Phytochrome B regulates resource allocation in *Brassica rapa*

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Abstract

Crop biomass and yield are tightly linked to how the light signaling network translates information about the environment into allocation of resources, including photosynthates. Once activated, the phytochrome (phy) class of photoreceptors signal and re-deploy carbon resources to alter growth, plant architecture, and reproductive timing. Most of the previous characterization of the light-modulated growth program has been performed in the reference plant *Arabidopsis thaliana*. Here, we use *Brassica rapa* as a crop model to test for conservation of the phytochrome–carbon network. In response to elevated levels of CO₂, *B. rapa* seedlings showed increases in hypocotyl length, shoot and root fresh weight, and the number of lateral roots. All of these responses were dependent on nitrogen and polar auxin transport. In addition, we identified putative *B. rapa* orthologs of *PhyB* and isolated two nonsense alleles. *BrphyB* mutants had significantly decreased or absent CO₂-stimulated growth responses. Mutant seedlings also showed misregulation of auxin-dependent genes and genes involved in chloroplast development. Adult mutant plants had reduced chlorophyll levels, photosynthetic rate, stomatal index, and seed yield. These findings support a recently proposed holistic role for phytochromes in regulating resource allocation, biomass production, and metabolic state in the developing plant.

Keywords: Brassicaceae, climate change, phytochromes, resource allocation.

Introduction

‘Functional equilibrium’ describes the metabolic balancing act whereby plants adjust the allocation of biomass between carbon-acquiring, photosynthetic shoots and nutrient-absorbing roots (Brouwer, 1963; Thornley, 1972; Iwasa and Roughgarden, 1984; Poorter et al., 2012). The biomass that gets invested in harvestable yield of crops is heavily influenced by this calculation. Light and CO₂ availability can limit photosynthetic productivity of aboveground tissue and thereby belowground carbon allocation (Rogers et al., 1994; Curtis and Wang, 1998; De Graaff et al., 2006; Litton et al., 2007; Dieleman et al., 2010). The availability of nitrogen to the roots plays a particularly significant role in constraining plant growth and crop yield worldwide (Epstein and Bloom, 2005; Hirel et al., 2011; Alvarez et al., 2012). Numerous species respond to changes in nitrogen availability by altering the root/shoot biomass ratio to maintain nutrient balance (Aerts et al., 1992; Scheible et al., 1997; Hermans et al., 2006; Grechi et al., 2007; Li et al., 2012).

The way in which plants respond to carbon and nitrogen supply is of particular interest in the context of increasing global atmospheric CO₂ levels. Since the Industrial Revolution, atmospheric CO₂ levels have increased from 280 ppm to >400 ppm at the time of writing, and are predicted to reach 730–1000 ppm by the end of the century (Intergovernmental Panel on Climate Change, 2007, 2014). CO₂ directly affects plants through impacts on photosynthetic gas exchange and downstream...
developmental processes (Ainsworth and Long, 2005; Gray and Brady, 2016). In a long-term field experiment, elevated CO₂ stimulated photosynthetic carbon assimilation rates by an average of ~30% across 40 species (Ainsworth and Long, 2005). A CO₂-driven increase in shoot biomass has been shown to lead to significant increases in seed yield in many crops, including soybean, rice, bean, wheat, and peanut (Hatfield et al., 2011). At the same time, increased carbon assimilation leads to higher nitrogen demand and often increased root growth. When carbon levels are increased by treatment with sugars or elevated CO₂ in laboratory conditions, Arabidopsis thaliana seedlings produce more lateral roots (MacGregor et al., 2008; Lilley et al., 2012; Hachiya et al., 2014). Natural C₃–C₄ grassland exposed to elevated atmospheric CO₂ shows significantly increased community root biomass (Anderson et al., 2010), and root biomass has been observed to increase significantly in response to elevated CO₂ in many crops (Madhu and Hatfield, 2013).

Biomass allocation calculations can vary dramatically by species and environment. In a meta-analysis of CO₂ responses, it was close to a 60:40 split between species that decreased versus increased their shoot/root ratio (Rogers et al., 1995). The progressive nitrogen limitation hypothesis posits that nitrogen additions should enhance CO₂ effects on plant productivity (Luo et al., 2004). Nitrogen scarcity may therefore limit ecosystem response to elevated CO₂ concentration (Oren et al., 2001; Hungate et al., 2003; Luo et al., 2004; Reich et al., 2006; Langley and Megenigal, 2010). Studies in Brassica show that yield improvements could be achieved with elevated CO₂ but only with increased nitrogen supplementation (Upreti and Mahalaxmi, 2000). Similarly, elevated CO₂ stimulates above-ground biomass in a grassland by up to 33%, but is dependent on water and nitrogen availability, with lower biomass stimulation observed in drier, lower nutrient conditions (Reich et al., 2014). Understanding the molecular targets of elevated CO₂, in addition to knowledge of how these targets impact biomass allocation, could provide guidance for crop breeding and management practices to optimize future yields.

Light signaling via photoreceptors acts at the intersection of plant growth control and metabolic homeostasis. The phytochrome family of photoreceptors responds primarily to red and far-red wavelengths, switching between Pr (inactive) and Pfr (active) isomeric forms (Fraser et al., 2016). Plants with reduced phyB function have altered leaf area and slower growth compared with wild-type plants (Halliday et al., 2003). One possible explanation for this retarded growth is reduced chlorophyll and, most probably, photosynthetic rate (Strasser et al., 2010; Hu et al., 2013). Phytochromes control chloroplast gene expression during photomorphogenesis as well as nuclear-encoded factors involved in chloroplast development (Waters et al., 2008, 2009; Oh and Montgomery, 2014). Phytochrome B is also required for the light-dependent development of stomata (Casson et al., 2009; Casson and Hetherington, 2014). Adult phy mutant plants in Arabidopsis have reduced CO₂ uptake and sizeable reductions in overall growth (Yang et al., 2016). In addition, phytochrome loss impacts core metabolism, notably the stress metabolites proline and raffinose. Mutants appear to be diverting resources from biomass production to improve resilience.

In this study, we tested the extent of PhyB’s effect on plants’ response to resource availability. We focused on Brassica rapa, as the Brassica genus includes species of worldwide economic importance, and their relatively recent divergence from Arabidopsis increased the likelihood of shared molecular pathways (Huang et al., 2016). We established that a number of growth parameters are significantly affected by elevated CO₂, including increased hypocotyl length, shoot and root fresh weight, and the number of lateral roots. All of these responses required adequate nitrogen and polar auxin transport, as has been observed in Arabidopsis. We also identified B. rapa orthologs of PhyB and found that loss of BrPhyB function abrogated seedling responses to high CO₂. Moreover, adult mutants had reductions in chlorophyll, photosynthetic rate, and stomatal index. Together, these results highlight the role of phytochrome photoreceptors in shaping plant architecture and biomass partitioning across the plant life cycle.

**Materials and methods**

**Plant materials and growth conditions**

**Seedlings**

The *B. rapa* wild-type R-0-18 and phyB mutant lines were obtained from the John Innes Centre’s RevGENUK resource (http://revgenuk.jic.ac.uk/) (Stephenson et al., 2010). Seeds were sterilized with Cl₂ gas for 4 h in a sealed bell jar (Clough and Bent, 1998), sown on plates, and stratified in the dark at 4 °C for 3 d. Standard medium was 0.5× Linsmaier and Skoog (LS) [LS03, Caisson Laboratories, Inc.; N sources were 1650 mg 1⁻¹ NH₄NO₃ and 1900 mg 1⁻¹ KNO₃] with 0.8% phytoagar (40100072-1, Plant Media: bioWORLD). Germinated seedlings were selected for similar developmental stage and moved to plates containing varying nitrogen levels or chemical treatment. For nitrogen-related experiments (Fig. 1; Supplementary Figs S1, S2 at JXB online), medium was prepared from 0.5× Murashige and Skoog without nitrogenous compounds (MS07, Caisson Laboratories, Inc.) and supplemented with either 0.5 mM KNO₃ (low N) or 0.5 mM KCl (no N). N₁-naphthylphthalamic acid (NPA, 100 mM) was added directly to 0.5× LS medium prior to pouring (Fig. 2). All other seedling experiments used the 0.5× LS (standard) medium. Plates were placed vertically at dawn in a Reliance LED growth chamber (www.reliancelabs.com) set at 20 °C. LED intensities were set at 9.23% for 470 nm, 11.64% for 660 nm, 9% for 730 nm, and 5.67% for 447 nm. Light intensity was 100 µmol m⁻² s⁻¹ with short-day conditions (8 h light, 16 h dark; 16 h dark). For CO₂ experiments, ambient CO₂ was set at 400 ppm for ambient (A) and 1000 ppm for high (H) CO₂. Chamber data were logged every 5 min. A mechanical issue led to elevated CO₂ levels being only 800 ppm in the experiments summarized in Fig. 3.

**Adult plants**

Seeds were sown directly onto our standard soil mix of 1:1 Sunshine Mix #4® (SunGro Horticulture):vermiculite. Plants were grown four per 2.6 liter square pots (McConkey Grower Products; Sumner, WA, USA). Plants were bottom-watered daily in long-day conditions Percival E-30B growth chamber set at 20 °C (16 h light, 8 h dark; ~115 µmol m⁻² s⁻¹) for 3 weeks.

**Plant measurements**

**Seedlings**

Hypocotyl lengths and lateral root number were measured from scans of vertical plates using ImageJ software (http://rsb.info.nih.gov/ij/) in at least four independent experiments of five seedlings. Plates were scanned daily from day 4 to day 10 for time course...
experiments. Lateral roots were counted in the first 4 cm of the primary root at day 7. Lateral roots were counted on day 7 to avoid lateral root entanglement between adjacent seedlings. At this stage, essentially all emerged lateral roots are found within the first 4 cm of the primary root. Hypocotyl lengths were measured at day 10. Biomass data were collected by harvesting immediately after the final scan on day 10. Five seedlings from each replicate plate were dissected, pooled, and weighed using an analytical balance, so that each biological replicate of five seedlings represents one fresh or dry weight data point. Fresh weight was recorded immediately; tissue was then dried at 70 °C for 4 d and measured again to determine dry weight.

**Results**

**Carbon and nitrogen availability alter development and biomass allocation in B. rapa.**

Increased carbon availability in the form of sucrose or elevated CO2 stimulates seedling growth in *A. thaliana*, but only in the presence of sufficient nitrogen (Stewart Lilley *et al.*, 2013). To test whether this same relationship held true for *B. rapa*, we exposed seedlings to high carbon environments under three nitrogen conditions (Fig. 1; Supplementary Fig. S1). Seedlings were grown for 10 days on standard growth medium, limited nitrogen (called low hereafter), or no nitrogen in either 400 ppm (ambient) or 1000 ppm (high) CO2. High CO2 conditions increased hypocotyl length for seedlings grown on standard medium by 37% (Fig. 1A; Supplementary Fig. S1; *P* < 0.001). This response was eliminated when plants were grown in low or no nitrogen (Fig. 1A; Supplementary Fig. S1; *P* < 0.001).

We dissected the seedlings at the end of the experiment to measure shoot and root fresh weights. When grown on standard media, average fresh weights of both tissues increased ~2-fold in response to high levels of CO2, with a root/shoot ratio increase from 0.26 to 0.29 (Fig. 1B, inset). The increased aboveground biomass response was significantly reduced when seedlings were grown on media containing low nitrogen and eliminated in no nitrogen media (*P* > 0.001 for both above- and belowground fresh weight).

In low nitrogen conditions, root/shoot ratios increased to 0.57 in ambient conditions and 0.65 in response to elevated CO2. The biomass difference between above- and belowground tissue was further reduced in the no nitrogen conditions (0.68–0.75). Dry weights showed similar trends (Supplementary Fig. S2).

**Sequence alignment and cluster analysis**

A phylogenetic tree was constructed using Geneious Pro 5.4.6 Biomatters Ltd. and sequences obtained from Arabidopsis.org and Phytozome.net. Sequence alignment was done using Phytozome.net and ClustalW (Larkin *et al.*, 2007; Goodstein *et al.*, 2012).

**RNA extraction and quantitative real-time PCR (qRT-PCR) analysis**

Expression analysis was performed on four whole seedlings per genotype collected at the end of the day on day 10. A 120 mg (FW) aliquot of each sample was immediately frozen in liquid nitrogen and stored at −80 °C until processing. Frozen tissue was ground in liquid nitrogen and stored of each sample was immediately frozen in liquid nitrogen and stored of each sample was immediately frozen in liquid nitrogen and stored of each sample was immediately frozen in liquid nitrogen and stored of each sample was immediately frozen in liquid nitrogen and stored of each sample was immediately frozen in liquid nitrogen and stored of each sample was immediately frozen in liquid nitrogen and stored of each sample was immediately frozen in liquid nitrogen and stored of each sample was immediately frozen in liquid nitrogen and stored of each sample was immediately frozen in liquid nitrogen and stored of each sample was immediately frozen in liquid nitrogen and stored of each sample was immediately frozen in liquid nitrogen and stored of each sample was immediately frozen in liquid nitrogen and stored.
Carbon-induced growth requires polar auxin transport

A number of lines of evidence demonstrate a link between sucrose and auxin in carbon-induced growth. Auxin biosynthesis and response genes are up-regulated by sugar treatment (Mishra et al., 2009; Lilley et al., 2012; Sairanen et al., 2012). Similarly, Arabidopsis seedlings grown under elevated CO₂
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Exhibit an increase of indole acetic acid (IAA) concentration in the shoots (Hachiya et al., 2014). Both sucrose and auxin alter the dynamics of A. thaliana seedling growth by amplifying the rate and duration of hypocotyl elongation (Stewart et al., 2011). To determine whether the increased hypocotyl length observed when B. rapa seedlings were grown in high CO₂ required auxin, we grew seedlings on medium containing NPA, an inhibitor of auxin transport from the shoot to the root. NPA treatment completely abolished CO₂-induced growth (Fig. 2A). This effect was mirrored in fresh weights. Whereas seedlings grown on standard medium had a root to shoot increase in response to high CO₂, seedlings exposed to NPA had a decreased root/shoot ratio (0.19 to 0.17; Fig. 2B, inset, P<0.001). Consistent with this trend, lateral root growth was also completely suppressed by NPA treatment (Fig. 2C).

Fig. 3. Response to elevated CO₂ requires BrPhyB. (A) Gene expression (qRT-PCR) in phyB mutants. Expression relative to PP2A control. Different letters denote statistical significance compared with the wild type (P<0.001, ANOVA and Tukey HSD multiple comparison test). Asterisks indicate a significantly different response to CO₂ compared with the wild type (**P<0.01, *P<0.05, ANOVA, n=3). (B) Average hypocotyl lengths of 10-day-old B. rapa seedlings on standard, low, or no nitrogen media in ambient (400 ppm) or high (800 ppm) CO₂. The bold black line is the median of at least four independent biological replicates with five seedlings per replicate. The inset shows the fold response to high CO₂ compared with ambient CO₂. Asterisks indicate that all mutants have a significantly different response to CO₂ than the wild type (**P<0.01, *P<0.05, ANOVA). (C) Average shoot and root weights of the same seedlings from (A); inset shows ln(root fresh weight, mg) on the x-axis and ln(shoot fresh weight on the y-axis, square= wild type, triangle=average of phyB-1 and phyB-3. Filled marker points indicate high CO₂; open marker points are ambient CO₂. CO₂ response in all mutants is significantly different from that in the wild type (P<0.05, ANOVA). (D) Number of lateral roots within the 4 cm of primary root near the crown of seedlings from (A), counted on day 7. CO₂ response in all mutants is significantly different from that of the wild type (P<0.001, ANOVA). Letters show significant differences of pairwise mean comparisons (ANOVA and Tukey HSD multiple comparison test).
**PhyB function is conserved across the Brassicaceae**

*Phy* genes are central regulators of carbon allocation (Yang *et al.*, 2016). Here, we identified putative *Phy B* *rapa* orthologs from analysis of the completed *B. rapa* genome (Lagercrantz and Lydiate, 1996; Trick *et al.*, 2009; Wang *et al.*, 2011). Using Phytozyome (Goodstein *et al.*, 2012), we queried all Arabidopsis *Phy* genes for possible *B. rapa* orthologs, in addition to other sequenced members of the Brassicaceae. *Brassica rapa* shares two paleotetraploidy events (beta and alpha) with Arabidopsis, and additionally underwent a whole-genome triplication thought to have occurred between 13 and 17 million years ago (Yang *et al.*, 1999; Town *et al.*, 2006; Beilstein *et al.*, 2010). The recent sequencing of the *B. rapa* genome confirmed the almost complete triplication of the genome compared with Arabidopsis and found that genes that underlie environmental adaptability are over-retained in *B. rapa* (Wang *et al.*, 2011).

A phylogenetic analysis of putative *Phy* genes across the sequenced Brassicaceae showed strong conservation among the five *Phy* clades represented by *Phy A–Phy E* in Arabidopsis (Supplementary Fig. S3). Within the *B. rapa* genome, only *Phy A* appears to have retained a duplicate homolog. There is probably only one *Phy B* ortholog and no likely ortholog for the closely related *AtPhyD*. Sequence alignment of *Brara.E02473* to Arabidopsis *PhyB* and other Brassicaceae orthologs shows high conservation of the GAF, phytochrome and histidine kinase related domain (HKRD) (Supplementary Fig. S4). A mutant screen of the Wisconsin Fast Plant (WFP) self-compatible *B. rapa* variety also identified *Brara.E02473* as a putative Arabidopsis *PhyB* ortholog (Procko *et al.*, 2014).

To test for functional conservation, we obtained multiple mutant alleles for *BrPhyB* (*Brara.E02473*) (Supplementary Fig. S4). The *BrphyB-1* and *BrphyB-3* alleles have nonsense mutations at amino acids 396 and 407, respectively (Supplementary Fig. S3). When grown in red light, seedlings with either mutation exhibited a nearly identical stereotypical *phyB* mutant phenotype with highly elongated hypocotyls and small, closed cotyledons (Supplementary Fig. S5A). Both mutants had a ~5-fold decrease in *BrphyB* transcript level compared with wild-type seedlings (Supplementary Fig. S5B).

**BrPhyB is involved in resource allocation**

Phytochromes are master regulators in organellar responses to environmental signals, controlling distinct aspects of nuclear and chloroplast gene expression (Chun *et al.*, 2001; Thum *et al.*, 2001; Oh and Montgomery, 2014). To examine whether transcriptional responses were affected in *BrphyB* mutant seedlings, we assayed the expression of genes known to be regulated by BrPhyB or involved in the high CO₂ response. In *BrphyB-1* and *BrphyB-3* mutant seedlings grown in ambient CO₂, transcript levels of *BrGH3-5* and *BrIAA19* were significantly higher than in wild-type seedlings; moreover, growth in high CO₂ had no effect on expression of either gene (Fig. 3A, *P* < 0.01 and *P* < 0.001, response to high CO₂ compared with wild-type seedlings, respectively, ANOVA). In Arabidopsis, *GOLDEN2-LIKE 1* (*GLK1*) affects the expression of nuclear photosynthetic genes involved in chloroplast development (Waters *et al.*, 2008, 2009) and its expression is significantly reduced in *phyAphyB* double mutants (Oh and Montgomery, 2014). Similarly, the chloroplast-targeted transcriptional regulator *SIG6* is involved in chlorophyll accumulation and plastid development (Kanamaru *et al.*, 2001; Ishizaki *et al.*, 2005), and is regulated by PhyB (Oh and Montgomery, 2014). We identified likely orthologs of *GLK1* and *SIG6* in *B. rapa*, each of which appears to be represented by a single gene. In *B. rapa* wild-type seedlings, *BrGLK1* expression increases 70% in response to high CO₂. In *BrphyB-1* and *BrphyB-3* seedlings, expression is significantly higher in ambient CO₂ compared with wild-type seedlings and decreases in response to high CO₂ (Fig. 3A, *P* < 0.001, ANOVA). CO₂ levels do not affect *BrSIG6* expression in wild-type plants; however, mutations in *BrphyB* seedlings significantly decrease expression of *BrSIG6* and lead to a negative response to elevated CO₂ conditions (Fig. 3A, *P* < 0.05 for *BrphyB-1*, ANOVA).

If the *Phy* genes are involved in the carbon-sensing network, as suggested by work in Arabidopsis (Lilley *et al.*, 2012; Yang *et al.*, 2016), then *B. rapa* *BrphyB* mutants should show a reduced seedling response to elevated CO₂. To test this hypothesis, we grew wild-type and *BrphyB* seedlings in elevated CO₂. To test this hypothesis, we grew wild-type and *BrphyB* seedlings in ambient and elevated CO₂ conditions (Fig. 3; Supplementary Fig. S6). In response to elevated CO₂, wild-type seedlings had a nearly 40% increase in average hypocotyl length. While significantly longer in ambient CO₂ than those of the wild type, hypocotyls of *BrphyB-1* and *BrphyB-3* showed essentially no response to elevated CO₂ (Fig. 3B, Supplementary Fig. S6 *P* < 0.01 and *P* < 0.001, respectively).

Other growth impacts of high CO₂ mirrored the hypocotyl results. Wild-type seedlings had an ~40% increase in shoot fresh weight when grown in elevated CO₂ compared with growth in ambient conditions (Fig. 3C). In comparison, increased CO₂ had essentially no impact on *BrphyB-1* and *BrphyB-3* shoot biomass (Fig. 3C; *P* > 0.01, ANOVA). As a result, root/shoot ratios were significantly altered in both alleles (Fig. 3C, inset). Carbon effects on root architecture were also dependent on *BrphyB*. The number of lateral roots in *BrphyB* mutants varied widely among individuals (particularly in *BrphyB-3*), but the average number did not significantly increase in response to high CO₂ (Fig. 3D, *P* < 0.001 for all three alleles, ANOVA).

**BrPhyB is required for normal photosynthetic function and seed yield**

Red light stimulates photosynthetic pigment production, and mutants with reduced phy function have significantly lower chlorophyll levels in Arabidopsis (Ghassemian *et al.*, 2006; Strasser *et al.*, 2010; Hu *et al.*, 2013). In addition, adult *phy* mutants have reduced CO₂ uptake and significant reductions in overall growth (Yang *et al.*, 2016). At 3 weeks old, *BrphyB-1* and *BrphyB-3* mutant plants are noticeably paler compared with their wild-type counterparts. Predictably, total chlorophyll is significantly reduced in both mutant alleles (Fig. 4A, ANOVA and Tukey HSD). Not surprisingly, photosynthetic
rates measured at ambient CO2 levels and a high light level are also significantly reduced in mutant plants (Fig. 4B; Supplementary Fig. S7, ANOVA and Tukey HSD).

Stomata are pores found on the surfaces of leaves. They regulate gas exchange, and are another component of photosynthetic productivity that is regulated by photoreceptors (Boccalandro et al., 2009; Casson et al., 2009; Kang et al., 2009). PhyB, specifically, is required for light-mediated systemic control of stomatal development (Casson and Hetherington, 2014). In another indication of conservation of photoreceptor function between Arabidopsis and B. rapa, the stomatal index was significantly reduced in BrphyB-1 and BrphyB-3 mutant plants compared with the wild type (Fig. 4C).

Adult BrphyB mutant plants also had significant reductions in weight and seed yield. Both phyB alleles had a 42% decrease in fresh weight compared with wild-type plants. Six-week-old BrphyB-1 and BrphyB-3 plants had a 53% and 55% reduction in dry weight compared with wild-type plants, respectively (Fig. 4D, E; ANOVA and Tukey HSD). These are similar effects to what has been observed in Arabidopsis phytochrome mutants (Halliday et al., 2003; Yang et al., 2016). In addition, BrphyB mutant plants had significant reductions in total seed yield. BrphyB-1 and BrphyB-3 plants had an 87% and 90% reduction in the number of seeds per plant, respectively, when compared with wild-type plants (Fig. 4F ANOVA and Tukey HSD).

Discussion
Climate change and the resultant shifts in temperature, atmospheric composition, and precipitation present a
critical challenge for plant life on Earth. Potential adaptive responses can take the form of altered initiation and/or timing of developmental events, and changes in the final form or architecture of organs and whole plants. How individual crop species respond to new and potentially more variable conditions will dramatically impact crop yield and global food security. In this work, we have shown that the agriculturally important species B. rapa demonstrates shifts in biomass allocation in response to CO2 and nitrogen availability (Fig. 1). When nitrogen is not limiting, an increase in CO2 leads to increased growth, in both shoots and roots. However, when CO2 levels are high but nitrogen is in short supply, the seedling allocates resources to roots at the expense of shoots (Fig. 1: Supplementary Fig. S1). The responses we observed are in line with those seen for other species in field conditions (Ainsworth and Long, 2005; Reich et al., 2014), highlighting the value of adding B. rapa as a laboratory crop model for studying the molecular mechanisms underlying functional equilibrium.

Phys have recently been proposed as regulators of carbon supply, metabolic status, and biomass production in Arabidopsis (Yang et al., 2016). Our results extend this model to B. rapa. We identified two mutant alleles of the B. rapa PhyB ortholog. Compared with wild-type plants, hypocotyls of BrphyB mutants were longer in ambient CO2, but showed impaired high CO2-induced elongation (Fig. 3B). This pattern is consistent with previously described phenotypes in Arabidopsis. The inability to respond to carbon availability may be attributed to other phytochrome mutant phenotypes reported in Arabidopsis and also observed here in B. rapa (Fig. 4), such as reduced chlorophyll levels (Ghassemian et al., 2006; Strasser et al., 2010; Hu et al., 2013; Yang et al., 2016) or a decreased stomatal ratio (Boccalandro et al., 2009). Mature BrphyB plants had reduced chlorophyll content, stomatal index, as well as photosynthetic rate when measured at high light levels (Fig. 4). Ectopic expression of Arabidopsis PhyB increases tuber yield, and cotton plant growth and yield at low light levels only (Schittenhelm et al., 2004; Rao et al., 2011), suggesting that the decrease in photosynthetic rate observed here may not be relevant in field conditions. However, the combined effect of chlorophyll levels, stomatal index, and auxin signaling—all affected by the phyB mutation in B. rapa and potentially working in concert—probably impact the whole plant response to high atmospheric CO2.

Detailed analysis of our BrphyB mutants across developmental time will be needed to elucidate the full extent of conservation of the Phy–carbon network across the Brassicaceae and into other plants of interest. Such work can lead to better models for plant growth that will facilitate improved predictions of how plants will respond to future climate conditions and can guide the selection of targets for engineering.

Supplementary data

Supplementary data are available at JXB online.

Fig. S1. Carbon and nitrogen availability shape seedling architecture.

Fig. S2. Dry weight of 10-day-old seedlings on variable nitrogen media.

Fig. S3. Amino acid alignment of Phy homologs in the Brassicaceae.

Fig. S4. Amino acid alignment of putative Brassicaceae PhyB orthologs.

Fig. S5. (A) PhyB expression in B. rapa mutants. (B) Wild-type and BrphyB mutant seedlings grown under red light for 4 d.

Fig. S6. Response to elevated CO2 requires PhyB.

Fig. S7. Photosynthetic and stomatal conductance time course of 3-week-old B. rapa wild-type plants

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