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Soil bacteria augment Arabidopsis photosynthesis by decreasing glucose sensing and abscisic acid levels *in planta*

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Summary

Photosynthesis is regulated by environmental factors as well as endogenous sugar signals. Whereas light-driven sugar biosynthesis is essential for terrestrial organisms, as well as belowground microflora, whether and how soil symbionts regulate photosynthesis has yet to be reported. Here, we show that the plant growth-promoting soil bacterium *Bacillus subtilis* GB03 augments photosynthetic capacity by increasing photosynthetic efficiency and chlorophyll content in Arabidopsis. Mechanistic studies reveal an elevation of sugar accumulation as well as the suppression of classic glucose signaling responses, including hypocotyl elongation and seed germination, with exposure to GB03. Compared with wild-type plants, two Arabidopsis mutants defective in hexokinase-dependent sugar signaling exhibit increased photosynthetic capacity, which is not further enhanced with GB03 exposure. Overlap in sugar/ABA sensing is observed in GB03-exposed plants, with a reduction of ABA-biosynthetic transcripts as well as downstream metabolite levels in leaves. Moreover, exogenous ABA abrogates GB03-triggered increases in photosynthetic efficiency and chlorophyll content. These results demonstrate that certain rhizobacteria elevate photosynthesis through the modulation of endogenous sugar/ABA signaling, and establish a regulatory role for soil symbionts in plant acquisition of energy.

Keywords: photosynthetic efficiency, hexokinase-dependent sugar signaling, plant growth promoting rhizobacteria (PGPR), volatile organic compounds (VOCs), *bacillus subtilis* (GB03).

Introduction

Photosynthesis converts light energy to chemical energy in the form of energy-rich sugar molecules. The sugars produced not only serve as carbon and energy sources, but also as pivotal signaling molecules for plant growth, development and stress responses. In particular, elevated sugar levels induce storage processes and confer feedback inhibition of photosynthesis (Jang and Sheen, 1994; Jang *et al.*, 1997; Moore *et al.*, 2003; Rolland *et al.*, 2006; Rook *et al.*, 2001; Sheen, 1994). Hexokinases are evolutionarily conserved glucose sensors in eukaryotes (Rolland *et al.*, 2006). To date, Arabidopsis hexokinase (HXK1) is the only identified sugar sensor *in planta* (Moore *et al.*, 2003), although there is also evidence for HXK1-independent signaling pathways (Chen and Jones, 2004; Johnston *et al.*, 2007; Xiao *et al.*, 2000). Hexokinase-dependent glucose signaling requires ABA signal transduction, as the two signaling pathways positively interact with each other (Rolland *et al.*, 2006; Smeekens, 2000).

Non-photosynthetic organisms usually obtain sugars either directly or indirectly from photosynthetic organisms. The survival of soil microorganisms is largely dependent upon the growth and productivity of the plant community. Plants not only supply organic matter for decomposers, but also release up to 30% of their photosynthetic output in the form of root exudates that attract and maintain fungal and bacterial soil colonies (Jones *et al.*, 2003; Smith *et al.*, 1993). The importance of exudates for the metabolic functions of microbes has been demonstrated by the girdling of forest trees, which reduces photosynthate supply to roots via the phloem, and subsequently substantially reduces soil respiration (Hogberg *et al.*, 2001; Johnsen *et al.*, 2007). Recent discovery of a monosaccharide transporter from symbiotic glomeromycotan fungi shed light on how carbohydrates are transported through the symbiotic interface (Schüβler *et al.*, 2006). Conversely, effects of soil microbes on plant photosynthesis are poorly understood.

Bacillus subtilis GB03 is a commercially available soil symbiont that emits a complex blend of volatile components that activates plant growth promotion (Ryu *et al.*, 2003, 2004;

Paré *et al.*, 2005). A bouquet of over 25 bacterial volatile odors from GB03 has been identified that triggers the differential expression of approximately 600 Arabidopsis transcripts related to cell wall modifications, primary and secondary metabolism, stress responses, hormone regulation and other expressed proteins (Ryu *et al.*, 2003; Farag *et al.*, 2006; Zhang *et al.*, 2007). Genetic and physiological studies have established that GB03 volatile organic compounds (VOCs) mediate auxin signaling specifically to allow for growth promotion via cell expansion, albeit that the changes in energy appropriation to provide for sustained plant growth promotion have not yet been identified. In this study, we examine how GB03 VOCs regulate photosynthetic capacity in Arabidopsis via the adjustment of glucose/ABA sensing *in planta*.

Results

GB03 increases photosystem II photosynthetic activity and chlorophyll content

The initial evidence of photosynthetic regulation by GB03 was that GB03-exposed plants were greener than controls (Figure 1a–b). Microscopic imaging of leaf sections exhibited visual increases in chloroplast numbers for GB03-exposed plants (Figure 1c–d), and mining of microarray data (Zhang *et al.*, 2007) identified several GB03-induced photosynthetic genes, such as chlorophyll *a*/*b* binding protein 165/180 (CAB2) and RuBisCO subunit binding proteins (Table S1). Consistent with these observations, GB03 treatment increased the photosynthetic efficiency of photosystem II (PSII) complexes (Figure 1e; Table 1). The maximum and effective quantum yields of PSII (F_v/F_m and Φ_{PSII}, respectively) in treated plants were significantly higher than in controls, implying an improvement of energy transfer within PSII. F_v and F_m represent the variable and maximal level of chlorophyll fluorescence respectively, which was measured for dark-acclimated leaves. On the other hand, the quantum yield of non-photochemical dissipation in PSII complexes was reduced by GB03 VOCs, and so was the fraction of Q_A, the primary electron acceptor of PSII, present in the reduced state (estimated as $1 - qP$), indicating improved electron transport downstream from PSII (Table 1). qP defines photochemical quenching of fluorescence. $qP = (F_m' - F_s)/(F_m' - F_o')$, where F_m' is the maximal level of fluorescence reached when saturating flash is applied during actinic illumination and F_s is the fluorescence immediately before a saturating flash was applied to measure F_m'. F_o' is the level of fluorescence measured after actinic illumination is off. Total chlorophyll (chlorophyll *a* + chlorophyll *b*) concentration is more abundant (88% increase) 2 weeks post-GB03 exposure, compared with control plants, whereas chlorophyll *a*/*b* ratios were unaltered (Table 1).

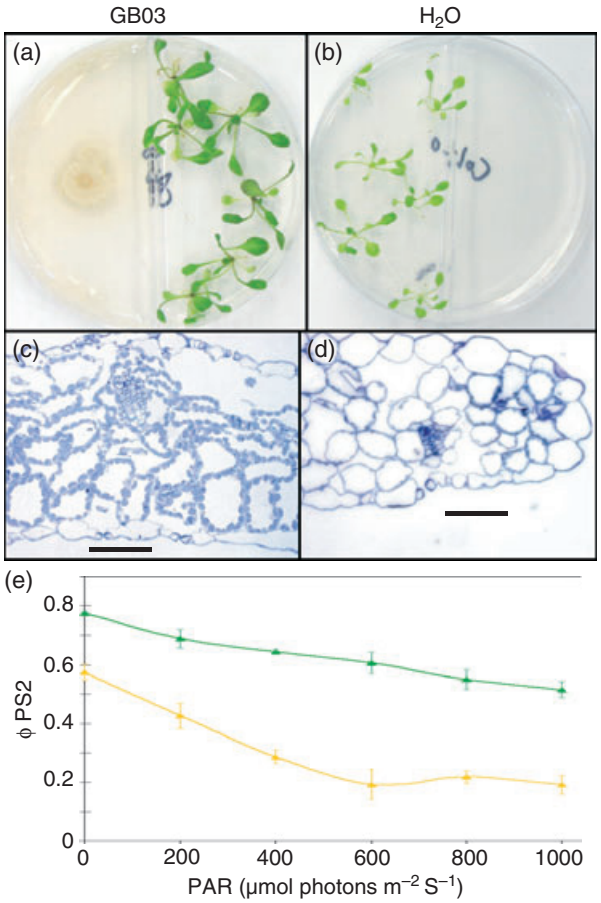

Figure 1. *Bacillus subtilis* GB03 augments photosynthetic machinery and increases photosynthetic efficiency in Arabidopsis.
GB03-exposed plants (a) have greener leaves compared with plants exposed to water as a control (b). Leaves of GB03-exposed plants have more chloroplasts (c) than controls (d) (the image of the leaf is centered on the vein closest to the leaf tip; scale bar, 50 μm).
Plants are 14 and 10 days post-exposure in (a, b) and (c, d), respectively. GB03 enhances photosynthetic efficiency (e), as compared by the effective quantum yield of photosystem II (Φ_{PSII}) for GB03-exposed (green triangle) and water control (yellow triangle); Arabidopsis illuminated with a range of photosynthetically active radiation (PAR) from 0–1000 μmol photons m^{-2} sec^{-1}. Error bars represent the standard deviation ($n = 4$).

GB03 represses plant sugar sensing

Photosynthesis is subject to feedback inhibition by elevated photosynthate levels that also trigger elevated storage activity. Expression of the *ApL3* gene, which encodes the large subunit of ADP-Glc pyrophosphorylase, and regulates starch biosynthesis, is an indicator of elevated sugar accumulation (Baier *et al.*, 2004; Rook *et al.*, 2001). With GB03 exposure, luciferase activity in *ApL3::LUC* transgenic plants revealed elevated sugar accumulation in the leaves (Figure 2a–b). Moreover, 8 and 16 days post-exposure, hexose levels in GB03-exposed plants are 50 and 62% higher, respectively, than those in control plants (Figure 2c). Although elevated photosynthate accumulation occurs in

Table 1 Photosynthetic activity and chlorophyll content increases with exposure to volatile organic compounds (VOCs) for 2 weeks

Parameters	Bacillus subtilis GB03	H_2O	*P*-value
F_v/F_m	0.76 ± 0.01	0.65 ± 0.04	1E-04
Φ_{PSII}	0.66 ± 0.01	0.56 ± 0.04	2E-03
Φ_N	0.25 ± 0.01	0.34 ± 0.05	1E-02
1 − qP	0.12 ± 0.01	0.16 ± 0.02	3E-04
Chl. a/b	3.32 ± 0.05	3.22 ± 0.15	0.26
Chl. a + b	1.88 ± 0.27	1.00 ± 0.12	8E-03

Mean ± SD (n = 8). A Student's t-test P value ≤ 0.05 indicates a statistically significant difference between parameters of treated and control plants. F_v/F_m, maximum quantum yield of photosystem II (PSII); Φ_{PSII}, effective quantum yield of PSII; Φ_N, quantum yield of non-photochemical dissipation in PSII complexes; 1 − *qP*, fraction of QA (the primary electron acceptor of PSII) present in the reduced state; Chl. *a*/*b*, ratio of chlorophyll *a* to *b*; Chl. *a* + *b*, total chlorophyll content expressed as mg per g fresh weight.

GB03-exposed plants, feedback inhibition is not observed, as indicated by chlorophyll accumulation, a quantitative indicator of sugar repression (Moore *et al.*, 2003), as well as by the increased photosynthetic efficiency with GB03 exposure (Table 1). The coexistence of enhanced photosynthesis and endogenous sugars suggests that sugar sensing is suppressed by GB03.

GB03 repression of sugar sensing was directly probed by assaying the inhibition of hypocotyl length and seed germination, both of which are responses attributed to sugar sensing rather than sugar metabolism or osmotic stress (Jang *et al.*, 1997; Price *et al.*, 2003). With increased exogenous glucose, the hypocotyl length was reduced by a greater extent without GB03 exposure (78%) than either with GB03 (55%) or for untreated glucose-insensitive mutants *gin2* (57%) and *gin1* (69%) (Figure 3a–c). As GB03-exposed and control seedlings accumulate similar levels of hexose (2.6 ± 0.1 and 2.5 ± 0.3 μmol g^{-1} fresh weight, respectively), as measured with 4% glucose in the media, reduced sugar sensitivity in GB03-exposed plants does not result from a reduction in sugar uptake. For dark-grown seeds exposed to GB03, a lowered sensitivity to elevated glucose was exhibited, compared with controls, with a greater than twofold increase in germination; glucose-specific delays in germination were also observed in light-grown seedlings exposed to GB03, albeit to a lesser degree (Figure 3d–e). These effects of rescuing sugar-inhibited developmental processes confirm that GB03 represses sugar sensing in plants.

Sugar signaling repression increases photosynthetic efficiency and chlorophyll content

To further characterize the role of sugar sensing in the GB03 regulation of photosynthesis, two Arabidopsis mutant lines defective in hexokinase-dependent glucose signaling, *gin2* (Moore *et al.*, 2003) and *gin1/ABA2* (Zhou *et al.*, 1998), were studied. Compared with wild-type plants, *gin1* and *gin2* plants have higher photosynthetic efficiency and chlorophyll content without exposure to GB03 (Figure 4a–b), thereby confirming the inhibitory role of sugar sensing in photosynthetic regulation. Moreover, exposure to GB03 did not increase photosynthetic efficiency or chlorophyll content in *gin2* and *gin1* plants (Figure 4a–b), indicating that GB03 releases sugar-induced photosynthetic inhibition through

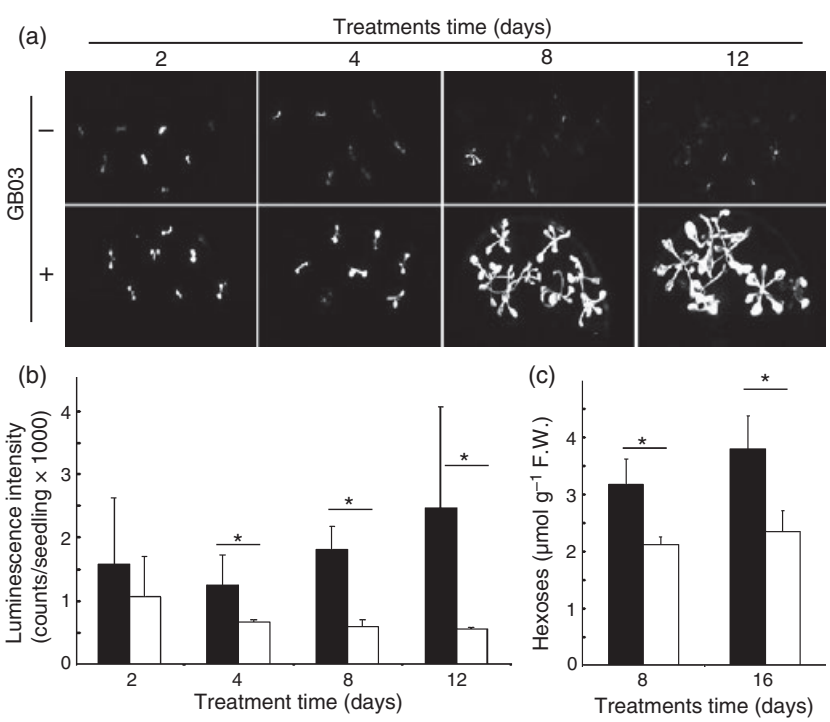

Figure 2. *Bacillus subtilis* GB03 elevates sugar levels in Arabidopsis. The elevated sugar level is indicated by increased luciferase activity in *ApL3::LUC* transgenic plants, as shown by luminescence imaging (a) and intensity quantification (b), as well as by comparison of plant hexose contents (c). Black bars indicate GB03 treatment; white bars indicate water controls; *P ≤ 0.05 treated versus controls [mean ± SD; n = 14 (b); n = 4 (c)].

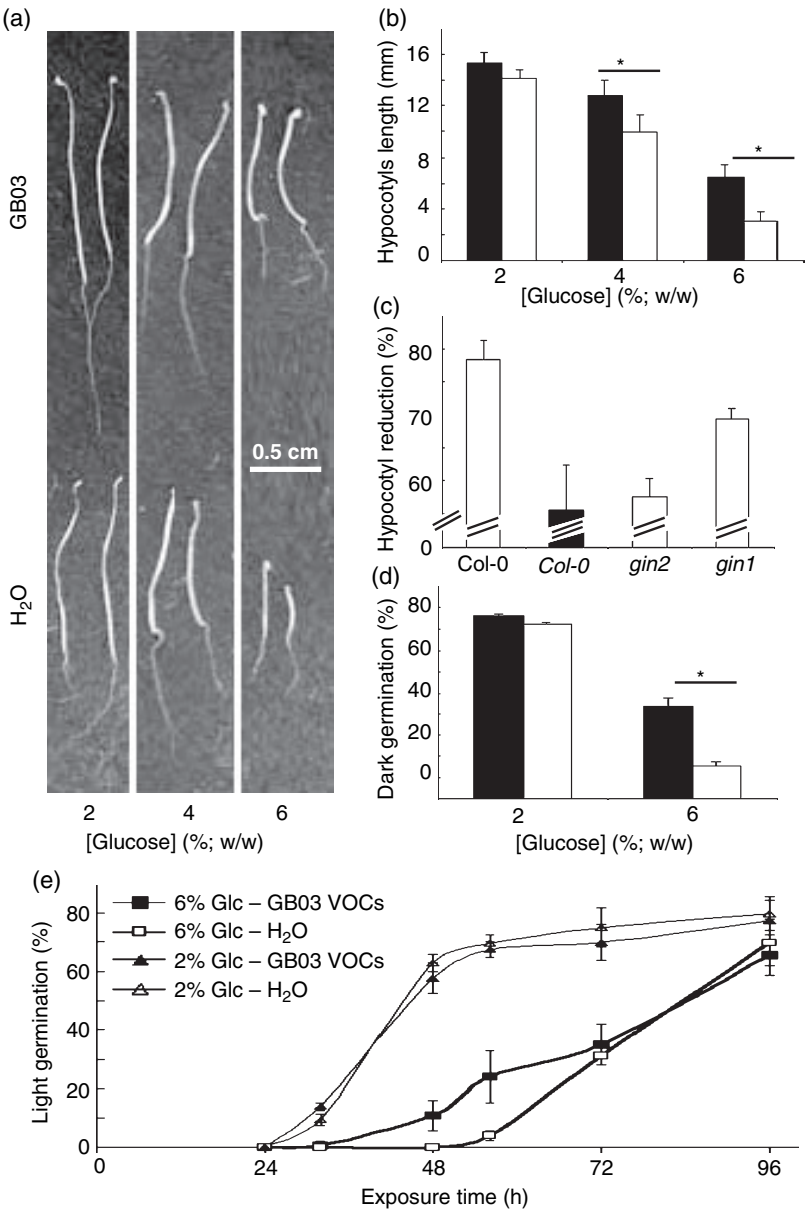

Figure 3. *Bacillus subtilis* GB03 releases sugar-specific inhibition of hypocotyl elongation and seed germination. (a) GB03 represses hypocotyl inhibition mediated by glucose signaling (scale bar, 0.5 cm; arrows indicate hypocotyl region). (b) Quantification of hypocotyl length ($n = 40$). (c) Reduction of hypocotyl length with 6% versus 2% glucose ($n = 4$). With 6% glucose, GB03-exposed seeds in the dark (d) and in light (e) exhibit higher germination rates than water controls; in the dark, germination rates were measured 6 days after seed stratification ($n = 3$), whereas in the light, germination was recorded at the indicated time points ($n = 4$). $^*P \leq 0.05$, treated versus controls, Student's *t*-test; mean ± SD; black bars, GB03 treatment; white bars, water control.

repressing HXK-dependent, rather than HXK-independent, glucose signaling. Meanwhile, the steady-state transcript levels of the hexose sensor HXK1 (Moore *et al.*, 2003), as well as GIN1, a known component in HXK-dependent sugar signaling (Zhou *et al.*, 1998), were not altered by GB03, as examined at 2 and 4 days post-exposure (Figure 4c). The transcript abundance of RGS1, a putative glucose receptor that mediates hexokinse-independent sugar signaling (Johnston *et al.*, 2007), was also examined, and showed no difference with or without exposure to GB03 (Figure 4c).

GB03 reduces ABA levels in Arabidopsis

Hexokinase-dependent sugar signal transduction requires ABA signaling (Rolland *et al.*, 2006; Smeekens, 2000). Mining microarray data revealed a cluster of ABA-synthesis and ABA-responsive genes that were downregulated with exposure to GB03 (Table S2), pointing to a potential role of ABA mediation in GB03-regulated plant sugar sensing and photosynthesis. Arabidopsis ABA synthesis genes, including the zeaxanthin epoxidase (ZEP/ABA1) and two 9-*cis*-epoxycarotenoid dioxygenases (NCED3 and NCED4), were downregulated within 24 h of exposure to GB03 VOCs (Figure 5a). ZEP catalyzes the epoxidation of zeaxanthin and antheraxanthin to violaxanthin, which is the first step in the ABA biosynthesis pathway (Xiong and Zhu, 2002); whereas NCEDs catalyze the cleavage of the C_{25} carotenoids 9-*cis*-neoxanthin or 9-*cis*-violaxanthin to the C_{15} ABA precursor xanthoxin, which has been proposed to be the rate-limiting step in ABA synthesis (Schwartz *et al.*, 1997). Consistent

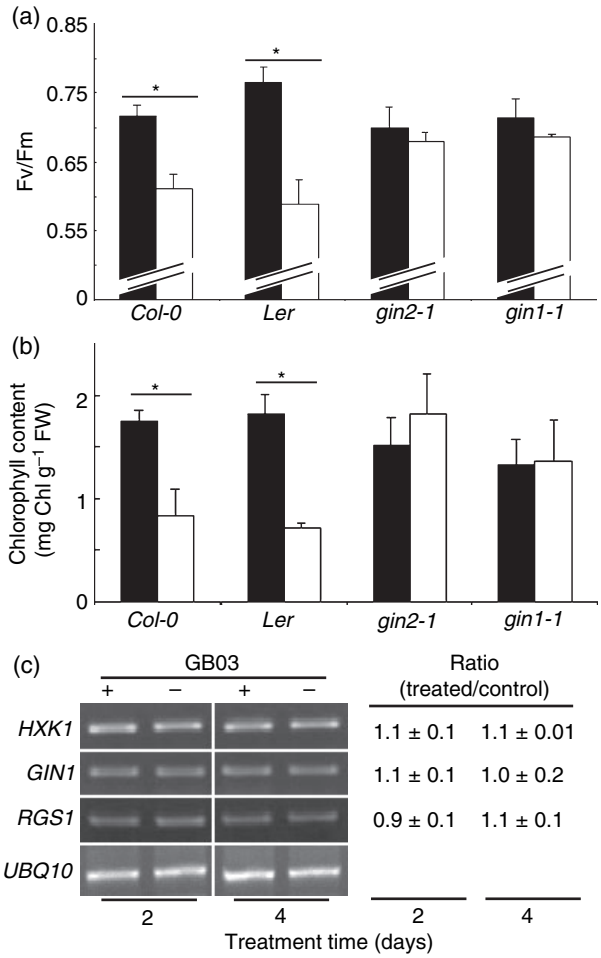

Figure 4. Blocking hexokinase (HXK)-dependent sugar sensing reverses the suppression of photosynthesis by sugar. *Bacillus subtilis* GB03 increases the photosynthetic efficiency (a) and chlorophyll contents (chlorophyll *a* + b) (b) in wild-type (*Col-0* and *Ler*) Arabidopsis compared with water controls at 14 days post-exposure. Genetic blocking of HXK-dependent sugar sensing in *gin2-1* and *gin1-1* mutants also results in greater photosynthetic efficiency (a) and chlorophyll contents (b) compared with their wild-type plants, whereas GB03 does not further increase the photosynthesis efficiency or chlorophyll content in these two mutants (a) and (b). Black bars indicate GB03 treatment; white bars indicate water controls; $*P \leq 0.05$, treated versus controls ($n = 4$, mean ± SD). GB03 does not transcriptionally regulate HXK1, GIN1 or RGS1 (c). Gene expression ratios are quantified from RT-PCR results, $n = 3$, mean ± SD.

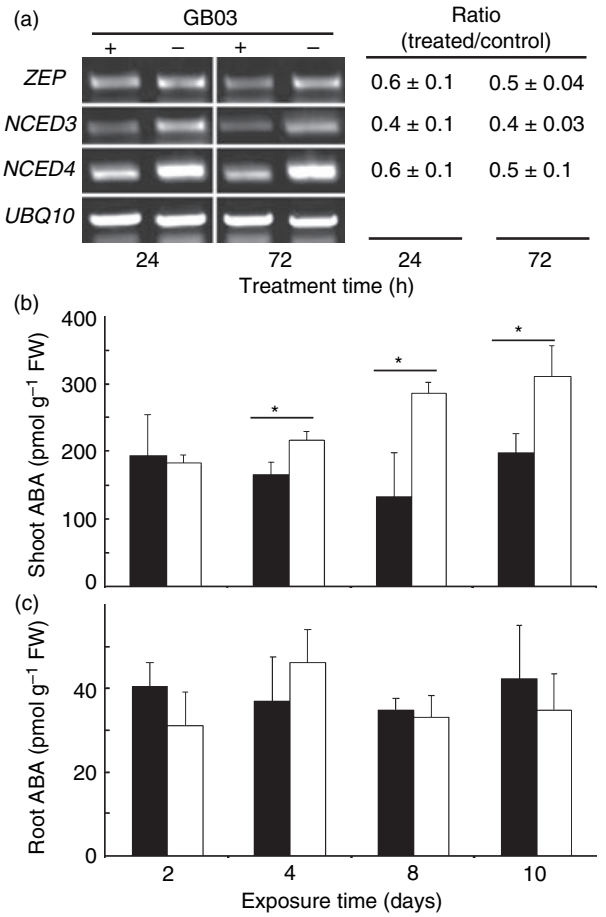

Figure 5. *Bacillus subtilis* GB03 volatile organic compounds (VOCs) downregulate ABA levels in Arabidopsis. Genes encoding key ABA biosynthesis enzymes are downregulated in plants with exposure to GB03, including *ZEP* (zeaxanthin epoxidase), *NCEDs* (9-*cis*-epoxycarotenoid dioxygenases) and *UBQ10* (house-keeping gene ubiquitin 10). Gene expression ratios are quantified from RT-PCR results ($n = 3$, mean ± SD) (a). The ABA levels in aerial portions of Arabidopsis plants are decreased by GB03 (b). GB03 does not change ABA accumulation in Arabidopsis roots (c). Black bars represent GB03 treatment; white bars represent water controls. $*P \leq 0.05$, treated versus controls ($n = 4$, mean ± SD).

with the transcriptional reduction, ABA content in the aerial portions is decreased by exposure to GB03 (Figure 5b). Compared with control plants, ABA levels in the shoots of GB03-exposed plants were 24, 53 and 37% lower, as measured at 4, 8 and 10 days post-exposure. In contrast, ABA levels in the roots are similar between GB03-exposed and control plants (Figure 5c). As reduced ABA levels inhibit hexokinase-dependent sugar signaling (Rolland *et al.*, 2006; Rook *et al.*, 2001; Smeekens, 2000), GB03-mediated ABA reduction can account for the repression in plant sugar sensing (Figure 3).

Reduced ABA levels are necessary for GB03-enhanced photosynthetic activity

To further characterize the role of reduced ABA levels in GB03-augmented photosynthesis, Arabidopsis plants were grown with exogenous ABA in the medium (Figure 6a). As exogenous ABA levels increase from 0 to 10 μM, the GB03-triggered enhancement of photosynthesis efficiency and chlorophyll content is impaired in a dose-dependent fashion (Figure 6b–c). A supplementation of 2 μM ABA in the media is sufficient to abrogate GB03-triggered increases in photosynthetic efficiency and chlorophyll content. Compared with an absence of exogenous hormone, 10 μM ABA in the media results in an 83 and 54% reduction in the photosynthetic

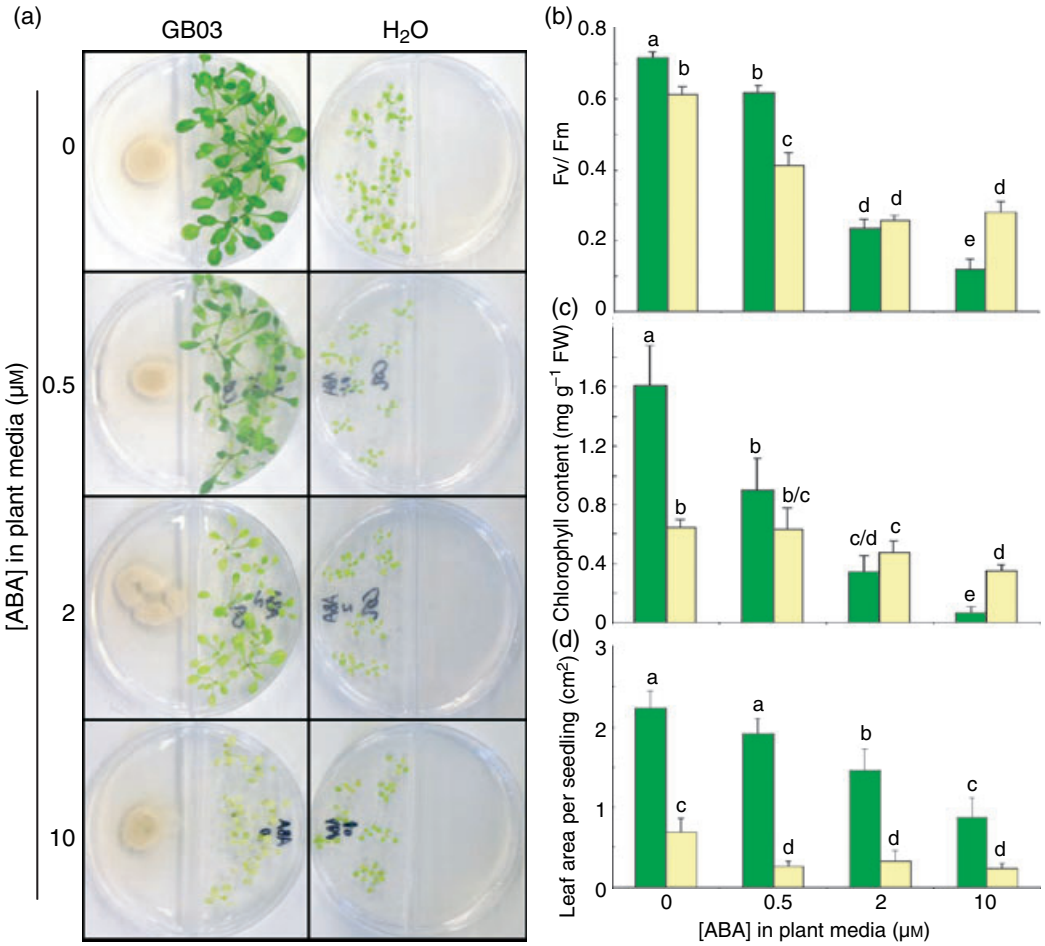

Figure 6. Reduction of ABA levels in Arabidopsis is critical for the volatile organic compound (VOC)-triggered increase in photosynthetic capacity. Arabidopsis plants were grown with different doses of exogenous ABA in the growth medium (a). Exogenous ABA dose-dependently impairs the *Bacillus subtilis* GB03-triggered increase in photosynthetic efficiency (b), chlorophyll content (c) and growth promotion, as determined by total leaf surface area per seedling (d). With 10 μM ABA, GB03-exposed plants exhibit leaf chlorosis, and thus do not readily show leaf edges; for quantification of whole leaf areas, see (d). Green bars represent GB03 treatment; yellow bars represent water controls. Different letters indicate significant differences between treatments according to least significance at $P \leq 0.05$ ($n = 5$, mean ± SD).

efficiency of GB03-exposed and control plants, respectively. Similarly, chlorophyll content in GB03-exposed plants drops by 95% from 0 to 10 μM exogenous ABA, whereas that in control plants decreases by 46%. These results collectively demonstrate that the GB03-triggered reduction of the ABA level is critical for the concomitant augmentation of photosynthesis. Whereas 10 μM, compared with 0 μM, exogenous ABA also reduced the leaf surface area, the percentage growth reduction at 61 and 65% for GB03-exposed and control plants, respectively (Figure 6d), is less disparate than either the percentage reductions of photosynthetic efficiency or chlorophyll content with the same ABA treatment (Figure 6b–c). With the leaf area for GB03-treated plants being more than twofold greater than that of controls, even at high exogenous ABA levels, it appears that leaf growth in plants exposed to GB03 can, at least in part, and in the short term, be decoupled from photosynthetic increases.

Discussion

Microbial symbionts in the rhizosphere require the plant-derived carbohydrates generated from photosynthesis (Schüβler *et al.*, 2006). Regulation of photosynthetic activities depends on the integration of endogenous signals and environmental stimuli (Leister, 2005; Rolland *et al.*, 2006). Herein, the soil bacterium *B. subtilis* GB03 demonstrates an ability to elevate photosynthesis through the modulation of plant endogenous sugar/ABA signaling. These results establish a regulatory role for plant symbionts in photosynthesis.

Chlorophyll fluorescence analysis is based on the detection of fluorescence signals originating from PSII; the analysis also provides information about the overall photosynthetic activity (Maxwell and Johnson, 2000). As a non-invasive method, chlorophyll fluorescence is well suited to

probe functional activity of the photosynthetic apparatus. The level of PSII fluorescence depends on the reduction state of Q_A, the primary quinone acceptor. Any interruption in electron transport downstream of Q_A, including decreased photosystem-I (PSI) activity or malfunction of the Calvin cycle, leads to a higher Q_A oxidation pool, and consequently to elevated chlorophyll fluorescence. The actual (effective) quantum yield of PSII photochemistry (Φ_{PSII}) correlates well with the quantum efficiency of CO_2 fixation (Edwards and Baker, 1993; Genty *et al.*, 1989), as well as with the quantum efficiency of PSI (Eichelmann and Laisk, 2000; Harbinson *et al.*, 1989).

GB03 enhances Arabidopsis photosynthetic capacity by improving the efficiency of the conversion of light energy, as well as by enhancing the photosynthetic apparatus, as evidenced by increases in photosynthetic efficiency and chlorophyll content, respectively. Consistent with elevated photosynthesis, transcripts of genes encoding chloroplast proteins known to be associated with photosynthesis are upregulated with GB03 exposure (Table S1). Expressing the chloroplast ferredoxin-NADP reductase antisense gene in transgenic tobacco has been shown to impair plant growth and restrict photosynthetic activity (Hajirezaei *et al.*, 2002). Arabidopsis chloroplast FtsH proteases have been reported to be involved in the repair cycle of PSII in thylakoid membranes (Sakamoto *et al.*, 2003). The identified GTP-binding protein (At1g02280) is a component of the chloroplast protein import machinery, and is required for the movement of NADPH-protochlorophyllide oxidoreductases B across the chloroplast membrane (Kim *et al.*, 2005), which is required for chlorophyll synthesis (Frick *et al.*, 2003; Runge *et al.*, 1996). Other genes encoding chloroplast proteins that are upregulated with GB03 VOCs play a more evident role in photosynthesis, including chlorophyll *a*/*b* binding protein and two RuBisCO subunit binding proteins (Table S1). In addition to concerted genetic action, biogenesis of the photosynthetic apparatus requires, and is sensitive to, iron availability (Spiller and Terry, 1980; Terry, 1980). Consistent with this study, GB03 transcriptionally enhances plant iron uptake (HZ and PWP, unpublished data).

The partition separating bacteria from the plant (Figure 1a–b) excludes the possibility that GB03 represses the glucose inhibition of hypocotyl elongation, or of germination through competition for plant sugar uptake. Moreover, the higher hexose level in GB03-exposed plants (Figure 2c) establishes that GB03 attenuates glucose inhibitory effects through the repression of sugar signaling, rather than by lowering sugar accumulation. Furthermore, studies using Arabidopsis mutants defective in hexokinase-dependent sugar signaling (Figure 3a–b) indicate that GB03 augments photosynthesis through repressing hexokinase-dependent, rather than hexokinase-independent, sugar signaling. Glucose signaling largely overlaps with ABA signal transduction, as revealed by the fact that Arabidopsis *ABA synthesis* (*aba*) and *ABA insensitive* (*abi*) mutants are, to varying degrees, sugar sensing mutants (Rolland *et al.*, 2006; Smeekens, 2000). Therefore, the reduction of ABA levels explains the repressed glucose signaling in GB03-exposed plants. Although ABA initiates various protective responses in plants upon biotic or abiotic stress (Finkelstein *et al.*, 2002), ABA can also suspend the post-germinative growth of seedlings, representing an early developmental checkpoint (Lopez-Molina *et al.*, 2001). Genetic and biochemical studies have provided enzymatic information for ABA biosynthesis, which is subject to complex regulation during plant development and in response to abiotic stresses (Finkelstein *et al.*, 2002; Nambara and Marion-Poll, 2005; Xiong and Zhu, 2002). Our results show that GB03 transcriptionally reduces plant ABA production (Figure 5). The future identification of cis-elements for ABA synthesis genes, and their respective transcriptional factors, will assist in the elucidation of how GB03 regulates ABA levels in plants.

In these split-plate Petri-dish studies, the partition separating the bacteria from the plants indicates that bacterial emissions are sufficient to regulate observed photosynthetic changes (Figure 1a,b). Volatiles from a non-growth-promoting *Escherichia coli* strain DH5α failed to augment photosynthetic efficiency via a non-specific microbial signal, such as the generation of elevated CO_2 (Figure S1). Chemical treatment with 2,3-butanediol also did not exhibit significant increases in photosynthetic efficiency (Figure S1). The characterization of the whole spectrum of GB03 volatile emissions, and biological testing of these chemical compounds, in the future is likely to reveal the bacterial elicitors that govern the photosynthesis changes that occur in plants.

Experimental procedures

Plant materials and treatments

Arabidopsis thaliana seeds were surface sterilized and then planted on one side of specialized plastic Petri dishes (100 $\times$ 15 mm) that contained a central partition (I plates; Fisher Scientific); both sides contained half-strength MS solid media with 0.8% (w/v) agar and 1.5% (w/v) sucrose. Before being placed in the growth room, seeds were vernalized for 2 days at 4°C in the absence of light. The growth room was set to a 16-h light/8-h dark cycle under metal halide and high pressure sodium lamps, with a total light intensity of 200 μmol m^{-2} sec^{-1}, a temperature of 21 $\pm$ 4°C and a relative humidity of 40 $\pm$ 10%.

One day before plant experiments, bacterial strains were streaked onto tryptic soy agar plates that were then incubated at 28°C in the absence of light for 24 h. *B. subtilis* GB03 cells were harvested from the agar plates in double-distilled water to yield 10^9 CFU ml^{-1}, as determined by optical density measurements and serial-dilution cell-plate counts. Two-day-old Arabidopsis seedlings were then inoculated with 20 μl of bacterial suspension culture or double-distilled water was applied dropwise to the non-plant side of the Petri dish. The quantification of the total leaf surface area was performed as described in Zhang *et al.* (2007).

Mutant *gin2-1* and *gin1-1* seeds were obtained from the ABRC (http://www.biosci.ohio-state.edu/pcmb/Facilities/abrc/abrchome.htm). For plates with ABA, ABA dissolved in MeOH or solvent alone was added into autoclaved plant growth medium. *Col-0* plants were allowed to germinate without ABA in the medium, and were then transferred to medium with ABA and were treated 2 days after germination.

Chlorophyll fluorescence measurements. Arabidopsis plants that had been exposed to GB03 or water for 2 weeks were used. Chlorophyll *a* fluorescence emission from attached leaves was measured with a pulse amplitude-modulated fluorometer (PAM 101/103; Heinz Walz GmbH, http://www.walz.com). The experimental protocol and nomenclature was based on the descriptions in Nambara and Marion-Poll (2005); also see the Supplementary Material.

Plants were kept in Petri dishes during photosynthesis measurements. F_o (minimal level of fluorescence in the dark-adapted state) measurements were performed on dark-adapted (30 min) leaves. The maximal level of fluorescence in the dark-adapted state (F_m) was measured by a 1-s pulse of saturating light (KL 1500 light source; Schott, http://www.schott.com). The ratio F_v/F_m ($F_v = F_m - F_o$) was used to estimate the potential quantum yield of PSII photochemistry. Leaves were then illuminated with actinic light (intensity as indicated in the Figure legends) in order to activate photosynthetic reactions. Illumination for 10 min at room temperature was sufficient for obtaining steady-state fluorescence parameters. Light-adapted leaves are also exposed to a saturating light pulse to obtain the maximal light-adapted fluorescence (F_m'). The actinic light was immediately switched off after the flash, and F_o' readings were taken following the short-term application of low-intensity far-red light. The following parameters were calculated: coefficient of photochemical quenching [$qP = (F_m' - F)/(F_m' - F_o')$, where F is the level of fluorescence just before the pulse of saturating light was applied], reflecting the portion of PSII complexes with reaction centers in the 'open' state; quantum efficiency of electron transport through PSII complexes [$\Phi_{PSII} = (F_m' - F)/F_m'$]; and quantum efficiency of non-photochemical dissipation in PSII complexes ($\Phi_N = F_o'/F_m'$). Φ_{PSII} represents the actual quantum yield of PSII photochemistry for light-acclimated samples under illumination, whereas Φ_N reflects the non-photochemical dissipation in light-acclimated samples, which have all of the PSII reaction centers in the 'open' state (Kornyeyev and Hendrickson, 2007).

Chlorophyll determination

Arabidopsis leaves were detached and measured 14 days after treatments. Tissue was homogenized in 80% aqueous acetone (1 ml) and centrifuged at 13 000 ***g*** for 5 min. Supernatant absorbance readings were taken at 470, 646.8 and 663.2 nm. The total chlorophyll content was calculated as $[(7.15 \times A_{663.2}) + (18.71 \times A_{646.8})]/[1000\times$ (fresh weight of leaves)], and was reported as mg Chl. per g FW, as described in (Lichtenthaler, 1987).

Microscopic measurement

The first true leaves of 12-day-old seedlings exposed to GB03 VOCs or water for 10 days were used for cell size measurements by microscopic analyses. Leaves were sectioned and prepared for light microscopic observations as described in Zhang *et al.* (2007). Leaves from different plants were measured for the three replicates for each treatment.

Luminescence imaging

ApL3::LUC transgenic plants were sprayed with 1 mM luciferin in 0.01% (V/V) Triton X-100, dark developed (5 min) and imaged with a thermoelectrically cooled charge-coupled camera (5-min exposure); to facilitate visualization, treated and control images were equally contrast adjusted for individual time points. The luminescence intensity was quantified by Andor IXON software (Andor Technology, http://www.andor.com).

Analysis of glucose sensitivity

The plant growth medium did not contain sucrose, but was supplemented with different quantities of glucose as indicated in the figure legends. The hypocotyl length for 6-day-old dark-grown seedlings with or without GB03 ($n = 4$, with 40 seedlings in total) was recorded for each glucose treatment. Germination was scored by radicle emergence from the seed coat ($n = 4$; 50–100 seeds in total).

Hexose measurements

The hexose content in aerial portions of Arabidopsis plants was extracted and measured as described by Hendrix *et al.* (1993). Enzymes (hexokinase, phosphoglucose isomerase and glucose-6-phosphate dehydrogenase) were added to use these carbohydrates as substrates to reduce NADP. The absorbance at 340 nm, which is specific to NADPH, but not to NADP, was measured.

Reverse-transcriptase PCR

RT-PCR was performed, and the results were quantified as described in Zhang *et al.* (2007).

ABA measurements

ABA was extracted as described by Rook *et al.* (2001). Grounded tissue was extracted in 1 ml of a 90% methanol solution, containing 20 ml L^{-1} acetic acid and 10 mg l^{-1} butylated hydroxytolunene (Sigma-Aldrich, http://www.sigmaaldrich.com) for 24 h at 4 °C. The ABA contents in the solution were determined using the Phytodetek ABA immunoassay kit (Agdia Inc., http://www.agdia.com). Mixed ABA isomers (Sigma-Aldrich) were used as standards.

Statistical analysis

Statistical analyses of the total leaf surface area and photosynthesis efficiency were performed with SAS software (SAS Institute, http://www.sas.com). Significant differences between treatments were based on P values of ≤ 0.05.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. An increase in the Arabidopsis effective quantum yield of photosystem II (Φ_{PSII}) is specific to *Bacillus subtilis* GB03-exposed seedlings.

Table S1. *Bacillus subtilis* GB03 regulation of genes encoding chloroplast proteins.

Table S2. *Bacillus subtilis* GB03-regulated ABA synthesis and ABA-responsive genes.

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