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Interaction of Brassinosteroids with Light Quality and Plant Hormones in Regulating Shoot Growth of Young Sunflower and *Arabidopsis* Seedlings

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Abstract Sunflower hypocotyls elongate as light quality changes from the normal red to far-red (R/FR) ratio of sunlight to a lower R/FR ratio. This low R/FR ratio-induced elongation significantly increases endogenous concentrations of indole-3-acetic acid (IAA) and also of three gibberellins (GAs): GA_{20}, GA_1, and GA_8. Of these, it is likely GA_1 that drives low R/FR-induced growth. Brassinosteroids are also involved in shoot growth. Here we tested three R/FR ratios: high, normal, and low. Significant hypocotyl elongation occurred with this stepwise reduction in R/FR ratio, but endogenous castasterone concentrations in the hypocotyls remained unchanged. Brassinolide was also applied to the seedlings and significantly increased hypocotyl growth, though one that was uniform across all three R/FR ratios. Applied brassinolide increased hypocotyl elongation while significantly reducing (usually) levels of IAA, GA_{20}, and GA_8, but not that of GA_1, which remained constant. Given the above, we conclude that endogenous castasterone does not mediate the hypocotyl growth that is induced by enriching FR light, relative to R light. Similarly, we conclude that the hypocotyl growth that is induced by applied brassinolide does not result from an interaction of brassinolide with changes in light quality. The ability of applied brassinolide to influence IAA, GA_{20}, and GA_8 content, yet have no significant effect on GA_1, is hard to explain. One speculative hypothesis, though, could involve the brassinolide-induced reductions that occurred for endogenous IAA, given IAA's known ability to differentially influence the expression levels of *GA20ox*, *GA3ox*, and *GA2ox*, key genes in GA biosynthesis.

Keywords Light quality · Brassinosteroids · Gibberellins · Auxin · Hypocotyl elongation · Growth

Introduction

Plants grown under canopy shade or in the shade of neighboring, proximate vegetation are subjected to sunlight having a lower than normal red to far-red (R/FR) ratio. Because of this they usually exhibit elongated (etiolated) stem tissues, that is, etiolated hypocotyls and internodes, and enlarged leaves (Smith 2000; Kurepin and others 2007c). Earlier work has shown that light quality-induced hypocotyl etiolation and leaf area growth in sunflower (*Helianthus annuus* L.) is regulated primarily by gibberellins (GAs) and auxin (IAA [indole-3-acetic acid]) (Kurepin and others 2007a, b). Additionally, ethylene (Kurepin and others 2007b, c), cytokinins (Kurepin and others 2007a), and possibly salicylic acid (Kurepin and others 2010) may be involved.

Brassinosteroids constitute another class of plant hormones that have been shown to be directly involved in the

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stem etiolation that occurs in the dark (Li and others 1996; Nagata and others 2000). However, the possible role of brassinosteroids in low R/FR ratio-mediated etiolation has not been examined. In this study we look at the effects of changing light quality on the levels of an endogenous brassinosteroid [castasterone (CS)] in sunflower hypocotyls and also on its interaction with applied brassinolide (BL). Here we assessed the effect of sunflower hypocotyl elongation under low, normal, and high R/FR ratios, with and without BL application, on concentrations of several endogenous GAs and also IAA, using the stable isotope dilution method for quantification. Finally, we also utilized the *Arabidopsis thaliana Col-0* wild-type (WT) and several *BRI* and *BAK* genotypes (mutants of the BR signal transduction pathway) to examine the effects of changing light quality on petiole growth, and also on endogenous GA_4 and IAA concentrations in *Arabidopsis* shoot tissue. Brassinosteroid-insensitive 1 (*BRI1*) is an essential component of the BR receptor complex (Wang and others 2001; Li and others 2002), and the extracellular domain of *BRI1* can bind BRs directly (Kinoshita and others 2005). BRI1-associated receptor kinase 1 (BAK1) is a second leucine-rich repeat receptor-like kinase. It interacts with BRI1 in vitro and in vivo, suggesting that receptor kinase heterodimerization may play an important role in BR signal transduction (Li and others 2002).

Materials and Methods

Sunflower seeds (6946, Pioneer Seeds, USA) were imbibed under running water (25°C) in dark conditions for 24 h. Germinated seeds were then planted in soil mix (2 parts peat moss, 1 perlite, 1 vermiculate and 0.25 terra green). Sunflower seedlings were watered daily with 0.25% strength Hoagland's solution (Hoagland and Arnon 1950). *Arabidopsis thaliana* seeds, prior to planting in 100-mm Petri dishes, were washed with 70% aqueous ethanol and then left for 4 days in the dark at 0°C in double-distilled H_2O. Seeds were then planted on gelrite (2.2 g/L, Sigma-Aldrich, USA) containing 0.5% Murashige and Skoog (1962) basal medium (Sigma-Aldrich, USA) plus 1% sucrose (EMB Chemicals, USA). Plants were grown in growth chambers (Model PGR15, Conviron, Manitoba, Canada) equipped with fluorescent (Sylvania cool white 160 W) and incandescent lights (Philips 60 W). The temperature was maintained at 20°C during 16 h of light and reduced to 16°C during an 8-h dark period.

The sunflower seedlings were harvested on day 7 following planting. Day 7 was chosen for hypocotyl collection because under our conditions it was the midpoint for elongation, between hook opening (day 4) and initiation of internode growth, when cessation of hypocotyl elongation occurred (day 10). The hypocotyls (excised just below the cotyledons and 5 mm above the roots) and internodes (first internodes between the cotyledons and first pair of leaves) were measured for length, fresh and dry weights, and endogenous CS, GA (GA_{20}, GA_1, GA_8), and IAA content. The *Arabidopsis* seedlings were harvested 14 days after planting, and the first leaf pair was measured for petiole length and leaf area by scanning on a flatbed scanner (LiDE20, Canon, China) using Scion Image for Windows software (Informer Technologies).

Combinations of fluorescent and incandescent light sources were used to alter the R/FR ratios, and the distance between the light bulbs and the seedlings was adjusted to maintain a constant level of photosynthetically active radiation (PAR). Both R/FR ratios (low of 0.87, normal of 1.23, and high of 4.31) and the PAR value (511 μmol m^{-2} s^{-1}) were measured with a LI-COR LI-1800/22 (LI-COR, Inc., Lincoln, NE, USA) quantum sensor. We utilized Tukey's ANOVA test for analysis of significance (at $p \leq 0.05$) and also the Spearman rank correlation test. Both were run on SPSS software ver. 15 (SPSS, Inc., Chicago, IL, USA).

For phytohormone analysis, the tissue was harvested and immediately frozen in liquid N_2, then freeze-dried. For analysis of endogenous GA and IAA levels, each sample (ca. 0.5 g dry weight [DW]) was ground with mortar and pestle with liquid N_2 as described by Kurepin and others (2007c). Then 250 ng of [$^2H_6$] IAA (a gift from Drs. L. Rivier and M. Saugy, University of Lausanne, Switzerland) and 20 ng each of [17,17-$^2H_2$] GA_1, GA_8, and GA_{20} for sunflower, and, additionally, 33 ng each of [17,17-$^2H_2$] GA_4, GA_9, and GA_{34} for *Arabidopsis*, were added to the extraction solvent as internal standards (deuterated GAs were obtained from Prof. L.N. Mander, Research School of Chemistry, Australian National University, Canberra, Australia). The identification and quantification of GAs and IAA were carried out using a gas chromatograph connected to a mass spectrometer (GC-MS) in the –selected ion-monitoring (–SIM) mode. For identification of the endogenous phytohormones, we utilized a comparison of GC retention times (Rt) of the endogenous hormone and its deuterated internal standard, as well as a comparison of the relative intensities of at least three characteristic *m/z* ion pairs, including the molecular ion (M^+) pairs. Details on GC-MS conditions and other methodologies used for hormone identification and quantification are described in Kurepin and others (2007c).

For analysis of endogenous brassinosteroids, including BL and CS, sunflower plants (4–8 g DW) were extracted with MeOH (300 ml × 3) and subsequently extracted again with $CHCl_3$ (200 ml × 3). The $CHCl_3$-soluble fraction was concentrated *in vacuo* and then partitioned between 80%

MeOH (200 ml) and *n*-hexane (200 ml) three times. The 80% MeOH-soluble fraction was reduced to an aqueous phase and partitioned between 0.1 M Na-phosphate buffer (pH 7.4) (200 ml) and ethyl acetate (200 ml). Then, 200 ng each of deuterium-labeled ([26,28-$^2H_6$]) CS and BL were added as internal standards. The obtained neutral ethyl acetate–soluble fraction was chromatographed on SiO_2 (30 g) eluted with mixtures of $CHCl_3$–MeOH by increasing the MeOH concentration (0, 2, 4, 6, 8, 10, 20, 50, 100%). The 4–8% MeOH-collected eluates in the $CHCl_3$–MeOH fraction were combined and then purified via SepPak (C_{18}) column chromatography eluted with aqueous MeOH (50, 60, 70, 80, 90, 100%; 5 ml each). The 80% MeOH-collected eluate fraction was purified via reversed-phase high-pressure liquid chromatography (HPLC) (Senshu-Pak C_{18}, 10 × 150 mm), eluted with aqueous methanol as a mobile phase (0–20 min: 45%, 20–40 min: gradient to 100%, 40–60 min: 100% MeCN) at a flow rate of 2.5 ml min^{-1}. Under the HPLC conditions eluted with the MeCN-water gradient, authentic CS and BL can be detected in fractions 19 to 21 and 13 to 15, respectively. Therefore, these fractions were analyzed by capillary GC-MS after bismethaneboronation. The amount of CS was calculated using [26,28-$^2H_6$] CS as an internal standard.

The GC-MS analyses were performed on a Hewlett-Packard 5973 mass spectrometer (electron impact ionization, 70 eV; Hewlett Packard, Palo Alto, CA) connected to a 6890 gas chromatograph fitted with a fused silica capillary column (HP-5, 0.25 mm, 30 m, 0.25-mm film thickness). The oven temperature was maintained at 175°C for 2 min, elevated to 280°C at a rate of 40°C min^{-1}, and then maintained at 280°C. Helium was used as the carrier gas at a flow rate of 1 ml min^{-1}, and samples were introduced using an on-column injection mode. Methaneboronation was performed by heating samples dissolved in pyridine containing methaneboronic acid (2 mg ml^{-1}) at 80°C for 30 min.

The BL was synthesized using the method of Back and others (1997). A putative inhibitor of BR biosynthesis, brassinazole (Asami and others 2000) was obtained from Professor Tadao Asami in University of Tokyo, Japan. For applications to the sunflower plants, BL and brassinazole were initially dissolved in an aqueous solution containing 1% DMSO at a concentration of 10^{-6} M and then further diluted with 1% DMSO solution to achieve concentrations of 10^{-7} and 10^{-8} M. The sunflower seedlings were then sprayed with a high volume of either BL solution or brassinazole solution at low pressure. This assured a uniform application because all of the shoot tissue became wet, with a small volume of spray solution dripping off the cotyledons. Sprays took place on days 4, 5, and 6 after planting. The control spray was an aqueous solution containing 1% DMSO.

Results

Sunflower hypocotyls gradually but significantly increased their elongation as the R/FR ratio decreased (see Figs. 1, 2, and 3, open bars) A decrease in the R/FR ratio has been previously shown to significantly increase endogenous GA and IAA levels in sunflower hypocotyls (Kurepin and others 2007a) and internodes (Kurepin and others 2007c). Thus, trends for endogenous IAA (Fig. 1, shaded bars) and endogenous GA_{20}, GA_1, and GA_8 (Fig. 3a–c, shaded bars) were expected, that is, decreased hormone levels (shaded bars) as the R/FR ratio increased, which for the GAs was approximately in parallel with the reduced hypocotyl elongation (open bars).

Analysis of endogenous brassinosteroids by GC-MS –SIM identified castasterone (CS), a brassinosteroid that has been demonstrated to be biologically active per se and which likely functions in most plants as the active BR in vegetative growth and development (Kim and others 2005).

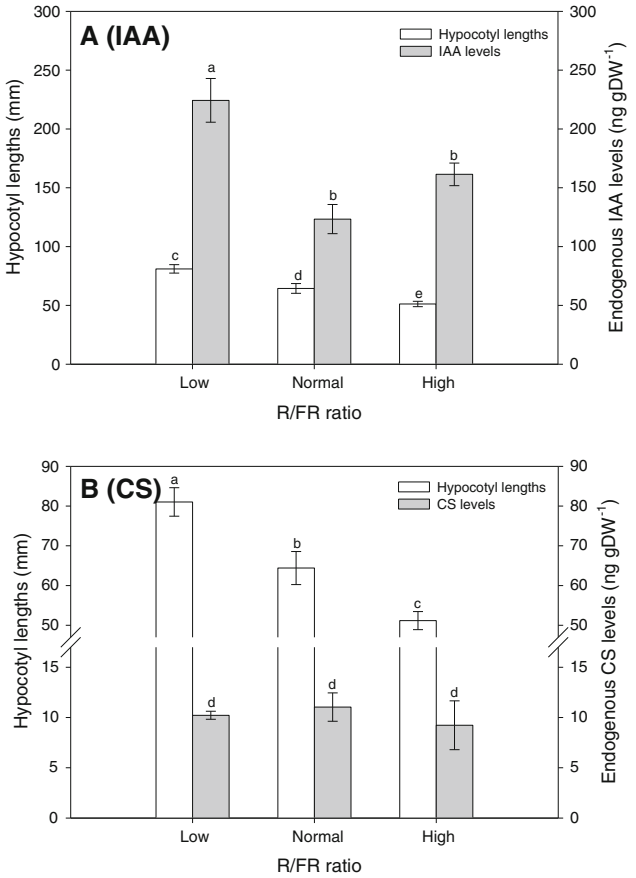

Fig. 1 Hypocotyl lengths (mm, *open bars*) and endogenous IAA (**a**) and CS (**b**) levels (ng gDW^{-1}, both IAA and CS as *shaded bars*) of 7-day-old sunflower seedlings grown under varied R/FR ratios with the same fixed PAR. The error bars indicate 1 SEM, and mean values with the same letter do not differ significantly at $p \leq 0.05$ based on Tukey's multiple-comparison test

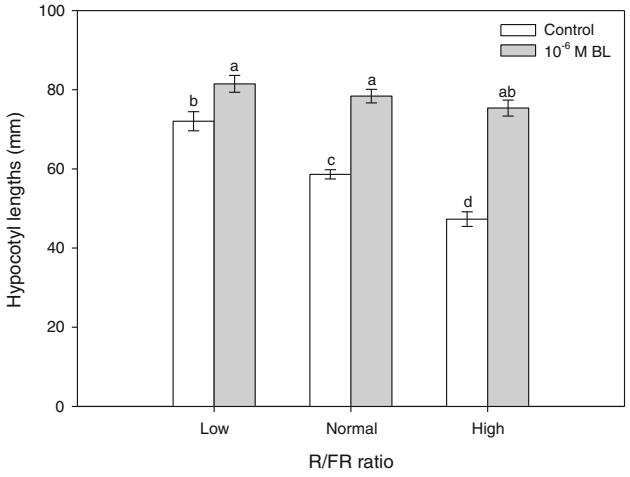

Fig. 2 Hypocotyl lengths (mm) of control (*open bars*) and BL-treated (10^{-6} M, *shaded bars*) 7-day-old sunflower seedlings grown under varied R/FR ratios with the same fixed PAR. The *error bars* indicate 1 SEM, and mean values with the same letter do not differ significantly at $p \leq 0.05$ based on Tukey's multiple-comparison test

CS can be converted to BL in cultured cells and seedlings of *Cataranthus roseus* (Sakurai 1999), and Kim and others (2005) have shown, using *Arabidopsis*, that the *CYP85A2* gene encodes a cytochrome P450 enzyme that catalyzes the biological oxidation of CS to BL. Endogenous BL, CS, dolichosterone, and norcastasterone have been previously identified in the pollen of sunflower (Gamoh and others 1990). However, in the hypocotyl tissues we found CS to be the only detectable BR, and its levels (Fig. 1b) were not significantly influenced by varying the R/FR ratio, though they tended to be lower (and more variable) under the high R/FR ratio treatment (Fig. 1b).

BL applied to young sunflower plants showed a significant growth-promoting effect on hypocotyl elongation only at the highest concentration: 10^{-6} M (Fig. 2). That same high concentration of BL also significantly promoted hypocotyl DW accumulation (data not shown). The effect of applied BL on hypocotyl elongation increased as the R/FR ratio increased, that is, the growth-retarding effect of

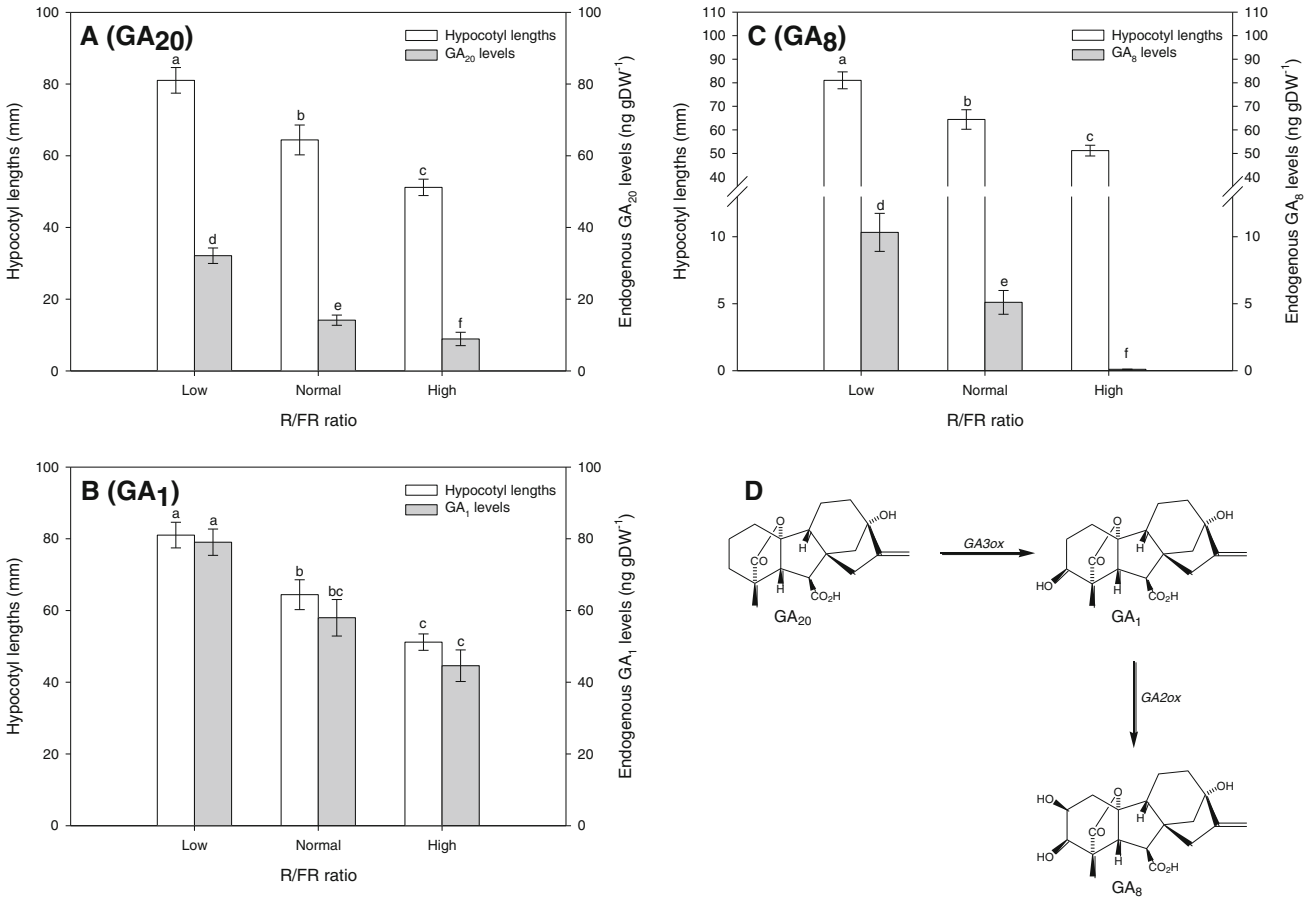

Fig. 3 Hypocotyl lengths (*open bars*) and endogenous gibberellin levels (*shaded bars*) (**a**) GA_{20}, (**b**) GA_1, (**c**) GA_8 (ng gDW^{-1}) for 7-day-old sunflower seedlings grown under varied R/FR ratios with the same fixed PAR. These plants were controls, that is, they were not treated with BL. The error bars indicate 1 SEM, and mean values with the same letter, as R/FR ratio changes, do not differ significantly at $p \leq 0.05$ based on Tukey's multiple-comparison test. A segment of the GA biosynthesis pathway is shown in **d**

increasing the R/FR ratio was effectively counteracted by applied BL, resulting in similar lengths for hypocotyls on BL-treated plants under all three R/FR ratios (Fig. 2). Application of brassinazole, however, yielded no statistically significant changes in hypocotyl elongation at any of the three concentrations tested (data not shown).

A similar set of sunflower seedlings were also sprayed with BL at 10^{-6} M and analyzed for endogenous IAA and GAs. There were significantly reduced IAA levels under two of the three R/FR ratios relative to IAA levels in control plants that received no BL (Fig. 4a). Interestingly, endogenous IAA levels were uniform at approximately 90 ng gDW^{-1} under all three R/FR ratios for plants treated with BL (Fig. 4a).

Endogenous GA levels were also influenced by applied BL (Fig. 4b–d). For the growth "effector" GA_1, hypocotyls of BL-treated seedlings showed slight but nonsignificant decreases in GA_1 [relative to hypocotyls of plants that received no BL (open bars)] across each of the three R/FR ratios (Fig. 4c). In contrast, the immediate precursor of GA_1, GA_{20}, was significantly reduced by BL treatment to a uniformly low level, about 4–6 ng gDW^{-1} (Fig. 4b) across all three light quality treatments. This is a very unusual pattern, one that we have never seen before. That said, one should note the very uniform growth promotion induced by applied BL across those same three light quality treatments (Fig. 2). For GA_8, the immediate 2β-hydroxylated (inactive) catabolite of GA_1, the effect of BL is even more unusual (Fig. 4d): a significant reduction in GA_8 concentration under the low R/FR ratio, a slight and nonsignificant reduction at the normal R/FR ratio, and a significant elevation of GA_8 by BL at the high R/FR ratio. Finally, as seen for GA_{20}, GA_8 levels in

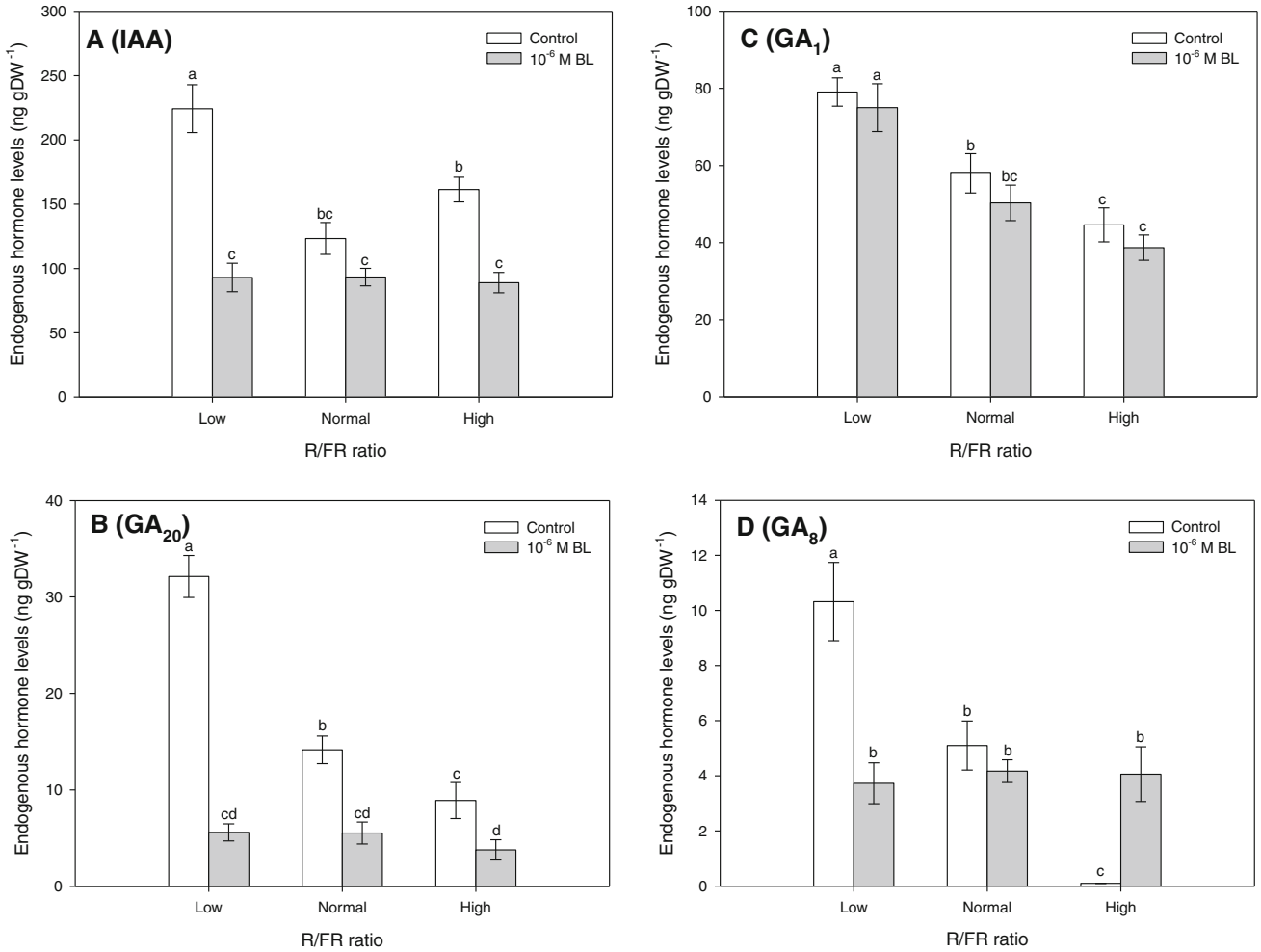

Fig. 4 Endogenous IAA and GA_{20}, GA_1, and GA_8 levels in hypocotyls of both controls and BL-treated sunflower seedlings grown under varied R/FR ratios with the same fixed PAR. BL-treated plants were sprayed to drip off at a 10^{-6} M concentration, and the hypocotyls of both controls and BL-treated plants were harvested for hormone analysis at age 7 days. The error bars indicate 1 SEM, and mean values with the same letter, as R/FR ratio changes, do not differ significantly at $p \leq 0.05$ based on Tukey's multiple-comparison test

hypocotyls of BL-treated seedlings were uniform (ca. 4 ng gDW^{-1}) across all three light quality treatments (Fig. 4d).

We also utilized four *Arabidopsis* mutants for testing the downstream signaling events of BR perception. These included (i) *bri1-201* (a BR-insensitive genotype with a dwarfed phenotype), (ii) *BRI1-GFP* (a BR-overexpression genotype, which has a phenotype that is characterized by stronger BL perception, that is, it possesses a high steady-state expression level of a BR receptor kinase, and increased shoot growth), (iii) *bak1* (a BR-insensitive genotype, also with a dwarfed phenotype), and (iv) *35S-BAK1* (a BR-overexpression genotype, with a phenotype similar to the WT line) (Wang and others 2001; Li and others 2002; Kim and others 2007). The *bri1-201*, *bak1*, and *35S-BAK1* genotypes responded to the reduction in the R/FR ratio in a manner similar to the background line, WT *Col-0* (Tables 1 and 2). However, the *BRI1-GFP* mutant from the same WT background line showed no changes in shoot growth (petiole length and leaf area) when low R/FR ratio treatments were compared with plants that were grown under a normal R/FR ratio (Tables 1, 2). We also analyzed the shoot tissue of WT *Col-0* and *BRI1-GFP* seedlings for endogenous IAA and GAs. Endogenous shoot

Table 1 Petiole lengths (mm) of first plus second leaves of 14-day-old *Arabidopsis* seedlings grown under varying R/FR ratios and fixed PAR

Petiole lengths (mm)	Low R/FR ratio	Normal R/FR ratio	High R/FR ratio
Col-0	7.0^{a}	4.6^{b}	4.0^{b}
bri1-201	2.9^{a}	2.3^{b}	2.4^{b}
BRI-GFP	6.4^{a}	6.3^{a}	5.7^{b}
bak-1	3.9^{a}	3.4^{b}	3.3^{b}
35S-BAK1	5.9^{a}	4.6^{b}	4.0^{c}

Mean values with the same letter do not differ significantly at $p \leq 0.05$ based on a Tukey's ANOVA test performed for each genotype

Table 2 Leaf areas (mm^2) of first plus second leaves of 14-day-old *Arabidopsis* seedlings grown under varying R/FR ratios and fixed PAR

Leaf areas (mm^2)	Low R/FR ratio	Normal R/FR ratio	High R/FR ratio
Col-0	17.6^{a}	13.0^{b}	9.8^{c}
bri1-201	10.1^{a}	8.2^{b}	8.3^{b}
BRI-GFP	11.4^{a}	11.4^{a}	11.7^{a}
bak-1	9.9^{a}	7.3^{b}	6.3^{b}
35S-BAK1	11.7^{a}	10.1^{b}	9.4^{b}

Mean values with the same letter do not differ significantly at $p \leq 0.05$ based on a Tukey's ANOVA test performed for each genotype

Table 3 Endogenous GA_4 and IAA levels (ng gDW^{-1}) in shoots of 14-day-old *Arabidopsis* seedlings grown under varying R/FR ratios and a fixed PAR

Genotype	GA_4 levels		IAA levels	
	Low R/FR ratio	Normal R/FR ratio	Low R/FR ratio	Normal R/FR ratio
Col-0	12.1^{a}	8.42^{b}	141^{a}	105^{b}
BRI-GFP	14.6^{a}	14.3^{a}	50.5^{a}	58.5^{a}

Mean values with the same letter do not differ significantly at $p \leq 0.05$ based on a paired *t*-test performed for each genotype and for each hormone

IAA concentrations were increased by the low R/FR ratio treatment in *Col-0* but not in *BRI1-GFP* plants. Additionally, *BRI1-GFP* shoot tissues had 50% lower IAA levels under a normal R/FR ratio relative to shoot tissues of *Col-0* (Table 3). Although no endogenous GA_1, GA_8, GA_9, GA_{20}, or GA_{34} levels were detected in the *Arabidopsis* shoot tissues, the native growth-active GA, GA_4, was identified. Endogenous GA_4 concentrations were increased in shoot tissues of *Col-0* plants by our low R/FR ratio treatment (Table 3). In contrast, plants of the *BRI1-GFP* genotype showed no significant response to low R/FR ratio treatment (Table 3). However, shoot tissues of *BRI1-GFP* plants had approximately 60% higher endogenous GA_4 concentrations under the normal R/FR ratio relative to *Col-0* plants (Table 3).

Discussion

First, hypocotyl growth response to varying light quality across three R/FR ratios behaved in a manner very similar to that shown by Kurepin and others (2007a), as did reductions in IAA and GA_{20}, GA_1, and GA_8 levels as R/FR ratio increased (Kurepin and others 2007a). This type of repeatability for light quality-induced changes in endogenous IAA and endogenous GAs gives us a reasonable level of confidence that our light quality treatment effects on endogenous brassinosteroid levels (Fig. 1b) are "real." Further, it also indicates that the sunflower hypocotyl system is a suitable one for investigating the possible physiological mechanisms by which applied BL might influence hypocotyl growth of intact plants grown under differing light quality treatments, that is, R/FR ratios that are lower or higher than the R/FR ratio seen with normal sunlight, which is approximately 1.2.

Castasterone, the only native brassinosteroid detected in sunflower hypocotyls, remains at a constant level, around 10 ng gDW^{-1}, as R/FR treatment progresses from low to high levels of R (Fig. 1b). Endogenous CS biosynthesis, therefore, appears to be totally disconnected from changes

in light quality as an influential environmental factor. Further, BL application yields a uniform hypocotyl growth response (promotion; Fig. 2) regardless of the R/FR ratio treatment. Again, there is a total disconnect: significant growth is induced by applied BL but with no influence of changing light quality on the response.

Finally, applied BL induced hypocotyl growth on young sunflower plants while (1) uniformly reducing, relative to controls, the endogenous IAA and GA_{20} concentrations across all light quality treatments (Fig. 4a, b), (2) having a negligible (nonsignificant) effect on the concentrations of GA_1 (Fig. 4c), and (3) reducing or increasing GA_8 relative to controls, thereby yielding a uniform GA_8 concentration across all three light quality treatments (Fig. 4d).

The above results of BL application are quite unusual, especially given the known involvement of BRs in light-dependent development, for example, the *Arabidopsis DET2*/*det2* and pea *LK*/*lkb* examples (Li and others 1996; Schultz and others 2001). Additionally, the literature shows several synergistic responses of applied BRs with IAA on stem segment growth and also of BRs with IAA and GAs on hypocotyl elongation (Cohen and Meudt 1983; Katsumi 1991; Tanaka and others 2003; Bajguz and Hayat 2009). In dark-etiolated pea seedlings, exposure to white light has been shown to increase an endogenous brassinosteroid, 6-deoxocastasterone (Symons and Reid 2003a). This increase accelerated from day 2 to day 4 following exposure to white light and was associated with accelerated increases in endogenous GA_1 levels and accelerated decreases in endogenous IAA levels (Symons and Reid 2003a, b).

Effects of BRs on the endogenous GA level have been discussed recently in Zhang and others (2009). Specifically, BR biosynthesis in rice can be promoted by *OsGSR1*. But, when *OsGSR1* is knocked out (Yang and others 2004), elevated levels of endogenous GA_4 were observed (Wang and others 2009). Or to paraphrase, reduced BR biosynthesis (due to "knocking out" *OsGSR1*) yields increased endogenous GA_4 levels. The results of Wang and others (2009) are somewhat similar to our findings where we see applied BL influencing levels of endogenous GA_{20} and GA_8, though not GA_1 (Fig. 4b–d).

Thus, the sunflower hypocotyl growth increases seen for untreated plants in response to FR enrichment (low R/FR ratio see Fig. 1a) do not appear to involve changes in CS, the only endogenous BR detected (see Fig. 1b). This conclusion is further reinforced by the growth increase induced by 10^{-6} M BL application (Fig. 2, shaded bars). In that case, applied BL totally counteracts the much reduced growth that occurs with R light enrichment, that is, when the R/FR ratio is increased (Fig. 2, compare open bars where plants had no BL treatment with the shaded bars). From these results we conclude that endogenous CS is not directly involved in R/FR ratio-mediated sunflower hypocotyl elongation.

There is evidence that IAA can influence the expression of genes that control the GA biosynthetic pathway (see below). Further, IAA concentration can be modified (increased) in both our sunflower hypocotyls and *Arabidopsis* shoots by reducing the R/FR ratio (Fig. 1a; Table 3). And, as discussed above, IAA levels are significantly reduced by applied BL (Fig. 4a). One could thus postulate that the applied BL has modified IAA biosynthesis or metabolism, thereby leading to significant reductions in endogenous IAA (Fig. 4a).

Further, in regard to the reductions that we see in concentrations of GA_{20} and GA_8 in response to applied BL, we could also postulate that BL functions as a direct inhibitor of at least some steps in GA biosynthesis. Alternatively, one could bring in a more complex explanation, one involving auxin. For example, the native auxin of pea, 4-chloro IAA, can enhance *GA20ox1* expression in the growing pod (Ngo and others 2002). Thus, the reduction in endogenous IAA in our sunflower hypocotyls by applied BL (Fig. 4a) might be the causal mechanism for the reduced levels of endogenous GA_{20} seen in hypocotyls of the BL-treated sunflower plants (Fig. 4b). Auxin has also been shown to regulate *GA3ox* expression in pea fruit (pods) and seeds (Ozga and others 2003).

IAA from the shoot apex is also known to suppress the expression of *GA2ox1* in the pea stem (O'Neill and Ross 2002). Thus, reduced IAA levels (from BL application) might be expected to yield an increase in GA_8 concentrations, which it does, but only for the high R/FR ratio treatment (Fig. 4d).

An explanation for the inability of applied BL to significantly influence hypocotyl GA_1 concentrations under any of the three R/FR ratios (Fig. 4c) is harder to come by. That said, one could postulate that there is an IAA-mediated "balance" between *GA20ox*, *GA3ox*, and *GA2ox* expression, thereby yielding a nil effect on GA_1 concentrations. However, our results do not provide direct evidence for such a speculative hypothesis.

Similar conclusions can be drawn from our *Arabidopsis* results. As would be expected from results obtained with the sunflower system (Kurepin and others 2007a, c), *Col-0* plants showed increased leaf and petiole growth as the light quality regimen was moved from a normal R/FR ratio to a low R/FR ratio. This increase in leaf area growth and petiole elongation was also associated with increased endogenous GA_4 and IAA concentrations in the shoot tissue of *Col-0* plants (Table 3). In contrast, for the *BRI1-GFP Arabidopsis* mutant there was a nil or negligible growth response to changes in light quality (Tables 1, 2), and coincidentally there was also no significant change in endogenous GA_4 or endogenous IAA levels in shoot tissues of *BRI1-GFP* plants grown under the different R/FR ratio regimens (Table 3). Thus, the *Arabidopsis BRI1-GFP*

mutant shows a pattern that is similar to that seen for our BL-treated sunflower hypocotyls (Fig. 4). Applied brassinolide was previously shown to regulate multiple genes in *Arabidopsis* shoots, including phytochrome- and auxin-related genes (Goda and others 2002).

The other *Arabidopsis* mutant with increased BR perception, *35S-BAK1*, responded to the changes in light quality in a manner similar to the *Col-0* WT line (Tables 1, 2). Although we expected the *35S-BAK1* genotype to show a response similar to *BRI1-GFP*, the fact that it did not is not novel. For example, 5-day-old plants of *BRI1-GFP* genotype show a greater root gravitropic curvature than WT plants when left in horizontal position for 24 h, whereas the response of the *35S-BAK1* genotype is similar to that seen for WT plants (Kim and others 2007). Similarly, a dose-dependent application of IAA to *Arabidopsis* roots increases the gravitropic response in both WT and *35S-BAK1* genotypes, whereas *BRI1-GFP* plants were not affected by applied IAA (Kim and others 2007).

We also tested hypocotyl elongation of *Col-0*, *BRI1-GFP*, and *bri1-201* genotypes under separate LED-generated light wavelengths (unpublished data). Hypocotyl elongation of *Col-0* was increased under R light relative to the response obtained under white light (low intensities of 10 μmol m^{-2} s^{-1} for both R and white light). In contrast, hypocotyl elongation was not affected by FR light relative to white light (FR also given at 10 μmol m^{-2} s^{-1}). Therefore, hypocotyls of *Arabidopsis* have a very different type of response to R and FR signalling than hypocotyls of sunflower. Neff and others (1999) showed that hypocotyls in the *BAS-1* antisense line (*BAS-1* is a gene that regulates brassinosteroid levels and light responsiveness in *Arabidopsis*) had increased elongation when grown under white or FR light at 10 μmol m^{-2} s^{-1} relative to the *Col-0* line.

Both of the BR mutant lines, *BRI1-GFP* and *bri1-201*, had their hypocotyl elongation increased under R light (but proportionally less than *Col-0*). In contrast, under FR light the hypocotyl elongation of both mutant lines was decreased. Therefore, although the mutant lines do show some differences in their response to R and FR light signalling compared to WT background line, these differences are the same for each of the two mutant lines. This again suggests that endogenous BRs are not involved in R/FR ratio-mediated hypocotyl elongation response.

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