [CO₂] also stimulated growth and photosynthetic parameters in Wild type (WT) plants at 21 dag, however, minor changes were observed in the level of primary metabolites. At 35 dag, elevated [CO₂] did not stimulate growth in WT plants and *gib-1* mutants showed their characteristic stunted growth phenotype.

Keywords *gib-1* . Tomato . Plant hormone . phase transition . cell division

Introduction

Plant growth and development involve many endogenous and environmental signals that interact with the plant's genetic program to determine plant architecture (Wang and Li 2008; Achard et al. 2009). Fundamental to this process are chemical regulators known as plant hormones (Santner et al. 2009). Among them, gibberellins (GAs) regulate major aspects of plant growth and development, including seed germination, stem elongation, leaf expansion, trichome development and flowering induction (Hedden and Thomas 2016). Many of these aspects are controlled by the capacity of GAs to stimulate cell division and elongation (de Lucas et al. 2008; Achard et al. 2009), through degradation of transcriptional repressor DELLA proteins (Dill et al. 2001; Alvey and Harberd 2005).

GAs are synthesized via terpenoid pathway by the action of terpene synthase, cytochrome P450 oxygenase and 2-oxoglutarate-dependent dioxygenases (2-ODDs) in plastids, the endomembrane system and the cytosol, respectively. 2-ODDs are 2-oxoglutarate dependent, the key intermediate of one the most fundamental biochemical pathways in carbon metabolism -the tricarboxylic acid (TCA) cycle- and a point of connection between carbon and nitrogen metabolism (Araújo et al. 2014). In this way, the dependence on 2-oxoglutarate (2-OG) links GA biosynthesis and homeostasis

53 directly with primary metabolism (Lancien et al. 2000), since most GA oxidases (GA_{ox})
54 are 2-OG dehydrogenase (2-ODDs) (GA_{3ox}, GA_{20ox}, GA_{2ox}).

55 Alterations in ambient [CO₂] expected for the next few years (IPCC, 2014) may
56 impact plant growth and development (Kimball 2016). Succinctly, [CO₂] directly
57 influences the rate of CO₂ assimilation by Rubisco, and consequently gas exchange
58 rates, which could influence plant growth and crop productivity (Campbell et al. 1988;
59 Igamberdiev 2015; Galmés et al. 2017). Particularly in C3 plants, CO₂ is a limiting
60 substrate for photosynthesis, and elevated [CO₂] usually leads to an increase in
61 photosynthetic assimilation rates and a decrease in photorespiration, stimulating
62 production of sugars and biomass accumulation (Ainsworth and Rogers 2007;
63 Ainsworth 2008; Högy et al. 2010). Higher sugar availability is a trigger for plant
64 growth at elevated [CO₂] (Taylor et al. 1994; Masle 2000; Ferris et al. 2001). However,
65 it is the sink capacity of the plant to use or store additional photoassimilate that
66 determines photosynthesis stimulation and growth at elevated [CO₂] (Arp 1991). In
67 soybean (*Glycine max*), for instance, growth habit was a determinant factor of
68 photosynthetic acclimation at elevated [CO₂] (Ainsworth et al. 2004). In this case, the
69 inability to form sufficient sinks in determinate-growth plants contributed to feedback
70 photosynthesis acclimation, suggesting the participation of sugar sensing and signaling
71 in the growth responses (Paparelli et al. 2013; Wang and Ruan 2013; Lastdrager et al.
72 2014).

73 Plant hormones act as chemical mediators to control growth and development in
74 response to elevated [CO₂]. Increase in GAs in response to elevated [CO₂] have
75 consistently been reported in several species such as *Ginkgo biloba* L.(Li et al. 2002),
76 *Arabidopsis* (Teng et al. 2006) and *Populus* (Liu et al. 2014). Furthermore, growth
77 reduction of *Arabidopsis* treated with the GA biosynthesis inhibitor paclobutrazol

(PAC) was reverted by elevated [CO₂] (Ribeiro et al. 2012). This suggests that plant growth at elevated [CO₂] may be partially coupled with the effects of GA (Ribeiro et al. 2012), and that elevated [CO₂] and GA act could in similar pathways related with plant growth. Despite this circumstantial evidence of association between elevated [CO₂] and plant hormones, little is known about how elevated [CO₂] coordinates plant growth together with GA.

Tomato (*Solanum lycopersicum* L.) is one of the most important horticultural crops in the world and has been widely used as a model organism in several fields of plant research (Kimura and Sinha 2008). The availability of monogenic mutant collections represents a powerful tool for the study of gene function and ecophysiological interactions (Carvalho et al. 2011). For example, three mutants in GA biosynthesis (*gibberellin deficient 1, 2 and 3*, *gib-1*, *gib-2* and *gib-3* respectively) were identified in tomato plants (Koornneef et al. 1990). The *gib-1* mutant shows reduction in ent-copalyl diphosphate synthase activity, the first enzyme involved in GA biosynthesis leading to a dwarf phenotype due to the drastic reduction in GA content (Bensen and Zeevaart 1990). Different to *gib-1*, both *gib-2* and *gib-3* show less conspicuous reductions in growth (Koornneef et al. 1990). Thus, the availability of these GA-related mutants makes tomato plants a useful model for the study of combinatorial effects of reduced GA content and elevated [CO₂] on the plant growth.

Since elevated [CO₂] influenced growth and metabolism of *Arabidopsis* treated with PAC (Ribeiro et al. 2012), here we investigated growth and metabolic responses in tomato plants with drastic reduction in GA content (*gib-1*) transferred to elevated [CO₂] at two different growth stages (21 and 35 days after germination, dag). Mutant *gib-1* plants cultivated in ambient [CO₂] showed stunted growth and reduced biomass accumulation, alterations in photosynthetic parameters and disruption in primary

metabolism. Transfer to elevated [CO₂] stimulated growth and most of primary metabolism of GA-deficient plants at 21 dag, but not 35 dag. We discuss the influence of elevated [CO₂] on the growth of tomato plants with reduced GA content and how growth can be influenced by developmental stage in tomato plants submitted to elevated [CO₂].

Material and methods

Growth conditions and experimental design

Seeds of tomato (*Solanum lycopersicum* L.) cv. Moneymaker and GA deficient *gib-1* mutants (kindly donated by M. Koornneef, Max Planck Institute for Plant breeding Research, Cologne, Germany) were germinated in Petri dish containing two layers of filter paper soaker in distilled water. After germination, the seedlings were transferred to pots (1,7L) containing commercial substrate (Tropstrato HT Hortaliças, Vida Verde), supplemented with NPK 20:5:20 fertilizer and cultivated as previously described in Vicente et al. (2015). Twenty-one and 35 days after germination, tomato plants were transferred to open top chamber under either ambient (400 µmol mol⁻¹) and elevated (750 µmol mol⁻¹) [CO₂]. Plants were maintained inside the open top chambers for 21 days. The experiment was conducted in greenhouse localized at Universidade Federal de Viçosa (20° 45'S, 42° 15'W).

Growth analysis

Stem length was measured every two days. At the end of the 21-day period at [CO₂] treatment, plants were harvested and divided into leaves, stem and roots. Leaf area was measured using a planimeter (Li-Cor Model 3100 Area Meter, Lincoln, NE, USA). Shoot and root biomass were measured from the dry weight of leaves, stem and

128 roots. Relative growth ratio (RGR) and specific leaf area (SLA) were determined as
129 described by Hunt (1982).

130

131 *Leaf anatomy*

132 Leaf discs were collected from the center of the third leaf and fixed in FAA50
133 (Formaldehyde, acetic acid and ethanol 50%) for 48 h, and then stored in ethanol 70%
134 according to Johansen (1940). Then the plant material was dehydrated in ethanolic
135 series and included in methacrylate (Historesin-Leica), according to the manufacturer's
136 recommendations. For light microscope observation (AX-70 TRF, Olympus Optical,
137 Tokyo, Japan), cross sections 5 μm thick were obtained with an automatic advanced
138 rotary microtome (model RM2155, Leica microsystems Inc., Deerfield, USA), were
139 stained with toluidine blue then photographed using a digital camera (Zeiss AxioCam
140 HRc, Göttinger, Germany). Anatomical features, as leaf thickness and thickness cell
141 layers, were evaluated using Image J (NIH, Bethesda, Ma).

142

143 *Gas exchanges and fluorescence measurements*

144 The net rate of carbon assimilation (A), stomatal conductance (g_s), internal CO_2
145 concentration (C_i) and fluorescence parameters were measured in third or fourth fully-
146 expanded leaf from the botton, using infrared gas analyzer (Li 6400XT, Li-Cor,
147 Lincoln, USA) equipped with integrated fluorescence chamber head (Li-6400-40; Li-
148 Cor Inc.). The measurements were conducted with $[\text{CO}_2]$ supply of 400 and 750 μmol
149 $\text{CO}_2 \text{ mol}^{-1}$ air with artificial photosynthetically active radiation level of 500 μmol of
150 photons $\text{m}^{-2} \text{s}^{-1}$, matching the greenhouse irradiance value. The rate of mitochondrial
151 respiration in darkness (R_D) was measured on dark-adapted leaves for at least 2 hours
152 after the end of the light period. Using the values of these parameter, mitochondrial

153 respiration in light (R_L) was estimated according to Lloyd et al. (1995) as $R_L = [0.5 -$
 154 $0.05 \ln(\text{PPFD})] \times R_D$. The photorespiratory rate of Rubisco (R_P) was estimated according
 155 Valentini et al. (1995), as $R_P = 1/12[\text{ETR} - 4(A + R_L)]$. A/C_i curves were measured at
 156 saturating irradiance ($1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) as described Barbosa et al. (2018).
 157 From these curves, maximum rate of carboxylation (V_{cmax}) and the maximum rate of
 158 electron transport (J_{max}) were calculated as proposed by Sharkey et al. (2007).

159

160 *Determination of metabolic levels*

161 Samples of third leaf, midpoint region of stem and root were collected,
 162 immediately frozen in liquid nitrogen, and stored at -80°C until further analysis.
 163 Glucose, fructose, sucrose and starch levels were determined as described by Fernie et
 164 al. (2001). Amino acids levels were determined as described by Gibon et al. (2004).
 165 Protein levels were measured as described by Bradford (1976), with modifications
 166 (Gibon et al. 2004). The levels of nitrate were measured as described by Fritz et al.
 167 (2006). Chlorophylls were extracted using acetone 80% and the content of them was
 168 determined as described by Lichtenthaler (1987). The levels of all others metabolites
 169 were quantified by gas chromatography mass spectrometry (GC-MS) following the
 170 protocol described by Lisec et al. (2006).

171

172 *Statistical analysis*

173 The experiments were designed in a completely randomized distribution.
 174 Differences described are systematically statistically grounded based on ANOVA,
 175 where $P < 0.05$ was considered significant. If ANOVA showed significant effects,
 176 Student's t test ($P < 0.05$) was used to determine differences between each treatment and

control. All statistical analyses were made using Statistical Package for the Social Sciences for Windows statistical software (SPSS).

Results

Elevated [CO₂] stimulates impaired growth of the gib-1 mutant

The *gib-1* tomato mutant shows impaired growth due to reduction in the activity of ent-copalyl diphosphate synthase, a key enzyme in the GA biosynthesis pathway (Bensen and Zeevaart 1990). Under ambient [CO₂], *gib-1* plants showed severe reductions in shoot (-82%) and root (-29%) biomass, as well as leaf area (-80%) and stem length (-77%), compared with WT plants (Fig. 1). In addition, SLA and RGR were lower in *gib-1* mutants, compared with WT plants at ambient [CO₂] (Supplementary Figure 1). Elevated [CO₂] did not affect shoot and root biomass of WT plants but led to increased leaf area (22%) and stem length (41%), compared with WT plants at ambient [CO₂] (Fig. 1). In the *gib-1* mutant, elevated [CO₂] restored shoot and root biomass, as well as leaf area, to similar values as WT plants at ambient [CO₂] (Fig. 1). Elevated [CO₂] doubled the stem length in the *gib-1* mutant, compared to *gib-1* at ambient [CO₂], but still fell short of the stem length of WT plants at ambient [CO₂] (Fig. 1 d). *gib-1* plants in elevated CO₂ also showed SLA and RGR values similar to those of ambient [CO₂] WT plants (Supplementary Figure 1).

Elevated [CO₂] restores leaf anatomy in gib-1 plants

Given the stimulatory effects of GAs and elevated [CO₂] on cell expansion and division (Taylor et al. 1994, 2005; Achard et al. 2009) we decided to analyze how elevated [CO₂] influences leaf anatomy of *gib-1* mutant and WT plants. Under ambient [CO₂], leaf cross-sections revealed considerable visual differences between *gib-1*

mutants and WT plants (Fig. 2 a and b). Leaf thickness was increased (35%) in *gib-1* compared to WT due to a 18% and 62% increase in the thickness of palisade and spongy parenchyma, respectively (Fig. 2 e, f and g). The greater thickness of spongy parenchyma resulted in the reduction of the palisade-to-spongy parenchyma ratio in *gib-1* (Fig. 2 h)

Under elevated [CO₂], *gib-1* leaf cross-sectional appearance was very similar to that of WT plants at ambient [CO₂] (Fig. 2 a and d). At elevated [CO₂], total leaf thickness, palisade and spongy parenchyma thickness, and the palisade-to-spongy parenchyma ratio of *gib-1* plants were similar to those of WT plants at ambient [CO₂] (Fig. 2 e, f, g and h). Thickness of either upper or lower epidermis was not altered in *gib-1* between [CO₂] levels (Fig. 2 i and j). As for WT plants, most leaf anatomical parameters were similar between treatments, except for upper epidermis thickness, which was reduced in elevated [CO₂] plants compared to ambient [CO₂] (Fig. 2).

Elevated [CO₂] restore photosynthetic function in the gib-1 mutant

We next investigated the combined effects of reduction in endogenous GA content and elevated [CO₂] on gas exchange in *gib-1* mutant tomato plants. Under ambient [CO₂], the *gib-1* mutant showed a marked reduction (~45%) in A, compared with WT plants (Fig. 3 a). In addition, the *gib-1* mutation led to reductions in R_D (-52%), V_{max} (-45%), J_{max} (-37%), R_P (-41%) and ETR (-44%) at ambient [CO₂]. Under ambient [CO₂], C_i was unaffected in the *gib-1* mutant, compared with WT plants (Fig. 3)

Under elevated [CO₂], A, R_D and C_i values were higher in both WT and *gib-1* compared to ambient [CO₂] WT plants (Fig. 3 a, b and g). Elevated [CO₂] increased J_{max} in *gib-1* but not in WT plants, compared to ambient [CO₂] WT plants (Fig. 3 d). R_P was

considerably reduced in both genotypes at elevated $[CO_2]$ (Fig. 3 e). Interestingly, g_s remained stable in WT and *gib-1* under both ambient and elevated $[CO_2]$ (Fig. 3 h). The F_v/F_m ratio was close to ideal values for non-stressed leaves (~0.83) in WT plants and *gib-1* mutant in both $[CO_2]$ regimes (Fig. 3 i)

231

232 *Nitrogen metabolism is altered by elevated $[CO_2]$ in *gib-1* mutant plants*

Elevated $[CO_2]$ typically reduces nitrogen content in the tissues of some plant species (Bloom et al. 2002, 2010, 2014; Taub and Wang 2008). We thus determined the content of some of the main nitrogenous compounds in leaves, stem and roots of WT and *gib-1* plants under ambient and elevated $[CO_2]$. Nitrate levels remained generally unchanged across treatments, except for an increase in *gib-1* at ambient $[CO_2]$ (Supplementary Fig. 2). Alterations in amino acids levels were found for the *gib-1* mutant at ambient $[CO_2]$. Whereas amino acids levels were reduced in leaf (35%), an increase was observed in the stem (76%) and roots (41%) (Supplementary Fig. 2). At elevated $[CO_2]$, amino acids levels remained unaltered in WT plants and *gib-1* mutants, compared with WT plants at ambient $[CO_2]$. At ambient $[CO_2]$, leaf protein content was reduced by 24%, while it increased approximately four-fold in stems of *gib-1* mutants (Supplementary Fig. 2). Leaf protein was lower in *gib-1* mutant and WT plants under elevated $[CO_2]$ (Supplementary Fig. 2). In the roots, protein levels were increased in both *gib-1* and WT plants at elevated $[CO_2]$ (Supplementary Fig. 2). Under ambient $[CO_2]$, chlorophyll analysis of *gib-1* mutants showed increases of 18% and 65% in the leaf and stem, respectively. Elevated $[CO_2]$ did not affect leaf chlorophyll content in WT plants.

250

251 *Changes in carbohydrate content and partitioning associated with GA content and* 252 *elevated [CO₂]*

253 Photosynthetic assimilation of CO₂ and GA are coupled to carbon metabolism
254 and consequently to carbohydrates content (Ainsworth et al. 2002; Ainsworth and Long
255 2005; Leakey et al. 2009; Paparelli et al. 2013). Since carbohydrates are essential to the
256 fundamental process required to plant growth (Eveland and Jackson 2012), we
257 investigated the levels of soluble sugars and starch in WT and *gib-1* mutant grown both
258 at ambient and elevated [CO₂]. Under ambient [CO₂], *gib-1* mutants showed reduced
259 levels of leaf and stem glucose (Supplementary Fig. 3) compared with WT plants at
260 ambient [CO₂]. By contrast, glucose level in roots increased in *gib-1* mutants at ambient
261 [CO₂] (Supplementary Fig. 3). Under ambient [CO₂], the reduction of leaf fructose in
262 *gib-1* mutants was accompanied by an increase in stem and roots in these plants,
263 compared with WT plants at ambient [CO₂]. The levels of sucrose showed a similar
264 pattern as those of fructose in *gib-1* plants grown at ambient [CO₂]. Growth of *gib-1*
265 mutant at ambient [CO₂] resulted in reduced leaf starch.

266 Elevated [CO₂] did not affect glucose levels in WT plants but restored them in
267 leaves of *gib-1* mutants. Although higher than *gib-1* at ambient [CO₂], the level of
268 glucose in stem was reduced in *gib-1* mutants grown at elevated [CO₂]. Moreover, the
269 level of glucose in the root increased in *gib-1* mutant at elevated [CO₂]. Although
270 elevated [CO₂] did not affect fructose content in leaf, it increased it in stem and roots of
271 WT plants, compared with WT plants at ambient [CO₂]. At elevated [CO₂], *gib-1*
272 mutants showed increased fructose content in all plant organs analyzed, compared with
273 WT plants at ambient [CO₂]. Elevated [CO₂] did not affect sucrose content in WT plants
274 but restored it in the *gib-1* mutant. The level of starch increased in WT plants and *gib-1*

275 mutants under elevated [CO₂], compared with WT plants at ambient [CO₂]
276 (Supplementary Fig. 3).

277

278 *Elevated [CO₂] modifies part of the metabolic profile in gib-1 mutant*

279 To investigate how the metabolism of each organ was modified by GA and
280 elevated [CO₂], we built a metabolic profile in WT and *gib-1* plants under ambient and
281 elevated [CO₂] (Fig. 4).

282 Under ambient [CO₂], the levels of 34 (out of 40) leaf metabolites were affected
283 in the *gib-1* mutant, of which 30 were reduced and 4 increased compared with WT
284 plants at ambient [CO₂] (Fig. 4). Among the metabolites whose level increased are 3-
285 PGA, trehalose, oxalic acid and leucine. Elevated [CO₂] affected few leaf metabolites in
286 WT plants and restored the levels of 26 of the 34 metabolites affected in the *gib-1*
287 mutant grown at ambient [CO₂]. The levels of galactinol, glyceric acid, glycerol,
288 mannose, 2-OG and proline were reduced in leaf both WT plants and *gib-1* mutants at
289 elevated [CO₂], while citric acid was increased. Elevated [CO₂] increased the level of
290 leaf GABA only in the *gib-1* mutant. Myo-inositol, lactate, glycine, ornithine, serine
291 and valine remained unchanged in WT plants and *gib-1* mutant under both ambient and
292 elevated [CO₂].

293 Thirty-three metabolites were detected in the stem by GC-MS. In *gib-1* mutant
294 grown at ambient [CO₂], 13 metabolites were reduced, while 11 were increased,
295 compared with WT plants at ambient [CO₂]. Most metabolites were not affected by
296 elevated [CO₂] in the stem of WT plants, compared with WT plants at ambient [CO₂].
297 However, the level of galactinol, mannose, malate and serine showed reduced and
298 fructose increased levels in WT plants grown at elevated [CO₂] in relation to WT plants
299 at elevated [CO₂]. Elevated [CO₂] restored 19 of 24 metabolites affected in stems of

300 *gib-1* mutants at ambient [CO₂]. The level of fructose, asparagine, glutamate, glutamine
301 remained increased, while malate and serine were reduced in *gib-1* mutants at elevated
302 [CO₂], compared with WT at ambient [CO₂]. No change was observed in myo-inositol,
303 lactate, pyruvate, GABA, glycine, leucine, valine and β-alanine (Fig. 4).

304 In roots, most amino acids were increased in *gib-1* mutants at ambient [CO₂]. In
305 addition, the level of fructose, glucose, 3-PGA, sucrose and 2-OG also increased in the
306 root of *gib-1* mutants, compared with WT at ambient [CO₂]. The level of fumarate,
307 malate and oxalacetate decreased in *gib-1* mutants grown at ambient [CO₂]. Elevated
308 [CO₂] led to increase in the level of fructose, galactinol, 3-PGA, succinate, glycine,
309 phenylalanine, tyrosine and β-alanine in WT plants and *gib-1* mutant, while reducing
310 the level of malate in these plants compared with WT plants at ambient [CO₂]. At
311 elevated [CO₂], an increase in glucose, citrate, isocitrate, glutamate and serine was
312 observed only in the roots of *gib-1* mutant, compared with WT plants at ambient [CO₂].
313 Lastly, no changes were observed in myo-inositol, aconitate, lactate, pyruvate, GABA,
314 ornithine and proline in the roots (Fig. 4).

315

316 *Elevated [CO₂] stimulates growth in tomato plants 21 dag, but not 35 dag*

317 The effect of elevated [CO₂] on plant growth and development was described as
318 age-dependent (Ainsworth 2008; Franks 2013). Thus, the influence of [CO₂] on growth,
319 gas exchange and primary metabolism was evaluated in WT and *gib-1* mutant submitted
320 at 35 dag at ambient and elevated [CO₂]. Under ambient [CO₂], *gib-1* showed a drastic
321 reduction in growth, biomass (~93%) and leaf area (~95%), compared to WT plants
322 (Fig. 5). Growth of WT and *gib-1* at elevated [CO₂] did not affect the growth
323 parameters evaluated at 35 dag (Fig. 5 b and c).

Under ambient [CO₂], reduction GA content in *gib-1* mutant decreases *A* (~35%), *R_D* (~19%) and *R_p* (~40%) at 35 dag, compared with WT (Fig 6 a, d and e). Growth in elevated [CO₂] increase *A* and *C_i*, while reduced *R_p* in both, WT and *gib-1* (Fig. 6 a, c and e). No alterations in WT plants was observed in *R_D*, however, these parameters were decreased in *gib-1* mutant at elevated [CO₂], compared to WT plants under ambient [CO₂]. *g_s* remained stable in WT and *gib-1* under both ambient and elevated [CO₂] (Fig. 6 b). The *F_v*/*F_m* ratio was close to ideal values for non-stressed leaves (~0.83) (Fig. 6 f).

Sugar evaluation of *gib-1* mutant submitted to ambient [CO₂] at 35 dag shows reduction in leaf glucose (~50%) and fructose (~87%) and increased in sucrose compared to WT plants at ambient [CO₂]. Under elevated [CO₂] WT plants did not differ in leaf sugar content when compared to WT plants at ambient [CO₂]. *gib-1* mutants kept reduction on glucose (~51%), fructose (~88%), and increase in sucrose (~27%) at elevated [CO₂] (Supplementary Fig. 4)

Discussion

Elevated [CO₂] stimulates growth at least in part in a GA-independent manner in *Arabidopsis* treated with GA synthesis inhibitor (PAC) (Ribeiro et al. 2012). Here, we evaluated the effect of [CO₂] in tomato mutants with drastic reduction in GA content. GA regulates major aspects of growth and here we show that elevated [CO₂] can directly stimulate mechanisms that compensate GA deficiency in *gib-1* mutants. However, this effect is strongly dependent on plant age.

Source-sink relationships determines carbohydrate allocation

Alterations in endogenous level of GAs change the pattern of growth and biomass allocation (Nagel et al. 2001). The concentration of GAs in the *gib-1* mutant is insufficient to maintain normal leaf and stem growth, however, a less drastic effect is observed on root growth (Fig. 1). The marked reduction in growth and shoot biomass allocation impaired RGR in *gib-1* mutants (Supplementary Fig. 1). RGR has a positive relation with leaf mass ratio and SLA (Gleeson and Tilman 1992; Poorter and van der Werf 1998), parameters that are strongly affected by GA. Elevated [CO₂] reestablished biomass allocation and SLA in *gib-1* mutants, which in turn influenced positively RGR.

Leaf anatomy influences photosynthetic capacity, determining the diffusion of CO₂ through the mesophyll (Terashima et al. 2011; Tomás et al. 2013). Reduction in GA content acts as a factor disrupting growth and leaf development, since GA acts in the cell division, cell expansion and mesophyll organization (Jiang et al. 2012). Impaired leaf expansion in GA-deficient plants leads to increased number of cells per unit leaf area (Jiang et al. 2012), giving *gib-1* the appearance of a highly packed mesophyll (Fig. 2). In addition, increased mesophyll thickness influenced the reduction of SLA in *gib-1* mutant under ambient [CO₂] (Supplementary Figure 1).

The increase in leaf thickness accompanied by the reduction in intercellular spaces in the *gib-1* mutant may have contributed to the reduction in *A*, since they restrict the diffusion of CO₂ through the mesophyll and to the carboxylation site of Rubisco (Evans and Caemmerer 1996; Terashima et al. 2001). Furthermore, reduced lamina size and overlapping leaves may cause self-shading in *gib-1*, which also impairs photosynthetic capacity.

Although *gib-1* presented reduced *A* (Fig. 3), CO₂ fixation probably exceeded the demand for growth, leading to the accumulation of carbohydrates in the stem and root (Supplementary Fig. 3). Stem storage of excess photoassimilate during periods of

low sink strength buffers against source-sink changes during the different stages of growth (Slafer 2003). Imbalance between source and sink can lead to downregulation of photosynthesis due to accumulation of non-structural carbohydrates (such as soluble sugars and starch) in leaves (Stitt and Krapp 1999; Ainsworth and Bush 2011; Sugiura et al. 2017). Thus, the allocation of carbohydrates to the stem could be a way of delaying photosynthetic inhibition in *gib-1* mutants. In addition, the larger fraction of carbohydrate allocated to the root supports root respiration, which is less affected by GA deficiency than shoot respiration (Nagel and Lambers 2002).

In general, elevated $[CO_2]$ increases carbon assimilation and the availability of carbohydrates, which contribute to increased plant growth (Ainsworth and Long 2005; Teng et al. 2006; Li et al. 2013). However, it is the ability to grow and produce new sinks that determines photoassimilates consumption, whose accumulation could result in the inhibition of photosynthesis. Dark respiration (R_D) is closely related to carbon balance and therefore the availability of carbohydrates can interfere in R_D . Elevated $[CO_2]$ accelerate the accumulation of carbohydrates, which leads to transcriptional up-regulation of genes associated with respiration pathways and R_D stimulus (Li et al. 2008; Leakey et al. 2009; Markelz et al. 2014; Watanabe et al. 2014). Thus, the increase in R_D observed in WT and *gib-1* mutants can be attributed to the increase in A and consequently to the increase of carbohydrates under elevated $[CO_2]$ (Fig. 3 and Supplementary Fig. 3).

Elevated $[CO_2]$ induces metabolic homeostasis in GA deficient plants

Plant growth is dependent on the interaction between carbon and nitrogen metabolism, which are linked by the tricarboxylic acid (TCA) cycle (Nunes-Nesi et al. 2010). Although the physiological function of genes regulated by GAs has been

398 addressed (Yamaguchi 2008), studies on the effect of GAs on energy metabolism are
 399 scarce. We did not observe any drastic differences in metabolites levels between
 400 ambient and elevated CO₂ treatments for WT plants (Fig. 4). Enhanced plant growth
 401 under elevated [CO₂] does not induce a massive remodeling of metabolism.
 402 Optimization of carbon and nitrogen acquisition under such conditions appears to be
 403 dependent mostly on fine-tuning of specific points of the metabolic network. GA-
 404 deficient plants, on the other hand, show a general reduction in levels of sugars and
 405 amino acids in the leaf and an increase in roots under ambient [CO₂] (Fig 4 and
 406 Supplementary Fig. 2, 3). This is probably a consequence of the alteration in carbon
 407 allocation in *gib-1*, whereby the mutation leads to increased root-to-shoot biomass ratio.

408 Elevated [CO₂] restored the levels of most metabolites in *gib-1* to the level of
 409 WT (Fig. 4). Alterations in the TCA cycle have been shown to influence GA levels
 410 (Margaretha et al. 2009; Araújo et al. 2012), as multiple enzymes in the GA
 411 biosynthetic pathway are dependent on a TCA cycle intermediate, 2-oxoglutarate. On
 412 the other hand, it is not clear if the reverse is true, *i.e.* how does altered GA impact
 413 primary metabolism?

414 GABA levels increased only in the leaves of *gib-1* under elevated [CO₂] (Fig. 4).
 415 Increases in GABA concentration occur in response to extreme conditions, like
 416 temperature, dehydration, salinity, oxygen stress (Kinnersley and Turano 2000; Bouché
 417 and Fromm 2004). GABA provides an alternative pathway for the conversion of alpha
 418 ketoglutarate to succinate in the TCA cycle, and compromising the enzymes of the TCA
 419 cycle involved in the steps up to the production of succinate alters GABA shunt activity
 420 (Lemaitre et al. 2007; Fait et al. 2008). Exogenous GABA application improved growth
 421 of *Zea mays*, *Stellaria longipes* and *Lemna*, possibly by inducing cell elongation and
 422 division or/and by maintaining metabolic balance within plant tissues (Kathiresan et al.

1998; Kinnersley and Lin 2000; Li et al. 2016). Thus, elevated [CO₂] in plants with reduction in GA can influence GABA content and consequently carbon flux, ameliorating *gib-1* growth at elevated [CO₂].

The effect of elevated [CO₂] on gib-1 is age-dependent

Both elevated [CO₂] and GA influence the expression of genes related to loosening and rearrangement of the cell wall, besides controlling the rate of cellular proliferation (Vogler et al. 2003; Yang et al. 2004; Achard et al. 2009; Ribeiro et al. 2012). We showed that GA, an essential hormone for the normal growth and development of plants, is dispensable for growth in tomato plants under elevated [CO₂]. Interestingly, we observed that the effects of elevated [CO₂] on tomato growth is age-dependent, regardless of GA content (Fig. 5). These results indicate the existence of a “sensitive phase” in which elevated [CO₂] is able to influence growth in plants. In addition to CO₂, the action of other environmental factors is also restricted to the “sensitive phase” in tomato plants. Calvert (1957) showed that the influence of temperature on tomato flowering occurs in the first two or three weeks from the seedling emergence. This stage corresponds to our treatment of plants submitted to elevated [CO₂] with 21dag.

In Arabidopsis the most pronounced growth under elevated [CO₂] was observed during the vegetative stage (Watanabe et al. 2014). As observed in Arabidopsis, the sensitivity of tomato plants to elevated [CO₂] may be linked to the juvenile phase. The efficiency of enzymes that promote cell expansion and, consequently, plant growth depends on factors controlled by the stage of development, which limits growth to specific periods (Sloan et al. 2009). Addition of new extracellular polymers and remodeling of existing components in the primary cell walls marks the exponential

448 phase of cell expansion. This is followed by cell wall thickening and rigidification to
 449 create secondary cell walls that enhance structural integrity, but reduce cell wall
 450 extension (Hall and Ellis 2013). Furthermore, the transition from the juvenile to the
 451 adult phase can determine plant architecture and growth pattern (Huijser and Schmid
 452 2011; Poethig 2013). Phase transition is preceded by a change in the competence of the
 453 shoot to respond to stimuli that induce reproductive development (Poethig 2013). The
 454 changes in meristem identity during phase transition are accompanied by genetic
 455 reprogramming that may trigger changes in leaf and stem morphology, as well as
 456 alteration in growth rate (Poethig 2010, 2013). With the development of floral organs
 457 most of the photoassimilates are destined for the production of flowers and fruits and
 458 for the maintenance of respiration of reproductive structures (Obeso 2002).

459 In conclusion, elevated [CO₂] favors photosynthesis and carbohydrate
 460 production, regardless of plant age. However, we showed here that plant age can
 461 indirectly influence carbon partitioning via changes in source-sink relationships. These
 462 changes are mostly driven by the growth phase, either juvenile or adult, whereby
 463 vegetative or reproductive structures will be favored as strong sinks. Plant hormones
 464 can act as integrators of growth and development, and GAs, in particular, control cell
 465 division and expansion. We have shown here that increased growth under elevated
 466 [CO₂] in tomato does not require a functional gibberellin biosynthetic pathway. In the
 467 juvenile phase, gibberellin-deficient mutants can grow to the same extent as wild-type
 468 plants. In the adult phase, however, elevated [CO₂] does not stimulate growth and
 469 gibberellin mutants show their characteristic stunted growth phenotype. This suggests
 470 that growth stimulation by [CO₂] is highly dependent on plant developmental stage,
 471 possibly linked to the juvenile-to-adult phase transition (Fig. 7). Further work should

472 explore the potential role of other hormones mediating growth stimulation by elevated
473 [CO₂].

474

475 **Author contribution statement**

476 KG conducted experiments and wrote the manuscript. LCC, performed the experiments
477 and statistical analysis. FALB, FBC and TMP performed the experiments. WLA
478 contributed reagents, materials, and analysis tools. DMR designed the experiments. AZ
479 finalized manuscript writing. All authors reviewed the final version of the manuscript
480 and approved it.

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486

487 **References**

- 488 Achard P, Gusti A, Cheminat S, et al (2009) Gibberellin Signaling Controls Cell
489 Proliferation Rate in Arabidopsis. *Curr Biol* 19:1188–1193. doi:
490 10.1016/j.cub.2009.05.059
- 491 Ainsworth EA (2008) Rice production in a changing climate: a meta-analysis of
492 responses to elevated carbon dioxide and elevated ozone concentration. *Glob*
493 *Chang Biol* 14:1642–1650. doi: 10.1111/j.1365-2486.2008.01594.x
- 494 Ainsworth EA, A. DP, J. BC, et al (2002) A meta-analysis of elevated [CO₂] effects
495 on soybean (*Glycine max*) physiology, growth and yield. *Glob Chang Biol* 8:695–
496 709. doi: 10.1046/j.1365-2486.2002.00498.x
- 497 Ainsworth EA, Bush DR (2011) Carbohydrate Export from the Leaf: A Highly
498 Regulated Process and Target to Enhance Photosynthesis and Productivity. *Plant*
499 *Physiol* 155:64–69. doi: 10.1104/pp.110.167684
- 500 Ainsworth EA, Long SP (2005) What have we learned from 15 years of free-air
501 CO₂enrichment (FACE)? A meta-analytic review of the responses of
502 photosynthesis, canopy properties and plant production to rising CO₂. *New Phytol*
503 165:351–372. doi: 10.1111/j.1469-8137.2004.01224.x
- 504 Ainsworth EA, Rogers A (2007) The response of photosynthesis and stomatal
505 conductance to rising [CO₂]: Mechanisms and environmental interactions. *Plant,*
506 *Cell Environ* 30:258–270. doi: 10.1111/j.1365-3040.2007.01641.x
- 507 Ainsworth EA, Rogers A, Nelson R, Long SP (2004) Testing the “source-sink”
508 hypothesis of down-regulation of photosynthesis in elevated [CO₂] in the field
509 with single gene substitutions in *Glycine max*. *Agric For Meteorol* 122:85–94. doi:

10.1016/j.agrformet.2003.09.002

Alvey L, Harberd NP (2005) DELLA proteins: integrators of multiple plant growth regulatory inputs? *Physiol Plant* 123:153–160

Araújo WL, Martins AO, Fernie AR, Tohge T (2014) 2-Oxoglutarate: linking TCA cycle function with amino acid, glucosinolate, flavonoid, alkaloid, and gibberellin biosynthesis. *Front Plant Sci* 5:1–6. doi: 10.3389/fpls.2014.00552

Araújo WL, Tohge T, Osorio S, et al (2012) Antisense Inhibition of the 2-Oxoglutarate Dehydrogenase Complex in Tomato Demonstrates Its Importance for Plant Respiration and during Leaf Senescence and Fruit Maturation. *Plant Cell* 24:2328–2351. doi: 10.1105/tpc.112.099002

Arp WJ (1991) Effects of source-sink relations on photosynthetic acclimation to elevated CO₂. *Plant Cell Environ* 14:869–875. doi: 10.1111/j.1365-3040.1991.tb01450.x

Barbosa MAM, Chitwood DH, Azevedo AA, et al (2018) Bundle sheath extensions affect leaf structural and physiological plasticity in response to irradiance. *Plant Cell Environ* 0:. doi: 10.1111/pce.13495

Bensen RJ, Zeevaart JAD (1990) Comparison of Ent-kaurene synthetase A and B activities in cell-free extracts from young tomato fruits of wild-type and gib-1, gib-2, and gib-3 tomato plants. *J Plant Growth Regul* 9:237–242. doi: 10.1007/BF02041969

Bloom AJ, Burger M, Asensio JSR, Cousins AB (2010) Carbon dioxide enrichment inhibits nitrate assimilation in wheat and arabidopsis. *Science* (80-) 328:899–903. doi: 10.1126/science.1186440

Bloom AJ, Burger M, Kimball BA, Pinter PJ (2014) Nitrate assimilation is inhibited by elevated CO₂ in field-grown wheat. *Nat Clim Chang* 4:477–480. doi: 10.1038/nclimate2183

Bloom AJ, Smart DR, Nguyen DT, Searles PS (2002) Nitrogen assimilation and growth of wheat under elevated carbon dioxide. *Proc Natl Acad Sci* 99:1730–1735. doi: 10.1073/pnas.022627299

Bouché N, Fromm H (2004) GABA in plants: Just a metabolite? *Trends Plant Sci* 9:110–115. doi: 10.1016/j.tplants.2004.01.006

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248–254. doi: 10.1016/0003-2697(76)90527-3

Calvert A (1957) Effect of the early environment on the development of flowering in tomato I. Temperature. *J Hortic Sci* 32:9–17. doi: 10.1080/00221589.1959.11513954

Campbell WJ, Allen LH, Bowes G (1988) Effects of CO₂ Concentration on Rubisco Activity, Amount, and Photosynthesis in Soybean Leaves. *Plant Physiol* 88:1310–1316. doi: 10.1104/pp.88.4.1310

- 550 Carvalho RF, Campos ML, Pino LE, et al (2011) Convergence of developmental
551 mutants into a single tomato model system: “Micro-Tom” as an effective toolkit
552 for plant development research. *Plant Methods* 7:18. doi: 10.1186/1746-4811-7-18
- 553 de Lucas M, Davière J-M, Rodríguez-Falcón M, et al (2008) A molecular framework
554 for light and gibberellin control of cell elongation. *Nature* 451:480–484. doi:
555 10.1038/nature06520
- 556 Dill A, Jung HS, Sun TP (2001) The DELLA motif is essential for gibberellin-induced
557 degradation of RGA. *Proc Natl Acad Sci U S A* 98:14162–14167
- 558 Evans JR, Caemmerer S Von (1996) Carbon Dioxide Diffusion inside Leaves. *Plant*
559 *Physiol* 339–346
- 560 Eveland AL, Jackson DP (2012) Sugars, signalling, and plant development. *J Exp Bot*
561 63:3367–3377. doi: 10.1093/jxb/err379
- 562 Fait A, Fromm H, Walter D, et al (2008) Highway or byway: the metabolic role of the
563 GABA shunt in plants. *Trends Plant Sci* 13:14–19. doi:
564 10.1016/j.tplants.2007.10.005
- 565 Fernie AR, Roscher A, Ratcliffe RG, Kruger NJ (2001) Fructose 2,6-bisphosphate
566 activates pyrophosphate: fructose-6-phosphate 1-phosphotransferase and increases
567 triose phosphate to hexose phosphate cycling in heterotrophic cells. *Planta*
568 212:250–263. doi: 10.1007/s004250000386
- 569 Ferris R, Sabatti M, Miglietta F, et al (2001) Leaf area is stimulated in *Populus* by free
570 air CO₂ enrichment (POPFACE), through increased cell expansion and production.
571 *Plant, Cell Environ* 24:305–315. doi: 10.1046/j.1365-3040.2001.00684.x
- 572 Franks PJ (2013) Tansley review Sensitivity of plants to changing atmospheric CO₂
573 concentration□: from the geological past to the next century. *New Phytol*
574 197:1077–1094
- 575 Fritz C, Palacios-Rojas N, Feil R, Stitt M (2006) Regulation of secondary metabolism
576 by the carbon–nitrogen status in tobacco: nitrate inhibits large sectors of
577 phenylpropanoid metabolism. *Plant J* 46:533–548. doi: 10.1111/j.1365-
578 313X.2006.02715.x
- 579 Galmés J, Molins A, Flexas J, Conesa MÀ (2017) Coordination between leaf CO₂
580 diffusion and Rubisco properties allows maximizing photosynthetic efficiency in
581 *Limonium* species. *Plant Cell Environ* 2081–2094. doi: 10.1111/pce.13004
- 582 Gibon Y, Blaessing OE, Hannemann J, et al (2004) A Robot-Based Platform to Measure
583 Multiple Enzyme Activities in *Arabidopsis* Using a Set of Cycling Assays:
584 Comparison of Changes of Enzyme Activities and Transcript Levels during
585 Diurnal Cycles and in Prolonged Darkness. *Plant Cell* 16:3304 LP-3325. doi:
586 10.1105/tpc.104.025973
- 587 Gleeson SK, Tilman D (1992) Plant allocation and the multiple limitation hypothesis.
588 *Am Nat* 139:1322–1343. doi: 10.3109/03630269109027902
- 589 Hall H, Ellis B (2013) Transcriptional programming during cell wall maturation in the
590 expanding *Arabidopsis* stem. *BMC Plant Biol* 13:. doi: 10.1186/1471-2229-13-14

- 591 Hedden P, Thomas SG (eds) (2016) The Gibberellins - Annual Plant Reviews 49. John
592 Wiley, Chichester, UK
- 593 Högy P, Keck M, Niehaus K, et al (2010) Effects of atmospheric CO₂ enrichment on
594 biomass, yield and low molecular weight metabolites in wheat grain. J Cereal Sci
595 52:215–220. doi: <https://doi.org/10.1016/j.jcs.2010.05.009>
- 596 Huijser P, Schmid M (2011) The control of developmental phase transitions in plants.
597 Development 138:4117–4129. doi: 10.1242/dev.063511
- 598 Hunt R (1982) Plant growth curves. The functional approach to plant growth analysis.
599 Edward Arnold Ltd., London
- 600 Igamberdiev AU (2015) Control of Rubisco function via homeostatic equilibration of
601 CO₂ supply. Front plant 6:1–9. doi: 10.3389/fpls.2015.00106
- 602 Jiang X, Li H, Wang T, et al (2012) Gibberellin indirectly promotes chloroplast
603 biogenesis as a means to maintain the chloroplast population of expanded cells.
604 Plant J 72:768–780. doi: 10.1111/j.1365-3113.2012.05118.x
- 605 Johansen DA (1940) Plant microtechnique. McGraw-Hill Publishing Company, Ltd.,
606 London
- 607 Kathiresan A, Miranda J, Chinnappa CC, Reid DM (1998) γ -aminobutyric acid
608 promotes stem elongation in *Stellaria longipes*: The role of ethylene. Plant Growth
609 Regul 26:131–137. doi: 10.1023/A:1006107815064
- 610 Kimball BA (2016) Crop responses to elevated CO₂ and interactions with H₂O, N, and
611 temperature. Curr Opin Plant Biol 31:36–43. doi: 10.1016/J.PBI.2016.03.006
- 612 Kimura S, Sinha N (2008) Tomato (*Solanum lycopersicum*): A Model Fruit-Bearing
613 Crop. CSH Protoc 2008:pdb.emo105
- 614 Kinnersley AM, Lin F (2000) Receptor modifiers indicate that 4-aminobutyric acid
615 (GABA) is a potential modulator of ion transport in plants. Plant Growth Regul
616 32:65–76. doi: 10.1023/A:1006305120202
- 617 Kinnersley AM, Turano FJ (2000) Gamma aminobutyric acid (GABA) and plant
618 responses to stress. CRC Crit Rev Plant Sci 19:479–509. doi:
619 10.1080/07352680091139277
- 620 Koornneef M, Bosma TDG, Hanhart CJ, et al (1990) The isolation and characterization
621 of gibberellin-deficient mutants in tomato. Theor Appl Genet 80:852–857. doi:
622 10.1007/BF00224204
- 623 Lancien M, Gadal P, Hodges M (2000) Enzyme Redundancy and the Importance of 2-
624 Oxoglutarate in Higher Plant Ammonium Assimilation. Plant Physiol 123:817–
625 824. doi: 10.1104/pp.123.3.817
- 626 Lastdrager J, Hanson J, Smeekens S (2014) Sugar signals and the control of plant
627 growth and development. J Exp Bot 65:799–807. doi: 10.1093/jxb/ert474
- 628 Leakey ADB, Ainsworth EA, Bernacchi CJ, et al (2009) Elevated CO₂ effects on plant
629 carbon, nitrogen, and water relations: six important lessons from FACE. J Exp Bot

- 630 60:2859–2876. doi: 10.1093/jxb/erp096
- 631 Lemaitre T, Urbanczyk-Wochniak E, Flesch V, et al (2007) NAD-Dependent Isocitrate
632 Dehydrogenase Mutants of Arabidopsis Suggest the Enzyme Is Not Limiting for
633 Nitrogen Assimilation. *Plant Physiol* 144:1546 LP-1558. doi:
634 10.1104/pp.107.100677
- 635 Li CR, Gan LJ, Xia K, et al (2002) Responses of carboxylating enzymes, sucrose
636 metabolizing enzymes and plant hormones in a tropical epiphytic CAM orchid to
637 CO₂ enrichment. *Plant, Cell Environ* 25:369–377. doi: 10.1046/j.0016-
638 8025.2001.00818.x
- 639 Li P, Ainsworth EA, Leakey ADB, et al (2008) Arabidopsis transcript and metabolite
640 profiles: Ecotype-specific responses to open-air elevated [CO₂]. *Plant, Cell*
641 *Environ* 31:1673–1687. doi: 10.1111/j.1365-3040.2008.01874.x
- 642 Li W, Liu J, Ashraf U, et al (2016) Exogenous γ -aminobutyric Acid (GABA)
643 Application Improved Early Growth, Net Photosynthesis, and Associated Physio-
644 Biochemical Events in Maize. *Front Plant Sci* 7:1–13. doi:
645 10.3389/fpls.2016.00919
- 646 Li X, Zhang G, Sun B, et al (2013) Stimulated leaf dark respiration in tomato in an
647 elevated carbon dioxide atmosphere. *Sci Rep* 3:2–9. doi: 10.1038/srep03433
- 648 Lichtenthaler HK (1987) Chlorophylls and Carotenoids: Pigments of Photosynthetic
649 Biomembranes. *Methods Enzymol* 148:350–382. doi: 10.1016/0076-
650 6879(87)48036-1
- 651 Lisec J, Schauer N, Kopka J, et al (2006) Gas chromatography mass spectrometry-based
652 metabolite profiling in plants. *Nat Protoc* 1:387–396. doi: 10.1038/nprot.2006.59
- 653 Liu J, Zhang J, He C, Duan A (2014) Genes responsive to elevated CO₂ concentrations
654 in triploid white poplar and integrated gene network analysis. *PLoS One* 9:1–11.
655 doi: 10.1371/journal.pone.0098300
- 656 Lloyd J, Wong SC, Styles JM, et al (1995) Measuring and Modelling Whole-Tree Gas
657 Exchange. *Funct Plant Biol* 22:987–1000
- 658 Margaretha JVDM, Osorio S, Moritz T, et al (2009) Decreased Mitochondrial Activities
659 of Malate Dehydrogenase and Fumarase in Tomato Lead to Altered Root Growth
660 and Architecture via. *Plant Physiol* 149:653–669. doi: 10.1104/pp.108.130518
- 661 Markelz RJC, Lai LX, Vosseler LN, Leakey ADB (2014) Transcriptional
662 reprogramming and stimulation of leaf respiration by elevated CO₂ concentration
663 is diminished, but not eliminated, under limiting nitrogen supply. *Plant, Cell*
664 *Environ* 37:886–898. doi: 10.1111/pce.12205
- 665 Masle J (2000) The effects of elevated CO₂ concentrations on cell division rates,
666 growth patterns, and blade anatomy in young wheat plants are modulated by
667 factors related to leaf position, vernalization, and genotype. *Plant Physiol*
668 122:1399–1415. doi: 10.1104/pp.122.4.1399
- 669 Nagel OW, Konings H, Lambers H (2001) The influence of a reduced gibberellin
670 biosynthesis and nitrogen supply on the morphology and anatomy of leaves and

- 671 roots of tomato (*Solanum lycopersicum*). *Physiol Plant* 111:40–45. doi:
672 10.1034/j.1399-3054.2001.1110106.x
- 673 Nagel OW, Lambers H (2002) Changes in the acquisition and partitioning of carbon and
674 nitrogen in the gibberellin-deficient mutants A70 and W335 of tomato (*Solanum*
675 *lycopersicum* L.). *Plant, Cell Environ* 25:883–891. doi: 10.1046/j.1365-
676 3040.2002.00871.x
- 677 Nunes-Nesi A, Fernie AR, Stitt M (2010) Metabolic and signaling aspects underpinning
678 the regulation of plant carbon nitrogen interactions. *Mol Plant* 3:973–996. doi:
679 10.1093/mp/ssq049
- 680 Obeso JR (2002) The costs of reproduction in plants. *New Phytol* 155:321–348. doi:
681 10.1046/j.1469-8137.2002.00477.x
- 682 Paparelli E, Parlanti S, Gonzali S, et al (2013) Nighttime Sugar Starvation Orchestrates
683 Gibberellin Biosynthesis and Plant Growth in Arabidopsis. *Plant Cell* 25:3760–
684 3769. doi: 10.1105/tpc.113.115519
- 685 Poethig RS (2010) The Past, Present, and Future of Vegetative Phase Change. *Plant*
686 *Physiol* 154:541–544. doi: 10.1104/pp.110.161620
- 687 Poethig RS (2013) Vegetative phase change and shoot maturation in plants. *Curr Top*
688 *Dev Bio* 105:125–152. doi: 10.1016/B978-0-12-396968-2.00005-1.Vegetative
- 689 Poorter H, van der Werf A (1998) Is inherent variation in RGR determined by LAR at
690 low light and by NAR at high light? Inherent Var plant growth *Physiol Mech Ecol*
691 consequences 309–336
- 692 Ribeiro DM, Araujo WL, Fernie AR, et al (2012) Action of Gibberellins on Growth and
693 Metabolism of Arabidopsis Plants Associated with High Concentration of Carbon
694 Dioxide. *Plant Physiol* 160:1781–1794. doi: 10.1104/pp.112.204842
- 695 Riou-Khamlichi C, Menges M, Healy JM, Murray J a (2000) Sugar control of the plant
696 cell cycle: differential regulation of Arabidopsis D-type cyclin gene expression.
697 *Mol Cell Biol* 20:4513–21. doi: 10.1128/MCB.20.13.4513-4521.2000
- 698 Rolland F, Moore B, Sheen J (2002) Sugar Sensing 2002 Rolland. *Plant Cell* 185–205.
699 doi: 10.1105/tpc.010455.S186
- 700 Santner A, Calderon-Villalobos LI a, Estelle M (2009) Plant hormones are versatile
701 chemical regulators of plant growth. *Nat Chem Biol* 5:301–307. doi:
702 10.1038/nchembio.165
- 703 Sharkey TD, Bernacchi CJ, Farquhar GD, Singsaas EL (2007) Fitting photosynthetic
704 carbon dioxide response curves for C3 leaves. *Plant, Cell Environ* 30:1035–1040.
705 doi: 10.1111/j.1365-3040.2007.01710.x
- 706 Slafer GA (2003) Genetic basis of yield as viewed from a crop physiologist's
707 perspective. *Ann Appl Biol* 142:117–128. doi: 10.1111/j.1744-
708 7348.2003.tb00237.x
- 709 Sloan J, Backhaus A, Malinowski R, et al (2009) Phased Control of Expansin Activity
710 during Leaf Development Identifies a Sensitivity Window for Expansin-Mediated

711 Induction of Leaf Growth. *Plant Physiol* 151:1844–1854. doi:
712 10.1104/pp.109.144683

713 Stitt M, Krapp A (1999) The interaction between elevated carbon dioxide and nitrogen
714 nutrition: The physiological and molecular background. *Plant, Cell Environ*
715 22:583–621. doi: 10.1046/j.1365-3040.1999.00386.x

716 Sugiura D, Watanabe CKA, Betsuyaku E, Terashima I (2017) Sink–source balance and
717 down-regulation of photosynthesis in *Raphanus sativus*: Effects of grafting, N and
718 CO₂. *Plant Cell Physiol* 58:2043–2056. doi: 10.1093/pcp/pcx132

719 Taub DR, Wang X (2008) Why are Nitrogen Concentrations in Plant Tissues Lower
720 under Elevated CO₂? A Critical Examination of the Hypotheses. *J Integr Plant Biol*
721 50:1365–1374. doi: 10.1111/j.1744-7909.2008.00754.x

722 Taylor G, Ranasinghe S, Bosac C, et al (1994) Elevated CO₂ and plant growth: cellular
723 mechanisms and responses of whole plants. *J Exp Bot* 45:1761–1774. doi:
724 10.1093/jxb/45.Special_Issue.1761

725 Taylor G, Street NR, Tricker PJ, et al (2005) The transcriptome of *Populus* in elevated
726 CO₂. *New Phytol* 167:143–154. doi: 10.1111/j.1469-8137.2005.01450.x

727 Teng N, Wang J, Chen T, et al (2006) Elevated CO₂ induces physiological, biochemical
728 and structural changes in leaves of *Arabidopsis thaliana*. *New Phytol* 172:92–103.
729 doi: 10.1111/j.1469-8137.2006.01818.x

730 Terashima I, Hanba YT, Tholen D, Niinemets U (2011) Leaf Functional Anatomy in
731 Relation to Photosynthesis. *Plant Physiol* 155:108–116. doi:
732 10.1104/pp.110.165472

733 Terashima L., Miyazawa S, Hanba YT (2001) Why are Sun Leaves Thicker than Shade
734 Leaves? - Consideration based on Analyses of CO₂ Diffusion in the Leaf. 93–
735 105

736 Tomás M, Flexas J, Copolovici L, et al (2013) Importance of leaf anatomy in
737 determining mesophyll diffusion conductance to CO₂ across species: quantitative
738 limitations and scaling up by models. *J Exp Bot* 64:2269–2281

739 Vicente MH, Zsögön A, Felicio A, et al (2015) Semi-determinate growth habit adjusts
740 the vegetative-to-reproductive balance and increases productivity and water-use
741 efficiency in tomato (*Solanum lycopersicum*). *J Plant Physiol* 177:11–19. doi:
742 10.1016/j.jplph.2015.01.003

743 Vogler H, Caderas D, Mandel T, Kuhlemeier C (2003) Domains of expansin gene
744 expression define growth regions in the shoot apex of tomato. *Plant Mol Biol*
745 53:267–272. doi: 10.1023/B

746 Wang L, Ruan Y-L (2013) Regulation of cell division and expansion by sugar and auxin
747 signaling. *Front Plant Sci* 4:1–9. doi: 10.3389/fpls.2013.00163

748 Wang Y, Li J (2008) Molecular Basis of Plant Architecture. *Annu Rev Plant Biol*
749 59:253–279. doi: 10.1146/annurev.arplant.59.032607.092902

750 Watanabe CK, Sato S, Yanagisawa S, et al (2014) Effects of elevated CO₂ on levels of

751 primary metabolites and transcripts of genes encoding respiratory enzymes and
752 their diurnal patterns in *Arabidopsis thaliana*: Possible relationships with respiratory
753 rates. *Plant Cell Physiol* 55:341–357. doi: 10.1093/pcp/pct185

754 Yamaguchi S (2008) Gibberellin Metabolism and its Regulation. *Annu Rev Plant Biol*
755 59:225–251. doi: 10.1146/annurev.arplant.59.032607.092804

756 Yang G-X, Jan A, Shen S-H, et al (2004) Microarray analysis of brassinosteroids- and
757 gibberellin-regulated gene expression in rice seedlings. *Mol Genet Genomics*
758 271:468–478. doi: 10.1007/s00438-004-0998-4

759

760 **Figure legends**

761 **Fig. 1** Effects of elevated [CO₂] on growth of wild type (WT) and *gib-1* mutant 21 dag.
762 a, phenotypes of WT and *gib-1* mutant grown at ambient and elevated [CO₂]. b, shoot
763 and root biomass. c, leaf area. d, stem length. Measurements were done in tomato plants
764 after 21 days of growing at 400 or 750 μmol CO₂ mol⁻¹. Asterisks indicate values
765 determined by Student's t test to be significantly different from WT plants in ambient
766 [CO₂] (P<0.05). Values are means ± standard error of 10 replicates.

767 **Fig. 2** Effects of GA and elevated [CO₂] at leaf anatomy of wild type (WT) and *gib-1*
768 mutant at 400 or 750 μmol CO₂ mol⁻¹. a, b, c and d cross section of the third leaf of the
769 wild type and *gib-1* plants at 400 or 750 μmol CO₂ mol⁻¹ (Scale bar: 100μm). e, total
770 leaf thickness. f, palisade parenchyma thickness. g, spongy parenchyma thickness. h,
771 palisade: spongy parenchyma ratio. i, upper epidermis thickness. j, lower epidermis
772 thickness. Asterisks indicate values determined by Student's t test to be significantly
773 different from WT plants at ambient [CO₂] (P<0.05). Values are means ± standard error
774 of 6 replicates. UE, Upper epidermis; LE, lower epidermis; PP, palisade parenchyma;
775 SP, spongy parenchyma.

776 **Fig. 3** Changes in gas exchange and fluorescence parameters in wild type (WT) and *gib-1*
777 mutant at 400 or 750 μmol CO₂ mol⁻¹. a, net rate of carbon assimilation (A). b, rate of
778 mitochondrial respiration in darkness (R_D). c, maximum rate of carboxylation (V_{cmax}). d,

779 maximum rate of electron transport ($J_{\max}$). e, photorespiratory rate of Rubisco (R_p). f,
780 electron transport rate (ETR). g, internal CO_2 concentration (C_i). h, stomatal
781 conductance (g_s). i, variable to maximum fluorescence ratio (F_v/F_m). Asterisks indicate
782 values determined by Student's t test to be significantly different from WT plants in
783 ambient $[\text{CO}_2]$ ($P < 0.05$). Values are means $\pm$ standard error of 10 replicates.

784 **Fig. 4** Relative metabolite level of leaf, stem and root from wild type (WT) and *gib-1*
785 mutant at 400 or 750 $\mu\text{mol CO}_2 \text{ mol}^{-1}$. Samples of leaf, stem and root were collected at
786 the end of the light period from plants growing for 21 days at ambient or elevated
787 $[\text{CO}_2]$. Asterisks indicate values determined by Student's t test to be significantly
788 different from wild type (WT) plants in ambient $[\text{CO}_2]$ ($P < 0.05$). Values are means $\pm$
789 standard error of 6 replicates. Data are normalized with respect to mean response
790 calculated for the wild type (WT) plants in ambient $[\text{CO}_2]$. nd, not detected. The full
791 dataset from the metabolite profiling study is available as Supplementary Table S1.

792 **Fig. 5** Effects of elevated $[\text{CO}_2]$ on growth of wild type (WT) and *gib-1* mutant 35 dag.
793 a, phenotypes of WT and *gib-1* mutant grown at ambient and elevated $[\text{CO}_2]$. b, total
794 biomass. c, leaf area. Measurements were done in tomato plants after 21 days of
795 growing at 400 or 750 $\mu\text{mol CO}_2 \text{ mol}^{-1}$. Asterisks indicate values determined by
796 Student's t test to be significantly different from WT plants in ambient $[\text{CO}_2]$ ($P < 0.05$).
797 Values are means $\pm$ standard error of 10 replicates.

798 **Fig. 6** Changes in gas exchange in wild type (WT) and *gib-1* mutant at 400 or 750 μmol
799 $\text{CO}_2 \text{ mol}^{-1}$, 35 dag. a, net rate of carbon assimilation (A). b, stomatal conductance (g_s). c,
800 internal CO_2 concentration (C_i). d, rate of mitochondrial respiration in darkness (R_D). e,
801 photorespiratory rate of Rubisco (R_p). f, variable to maximum fluorescence ratio
802 (F_v/F_m). Asterisks indicate values determined by Student's t test to be significantly

different from WT plants in ambient [CO₂] (P<0.05). Values are means ± standard error of 10 replicates.

Fig. 7 Proposed model of growth regulation by elevated [CO₂] in tomato plants. Plants at 21 dag (a) and 35 dag (b) grown under ambient and elevated [CO₂] and their respective meristem development stage. Elevated [CO₂] favors photosynthesis and carbohydrate production independently of the plant age. In the juvenile phase (21 dag), gibberellin-deficient mutants can grow to the same extent as wild-type plants as a response of the cell division and expansion capacity. In the adult phase (35 dag), however, elevated [CO₂] does not stimulate growth and gibberellin mutants show their characteristic stunted growth phenotype. This suggests that growth stimulation by [CO₂] is highly dependent on plant developmental stage, possibly linked to the juvenile-to-adult phase transition. However, which hormones are involved in the control of these events remains an open question.

Supplementary material

Fig. S1 Effects of elevated [CO₂] on specific leaf area (SLA) and relative growth rate (RGR) of wild type (WT) and *gib-1* mutant.

Fig. S2 Levels of nitrate, amino acids, protein and chlorophyll in wild type (WT) and *gib-1* mutant at 400 or 750 μmol CO₂ mol⁻¹.

Fig. S3 Levels of carbohydrate in wild type (WT) and *gib-1* mutant at 400 or 750 μmol CO₂ mol⁻¹.

Fig. S4 Levels of leaf soluble sugars in wild type (WT) and *gib-1* mutant at 400 or 750 μmol CO₂ mol⁻¹, 35 dag.

825 **Table S1** Relative metabolite level of leaf, stem and root from wild type (WT) and *gib*-
826 *I* mutant at 400 or 750 $\mu\text{mol CO}_2 \text{ mol}^{-1}$.

827

828

829

A

Ambient [CO₂]Elevated [CO₂]

B

C

Ambient [CO₂]

Elevated [CO₂]

A

Ambient [CO₂]Elevated [CO₂]

WT

gib-1

WT

gib-1

B

C

D

A

Sugars and sugars derivatives

Leaf

B

Stem

C

Root

D

Organic acids

E

F

G

Amino acids

H

I

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WT 400
gib-1 400
WT 750
gib-1 750