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Red/Far Red Light Controls Arbuscular Mycorrhizal Colonization via Jasmonic Acid and Strigolactone Signaling

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Regular Paper

Establishment of a nitrogen-fixing symbiosis between legumes and rhizobia not only requires sufficient photosynthate, but also the sensing of the ratio of red to far red (R/FR) light. Here, we show that R/FR light sensing also positively influences the arbuscular mycorrhizal (AM) symbiosis of a legume and a non-legume through jasmonic acid (JA) and strigolactone (SL) signaling. The level of AM colonization in high R/FR light-grown tomato and *Lotus japonicus* significantly increased compared with that determined for low R/FR light-grown plants. Transcripts for JA-related genes were also elevated under high R/FR conditions. The root exudates derived from high R/FR light-grown plants contained more (+)-5-deoxystrigol, an AM-fungal hyphal branching inducer, than those from low R/FR light-grown plants. In summary, high R/FR light changes not only the levels of JA and SL synthesis, but also the composition of plant root exudates released into the rhizosphere, in this way augmenting the AM symbiosis.

Keywords: Arbuscular mycorrhizal symbiosis • Jasmonic acid • *Lotus japonicus* • Red/far red light ratio • *Solanum lycopersicum* cv. Micro-Tom • Strigolactone.

Abbreviations: AM, arbuscular mycorrhizal; CE/TOF MS, capillary electrophoresis time-of-flight mass spectrometry; ESI-MS, electrospray ionization-mass spectrometry; JA, jasmonic acid; PAR, photosynthetically active radiation; R/FR, red/far red; RT–PCR, reverse transcription–PCR; SAS, shade avoidance syndrome; SL, strigolactone.

Introduction

Arbuscular mycorrhizal (AM) fungi are soil microorganisms that establish a phosphate (P)-acquiring symbiosis with the majority of higher plant families except Brassicaceae and Chenopodiaceae (Harrison 2005). This ancient symbiosis (dating from >460 million years ago) (Simon et al. 1993, Remy et al. 1994, Redecker et al. 2000, Heckman et al. 2001) is the most widespread association between plant roots and fungi in both natural and agricultural ecosystems. AM fungal hyphae penetrate and colonize root cortical cells of host plants where they differentiate into highly branched, plant membrane-bound structures known as arbuscules, which are the principal sites of nutrient exchange between host plants and AM fungi. Host plant roots release signal molecules, strigolactones (SLs), which promote the branching of AM fungal hyphae as they encounter the root (Akiyama et al. 2005). Thus, the development of the AM symbiosis is controlled by complex signal perceptions and exchanges, and considerable information about the genes and signals involved in this important symbiosis has been obtained over the years.

It is well known that plants require light for photosynthesis, and that both light quality and quantity influence plant development and survival. Plants also sense the presence of their neighbors, which may compete for photosynthetically active radiation (PAR). They do this via photoreceptors such as phytochrome, which detect the ratio of red (R) and far red (FR) light. This sensing initiates the shade avoidance syndrome (SAS), where crowding causes plants to grow taller or bend towards the light to avoid shade (Smith and Whitelam 1997, Neff et al. 2000, Franklin 2008, Franklin and Quail 2010, Ballaré et al. 2012). Earlier, we reported that light quality influences root nodule formation in the legume *Lotus japonicus*; low R/FR light conditions significantly reduced root nodule formation (Suzuki et al. 2011). We further demonstrated that nodule development is photomorphogenetically controlled via the photoreceptor phytochrome, which perceives the R/FR light ratio and affects downstream jasmonic acid (JA) signaling (Suzuki et al. 2011).

JA is a crucial molecule involved in many aspects of plant life, including defense against pathogens and environmental tolerance. Many reports show that JA acts positively on the mycorrhizal symbiosis (Hause and Schaarschmidt 2009, Ballaré 2011, León-Morcillo et al. 2012). Our previous study demonstrated

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that an active JA derivative, responsible for increased nodule numbers, was present in higher concentrations in *L. japonicus* MG20 than in *phyB* mutant plants, even when the latter was grown under white light conditions (Suzuki et al. 2011). Based on these findings, we predicted that the AM symbiosis would be enhanced under light conditions that promoted nodule numbers in *L. japonicus*. Here, we have expanded upon our earlier study by including both a legume, *L. japonicus* MG20, and a non-legume, *Solanum lycopersicum* cv. Micro-Tom (Ariizumi et al. 2011), in an investigation of how light quality affects the AM symbiosis and the regulatory mechanisms of its establishment. The vast majority of flowering plants, with a few exceptions, establish AM symbioses, whereas only a limited number form nitrogen-fixing nodules. Thus, studying the AM symbiosis in a legume and non-legume should help us determine whether paradigms regarding the role of light quality in plant–microbe interactions are applicable to both groups of plants.

Results

AM fungal colonization under low R/FR and high R/FR light conditions

Solanum lycopersicum cv. Micro-Tom plants were grown under continuous white light for 28 d and either inoculated with *Rhizophagus irregularis* (**Fig. 1A**; top row) or left uninoculated to assess mycorrhizal fungal colonization. **Fig. 1B** illustrates the increase in shoot length of *S. lycopersicum* cv. Micro-Tom in response to AM fungal inoculation (myc +) compared with the uninoculated (myc −) control. Plants of *S. lycopersicum* cv. Micro-Tom and *L. japonicus* MG20 were also grown for 14 d in continuous white light to ensure that the photosynthetic apparatus was sufficiently well developed, and then subsequently transferred to either continuous high R/FR or low R/FR light conditions. The plants were either inoculated or left uninoculated at the time of transplantation (**Fig. 1A**; bottom two rows). The photosynthetic photon flux density (PPFD) was kept constant under both R/FR light conditions to maintain photosynthesis. Under these conditions, we found that the extent of AM colonization of both *L. japonicus* and *S. lycopersicum* grown in low R/FR light significantly decreased compared with plants grown under high R/FR light conditions (**Fig.1C, D**). However, the level of AM colonization did not significantly differ in *S. lycopersicum* cv. Micro-Tom grown under low vs. high R/FR light conditions if examined 5 weeks after infection of AM fungi (**Supplementary Fig. S1**). This result suggests that AM colonization is the most sensitive to the effects of the R/FR light ratio at the earlier (up to 4 weeks post-infection) stages of mycorrhizal symbiosis.

The fresh weights of the plant aerial parts were measured in uninoculated *S. lycopersicum* cv. Micro-Tom and *L. japonicus* MG20 grown under continuous high R/FR or low R/FR light. We found that the fresh weights of the host plants did not differ significantly in response to the level of R/FR light (**Supplementary Fig. S2A**, **B**). When *L. japonicus* MG20 was AM inoculated, no significant difference in fresh weight between high R/FR and low R/FR light-grown plants was observed (**Supplementary Fig. S2C**). In contrast, the fresh weight of the AM-inoculated *S. lycopersicum* cv. Micro-Tom grown under high R/FR light was significantly higher than that measured in the low R/FR light-grown plants (**Fig. 1E**). This result occurred in spite of the fact that for *S. lycopersicum* cv. Micro-Tom, no significant difference in photosynthetic rate was observed between low R/FR light-grown plants and high R/FR light-grown plants (**Supplementary Fig.S2D**, **E**). We therefore conclude that the enhanced growth, as measured by fresh weight increase, of the high R/FR light-grown tomato plants was not due to a difference in photosynthetic rate, but rather was due to increased AM colonization.

To confirm this observation, we measured the fresh weights of myc + vs. myc − *S. lycopersicum* cv. Micro-Tom plants grown under white light and saw a significant increase in response to inoculation (**Fig. 1F**), which was strongly correlated with the extent of AM colonization (**Fig. 1G**). Earlier, Beltrano et al. (2013) and Nadeem et al. (2014) reported that mycorrhization has a beneficial effect on *S. lycopersicum* cv. Micro-Tom growth, and we confirmed this response (**Supplementary Fig. S3**). Thus, the R/FR light ratio has a significant effect on AM colonization in plants, although *Lotus* MG20 plants did not exhibit a significant change in fresh weight under the experimental conditions employed (**Supplementary Fig. S2**). We next investigated whether light quality could directly influence the amount of AM colonization by examining the R/FR light responses of an *L. japonicus phytochrome B* (*phyB*) mutant. PhyB is the major red light photoreceptor that senses the R/FR ratio and also controls the SAS responses (Franklin 2008). In *L. japonicus phyB* mutants, mycorrhizal colonization was clearly suppressed in comparison with wild-type *L. japonicus* MG20 plants grown under continuous white light (**Fig. 2A**). Under these conditions, the fresh weight of AM-inoculated MG20 was slightly (but not significantly) larger than that of *phyB* mutant plants (**Fig. 2B**). In addition, the level of mycorrhizal colonization of the *phyB* mutants was very low under both R/FR light conditions, albeit not very significantly different from each other, although a trend towards greater colonization in high R/FR light was observed ($P = 0.16$, **Supplementary Fig. S4**). To examine this difference further, we measured the expression of several AM symbiosis marker genes in both high and low R/FR light-grown plants by real-time reverse transcription–PCR (RT–PCR). The gene encoding a subtilisin-like serine protease (*SbtM1*) is strongly and specifically induced during the AM symbiosis in *L. japonicus* (Takeda et al. 2009, Takeda et al. 2011). We found that *LjSbtM1* expression was significantly increased in roots of AM-inoculated, high R/FR light-grown plants compared with AM-inoculated, low R/FR light-grown plant roots (**Fig. 2C**). *LjSbtM1* expression in AM-inoculated *L. japonicus* MG20 plants was also higher than in AM-inoculated *phyB* mutants grown under continuous white light (**Fig. 2D**). In addition, we found that the expression of *LjPT4* was increased in AM-inoculated, high R/FR light-grown *L. japonicus* MG20 compared with AM-inoculated, low R/FR light-grown plants, similar to *LjSbtM1* expression levels (cf. **Fig.**

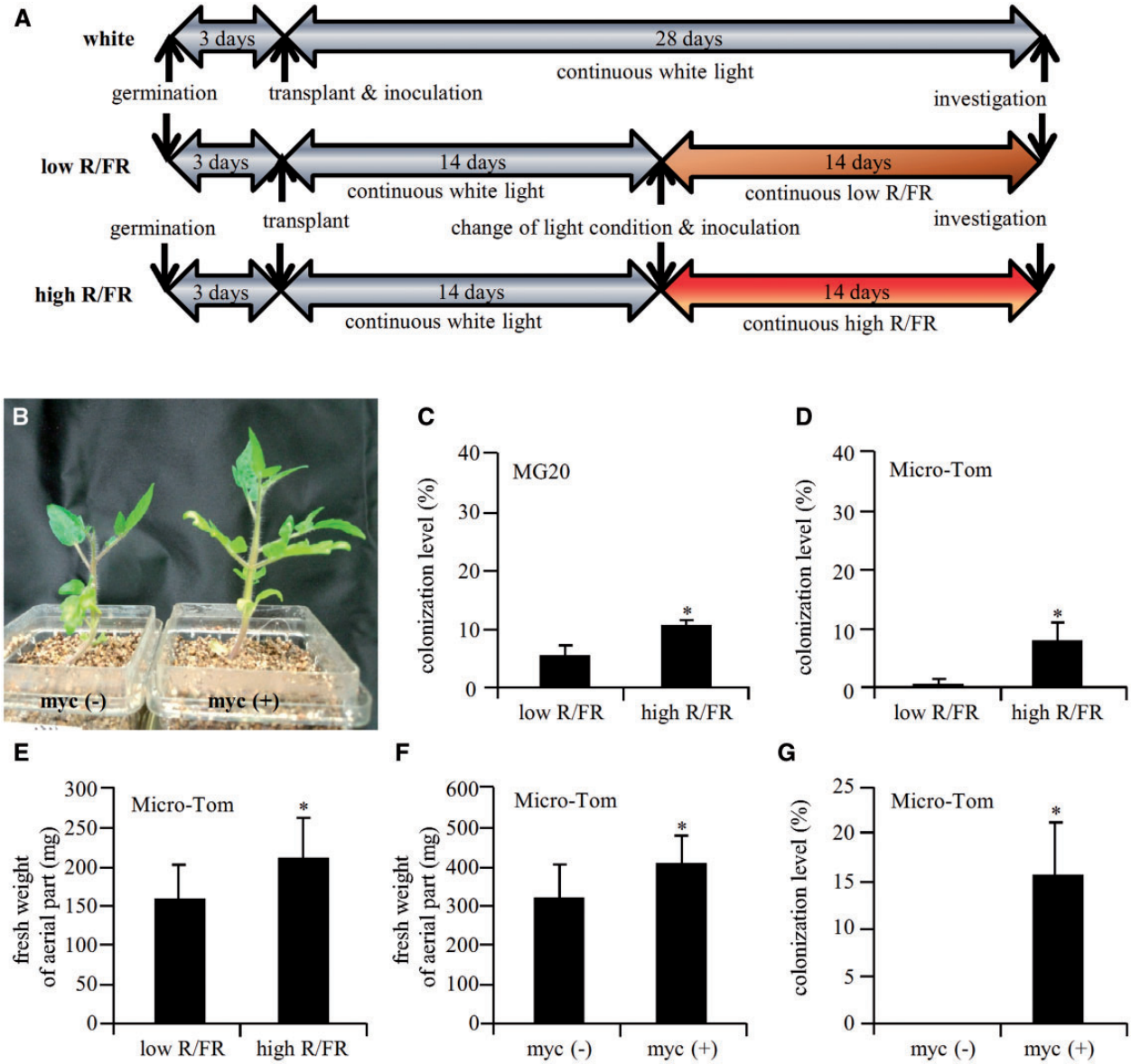

Fig. 1 Cultivation and lighting regime of plants and their responses when grown under different light conditions. (A) Schematic representation of the experimental procedure under different light and inoculation conditions. (B) *S. lycopersicum* cv. Micro-Tom inoculated with *R. irregularis* at 28 days after inoculation (DAI; myc +) is significantly taller than the uninoculated control (myc −). (C) *L. japonicus* MG20 ($n = 16$) and (D) *S. lycopersicum* cv. Micro-Tom ($n = 18$) were inoculated with AM fungi 14 DAI after being in continuous white light and harvested after 14 more days of growth in either type of R/FR light condition. (E) The fresh weights of the aerial parts of Micro-Tom plants were measured 28 DAI after growth in continuous white light ($n = 15$). (F) The fresh weights of the aerial parts of Micro-Tom plants inoculated with *R. irregularis* after 14 d of growth in white light and grown for an additional 14 d in either low R/FR or high R/FR light ($n = 16$). (G) The level of AM colonization in uninoculated (myc −) vs. inoculated (myc +) Micro-Tom ($n = 15$). The data represent averages ± SE of three independent experiments. Error bars represent the SE, and a statistically significant difference is indicated by asterisks ($P < 0.05$ by Student's *t*-test). *n* was the number of tested plants.

2C, E) as reported earlier (Guether et al. 2009). *LjPT4* expression levels in AM-inoculated *L. japonicus* MG20 were also higher than those in AM-inoculated *phyB* mutants grown under continuous white light (Fig. 2F). Another gene reported as being up-regulated upon AM fungal colonization is *S. lycopersicum SlPT4*, which encodes phosphate transporter 4 (Xu et al. 2007). We found that *SlPT4* expression was dramatically increased in AM-inoculated, high R/FR light-grown *S. lycopersicum*, but was negligible in AM-inoculated, low R/FR light-grown plants (Fig. 2G).

Expression of JA-related genes under low R/FR light conditions

JA and its derivatives play a major role in the AM symbiosis. For example, JA accumulates to high levels in AM-colonized roots compared with non-AM colonized roots (Hause et al. 2002).

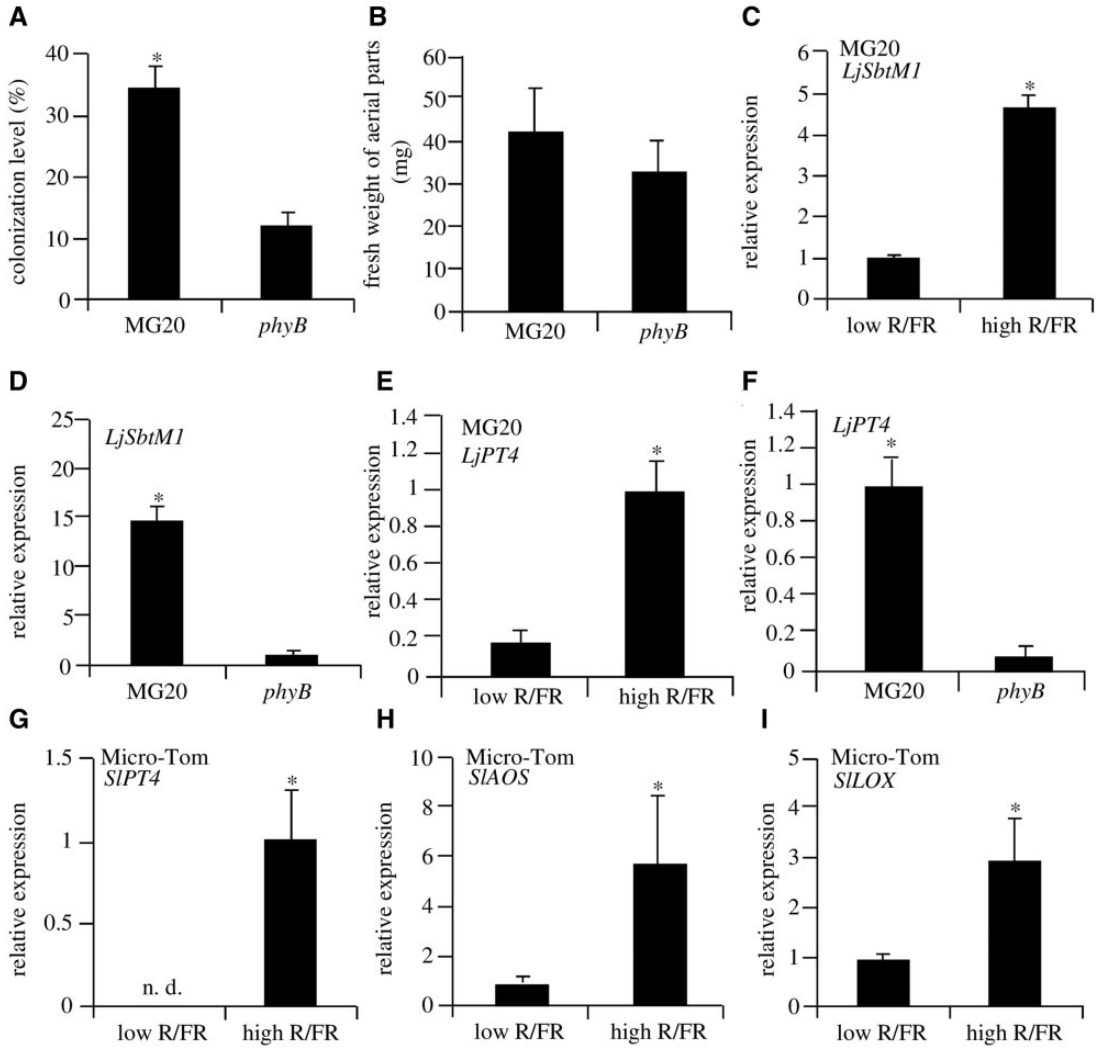

Fig. 2 AM colonization levels in wild-type and *phyB* mutant *L. japonicus* and expression of JA-related genes in plants grown in continuous white light or either continuous low or high R/FR light. (A) *L. japonicus* MG20 and *phyB* mutants were inoculated with AM fungi just before transfer to continuous white light for 28 d ($n = 10$). The levels of AM colonization were counted using the grid intersect method, and the data represent averages ± SE of independent experiments. Error bars represent the SE, and a statistically significant difference is indicated by asterisks ($P < 0.05$ by Student's *t*-test). (B) Fresh weight of aerial parts of AM-inoculated *L. japonicus* MG20 and *phyB* mutants were measured under continuous white light ($n = 12$). (C) Relative expression of the AM fungal-induced gene *LjStbM1* in AM-inoculated *L. japonicus* MG20 plants grown in either low or high R/FR light ($n = 6$). Transcript amounts were normalized against *ATP synthase* (internal control) transcripts. The mean value of expression in MG20 grown in low R/FR light was set as one. (D) Relative transcript amounts of *LjStbM1* in AM-inoculated *L. japonicus* MG20 vs. *L. japonicus phyB* mutant plants grown under continuous white light ($n = 8$). Transcript amounts were normalized against *ATP synthase* transcripts, and the mean value in *phyB* mutants was set to one. (E) Relative expression of the AM fungal-induced gene *LjPT4* in AM-inoculated *L. japonicus* MG20 plants grown in either low or high R/FR light ($n = 18$). (F) Relative transcript amounts of *LjPT4* in AM-inoculated *L. japonicus* MG20 vs. AM-inoculated *L. japonicus phyB* mutant plants grown under continuous white light ($n = 18$). Transcript amounts were normalized against *ATP synthase* transcripts. (G) Relative expression of the AM fungal-induced gene *SlPT4* in AM-inoculated *S. lycopersicum* cv. Micro-Tom grown in either low or high R/FR light ($n = 6$). (H) Comparison of transcript amounts of the JA-related gene *SlAOS* from plants grown in either low or high R/FR light for 14 d ($n = 6$). (I) Relative transcript amounts of the JA-related gene *SlLOX* in both low and high R/FR light-grown AM-inoculated *S. lycopersicum* cv. Micro-Tom ($n = 6$). Transcript amounts were normalized against *SlEF-1α* (internal control) transcripts. The mean value of expression in Micro-Tom in low R/FR light (G–I) was set as one. The data represent averages ± SE of three independent experiments. *n* was the number of tested plants. Error bars represent the SE, and a statistically significant difference is indicated by asterisks ($P < 0.05$ by Student's *t*-test). n. d., not detected.

Suppression of JA biosynthetic gene expression in *Medicago truncatula* roots reduced JA levels and concomitantly negatively affected mycorrhizal colonization (Isayenkov et al. 2005). In addition, mutants defective in JA biosynthesis were reported to show reduced mycorrhizal intensity and colonization although JA deficiency did not affect arbuscule formation (Tejeda-Sartorius et al. 2008). Similarly, we found that the expression of JA-responsive genes decreased in both low R/FR

light-grown *L. japonicus* MG20 plants and white light-grown *phyB* mutants, and was correlated with decreased jasmonoyl-isoleucine (JA-Ile) content in the *phyB* mutants (Suzuki et al. 2011). Adding JA back to the *phyB* mutants increased the number of root nodules (Suzuki et al. 2011). Based on these collective data, we predicted that the expression levels of JA-responsive genes would be higher in mycorrhizal-colonized plants grown under high R/FR light conditions. To assess whether the R/FR ratio controls the AM symbiosis through JA signaling by the same regulatory mechanisms in a non-legume as in a legume, we quantified the expression pattern of JA-responsive genes by real-time RT–PCR. In AM-inoculated, *S. lycopersicum*, the expression of *SlAOS* (allene oxide synthase) and *SlLOX* (lipoxygenase), which are involved in JA biosynthesis, increased in the roots of high R/FR light-grown plants compared with those of low R/FR light-grown plants (**Fig. 2H, I**).

The rescue by JA treatment of AM colonization in plants grown under low R/FR light conditions

If reduced AM colonization in low R/FR light-grown plants is due to an inhibition of JA production, we hypothesized that mycorrhizal colonization would be rescued by adding JA back. To test this hypothesis, *L. japonicus* and *S. lycopersicum* were each inoculated with AM fungi, with or without 0.5 μM JA added, and grown in low R/FR light. For these experiments, the infection method was changed to include a longer time interval between germination and inoculation, which increases the level of AM colonization (see the Materials and Methods and the legend of **Fig. 3**). In JA-supplemented plants grown under low R/FR light conditions, the level of AM colonization increased over that of the untreated control for both *L. japonicus* and *S. lycopersicum* (**Fig. 3A, B**). In general, the colonization levels of *S. lycopersicum* were always lower than those for *L. japonicus*, but the overall trends in plant response were the same. The level of AM colonization in low R/FR light-grown *L. japonicus phyB* mutant plants was also rescued by 0.5 μM JA treatment (**Fig. 3C**). On the other hand, AM colonization in *S. lycopersicum* and *L. japonicus* MG20 plants grown under high R/FR light conditions was not influenced by JA treatment (**Supplementary Fig. S5A**, **B**).

We also investigated whether JA-related gene expression in AM-inoculated plants was influenced by JA treatment under low R/FR light conditions. We found that the expression of JA-related genes was significantly increased by JA treatment in AM-inoculated *S. lycopersicum* (**Fig. 3D**; **Supplementary Fig. S5C**). The expression of marker genes for the AM symbiosis, namely *LjSbtM1* and *SlPT4*, in AM-inoculated, low R/FR light-grown plants was also significantly elevated by JA treatment

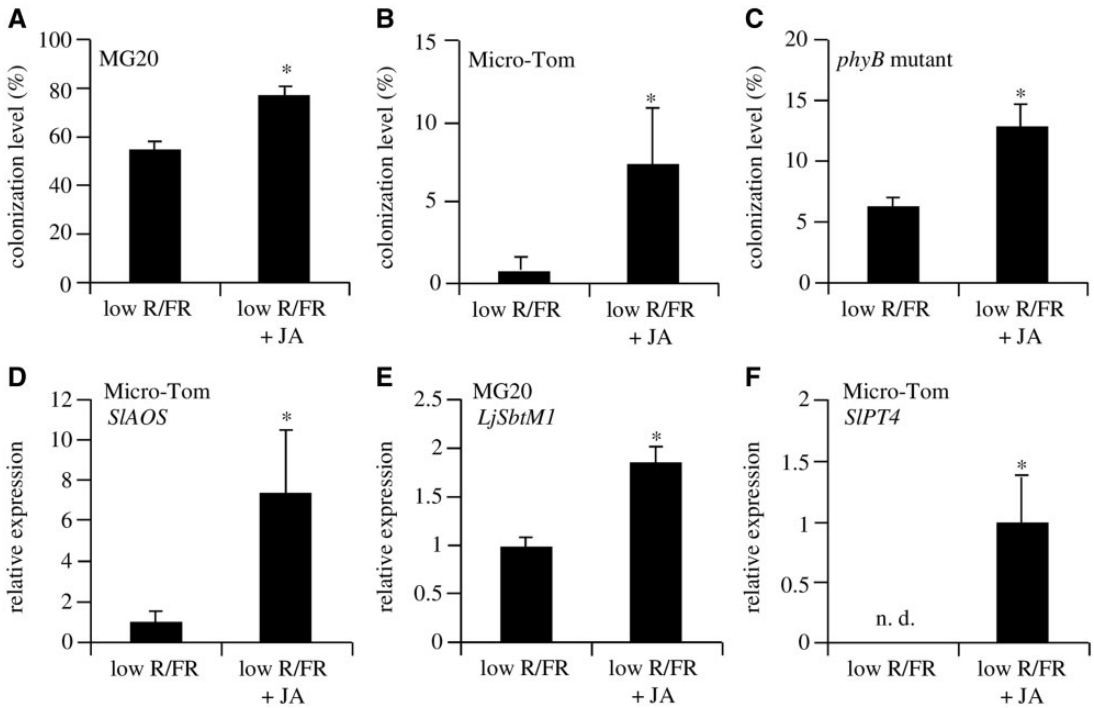

Fig. 3 Effect of JA on AM colonization and JA-related gene expression in plants grown in continuous low R/FR light. (A) *L. japonicus* MG20 ($n = 12$), (B) *S. lycopersicum* cv. Micro-Tom ($n = 12$) and (C) *L. japonicus phyB* mutants were inoculated with AM fungi, treated or not with 0.5 μM JA and grown in continuous low R/FR light ($n = 12$) (see the Materials and methods with regard to changes in interval between germination and transplantation for JA experiments). The levels of AM colonization was counted by the grid intersect method. Comparison of transcript amounts of (D) the JA-related gene *SlAOS* ($n = 3$), (E) the AM fungal-induced gene *LjStbM1* ($n = 15$) and (F) the AM fungal-induced gene *SlPT4* in low R/FR light conditions with or without 0.5 μM JA addition ($n = 3$). Transcript amounts were normalized against *SlEF-1α* (D, F) and *LjATP synthase* (E) transcripts. The mean value of expression in plants grown in low R/FR light (D, E) or in low R/FR light with 0.5 μM JA addition (F) were set as one. The data represent averages ± SE of three independent experiments. *n* was the number of tested plants. Error bars represent the SE, and a statistically significant difference is indicated by asterisks ($P < 0.05$ by Student's *t*-test).

(**Fig. 3E, F**). Collectively, these results demonstrate that JA levels are low in low R/FR light-grown plants and that exogenous JA increases both colonization levels and AM marker gene expression. The increase of AM marker gene expression by adding JA to low R/FR light-grown plants strongly suggests that this is the reason for the rescue of the AM fungal colonization level.

In establishing the mycorrhizal symbiosis, SLs, terpenoid lactones secreted from roots, are also very important signal molecules because they influence AM fungal hyphal branching at very low concentrations (Akiyama and Hayashi 2006, Steinkellner et al. 2007, Akiyama et al. 2010). Accordingly, SL production was predicted to increase under high R/FR light conditions compared with low R/FR light conditions. One particular SL, (+)-5-deoxystrigol, which is present in root exudates of *L. japonicus*, has been identified as an inducer of hyphal branching. To investigate whether (+)-5-deoxystrigol content in *L. japonicus* is influenced by R/FR light conditions, we analyzed the expression levels of two SL biosynthesis genes that encode carotenoid cleavage dioxygenases, *LjCCD7* and *LjCCD8* (Vogel et al. 2010, Kohlen et al. 2012), in uninoculated roots of *L. japonicus* grown under both R/FR light conditions. We found that the expression of the *CCD* genes was higher in high R/FR light-grown plants compared with low R/FR light-grown plants (**Fig. 4A**). In addition, we estimated the relative amount of (+)-5-deoxystrigol exuded from both types of uninoculated R/FR light-grown plant roots. In both MG20 and *phyB* mutant *L. japonicus*, the amount of SL in root exudates derived from uninoculated plants grown in high R/FR light was significantly higher than the levels detected in the root exudates obtained from uninoculated, low R/FR light-grown plants. Moreover, the amount of SL exudated from WT MG20 roots was significantly higher than that from *phyB* mutant roots under the same light conditions (**Fig. 4B**). These results demonstrate that the R/FR ratio controls SL production in roots and has an influence on SL secretion into the rhizosphere.

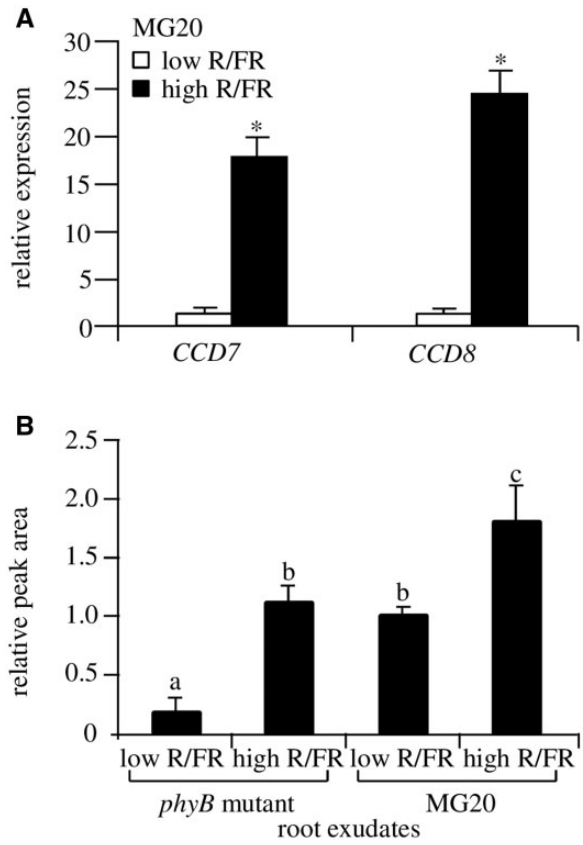

Fig. 4 Effect of the R/FR ratio on strigolactone biosynthesis in plants grown in low and high R/FR light. (A) Estimate of the expression of two strigolactone biosynthesis-related genes, *LjCCD7* and *LjCCD8*, in both low and high R/FR light-grown plants without AM inoculation ($n = 9$). Transcript amounts were normalized against *ATP synthase* transcripts. The mean value of expression in MG20 grown in low R/FR light was set as one. The data represent averages ± SE of three independent experiments. Error bars represent the SE, and a statistically significant difference is indicated by asterisks ($P < 0.05$ by Student's *t*-test). (B) Measurement of the amount of strigolactones by the CE/TOF MS system in root exudates from either low or high R/FR light-grown plants without AM inoculation ($n = 9$). The amount of strigolactones in root exudates from low R/FR light-grown MG20 was set as one. The data represent averages ± SE of three independent experiments. *n* was the number of tested plants. Different letters indicate significant differences among treatments ($P < 0.05$ by Tukey's multiple comparison test).

Discussion

In this report, we investigated the effect of light quality on AM colonization in both a legume and a non-legume. We predicted and found that the AM colonization of both *L. japonicus* and *S. lycopersicum* grown in low R/FR light significantly decreased compared with plants grown under high R/FR light conditions. Likewise, in an interaction between *Festuca rubra* L. and the AM fungus *Claroideoglomus etunicatum*, mycorrhizal colonization was significantly higher when plants were grown under high R/FR light compared with plants grown under low R/FR light, although it is unclear whether the PPFD was constant in both R/FR light conditions (see Skálová and Vosátka 1998). On the other hand, in the interaction between *Pinus sylvestris* and the ectomycorrhizal fungus *Cenococcum geophilum*, neither the mycorrhizal morphotypes nor the amount of fungal biomass in roots was affected by light quality (de la Rosa et al. 1999). However, de la Rosa et al. (1999) suggested that FR irradiation might influence mycorrhizal development because the percentage of dichotomous fungal ramifications and P and N nutrition in low R/FR light-grown seedlings slightly increased compared with that in control plants. The reasons for the differences between the fungal systems are unclear, but they might also be explained by the different lifestyles of ectomycorrhizal (external) vs. AM (internalized) fungi.

In the current study, we focused on AM colonization and the involvement of the plant hormones JA and SL in both roots and the rhizosphere under low and high R/FR light conditions. We conclude that the R/FR ratio influences AM colonization in a legume and a non-legume during the early stages of the mycorrhizal symbiosis based on the fact that 5 weeks after infection, AM colonization levels no longer

significantly differed in *S. lycopersicum* cv. Micro-Tom grown under low vs. high R/FR light conditions (**Fig. 1C, G**; **Supplementary Fig. S1**). In contrast, the colonization level of the *L. japonicus phyB* mutant was significantly decreased under the same light conditions 4 weeks after infection. Thus, AM colonization and the expression of JA biosynthesis genes increased in roots of high R/FR light-grown AM-inoculated plants compared with those of low R/FR light-grown AM-inoculated plants (**Fig. 2**). Moreover, the application of exogenous JA to AM-inoculated host plants grown under low R/FR light enhanced AM colonization and JA-responsive gene expression (**Fig. 3**). The data indicate that the host plants employ SAS to survive under low R/FR conditions, attenuating an AM–plant association if not enough photosynthetic products are available as an energy source to maintain the symbiosis. Interestingly, SL production and SL-biosynthetic gene expression levels increased in the roots of uninoculated host plants grown under high R/FR light conditions (**Fig. 4**). SLs are known to induce AM fungal hyphal branching (Akiyama and Hayashi 2006, Akiyama et al. 2010). The fact that the R/FR ratio changes SL production in uninoculated host plants and also controls the amounts of SL secreted into the rhizosphere strongly suggests that light quality facilitates the establishment of the AM symbiosis.

Taken together, a model representing the proposed mechanisms whereby JA and SL contribute to shade perception and AM colonization is depicted in **Fig. 5**. In high R/FR light, JA and SLs are produced and SLs are secreted from roots to the rhizosphere at higher levels compared with plants growing under low R/FR light conditions. Under low R/FR light conditions, the levels of both synthesized and secreted SL are reduced compared with those found in high R/FR light-grown plants.

Recently, SLs and light have been reported to interact in the non-mycorrhizal plant *Arabidopsis thaliana* (Tsuchiya et al. 2010). Cotylimides, a new group of small molecules, have been identified as increasing SL accumulation in *A. thaliana* and causing a bleached cotyledon phenotype. However, *A. thaliana hy1* mutants—the loss-of-function mutants of *ELONGATED HYPOCOTYL1* (*HY1*)—show reduced SL levels and also resistance to cotylimides. *HY1* encodes a heme oxygenase involved in the first biosynthetic step of the linear tetrapyrrole chromophore (3E)-phytochromobilin, strongly suggesting that R and FR light are important for SL synthesis (Tsuchiya et al. 2010). In *A. thaliana*, SL has a function similar to that of light in promoting germination and greening. In addition, recent studies show that light intensity is a positive regulator of SL levels and *CCD7* transcription, although it is not clear exactly how light intensity affects SL levels in *S. lycopersicum* cv.

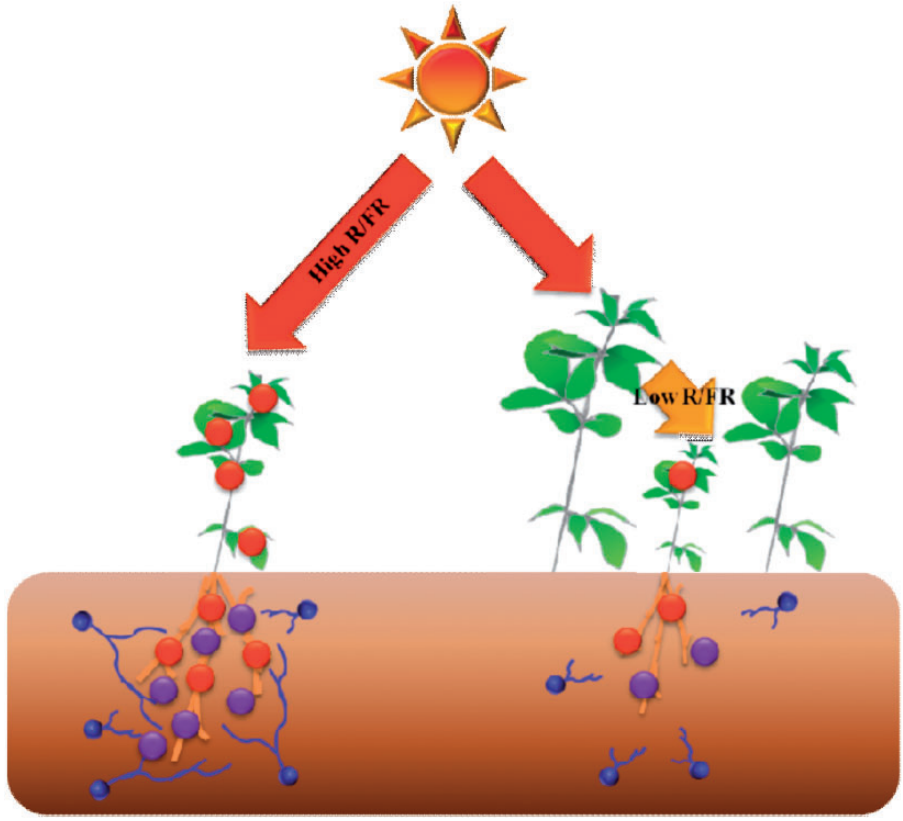

Fig. 5 Model representing the proposed mechanism of JA signaling for shade perception and AM colonization. Under low R/FR light conditions, AM colonization is suppressed because of reduced JA and SL. In high R/FR light conditions, AM colonization is increased due to greater amounts of JA and SL. JA and SLs are produced in host plants. Then, SL is secreted from roots to the rhizosphere and works outside host plants. In addition, high R/FR light suppresses SAS. The conclusion is that the R/FR ratio controls AM colonization through JA signaling in higher plants. Red circles show JA, purple circles show SL and blue circles show AM fungi.

M82 (Koltai et al. 2011). Furthermore, SL accumulation in plant roots is a time-dependent process and is correlated with strong light intensity (763.9 ± 2.8 μmol $m^{-2} s^{-1}$) (Koltai et al. 2011). Together, these reports support the idea that an interaction occurs between light and SLs in plants.

In addition, supporting the idea that light is involved in SL synthesis is the finding that reduced amounts of SL were detected in *phyB* mutant root exudates compared with those derived from *L. japonicus* MG20 (**Fig. 4B**). It is also possible that other phytochromes in addition to PHYB may be involved in SL synthesis. This is suggested by the difference in SL amounts in the *phyB* mutant when grown under either low or high R/FR light (**Fig. 4B**). Also AM colonization levels tend to be lower, but are still detectable, in *phyB* mutants grown under low R/FR light compared with high R/FR light (**Fig. 3C**).

Taken together, these data tell us that by monitoring the R/FR ratio, mycorrhizal host plants maintain an optimal symbiotic interaction with AM fungi, not only by changing the concentrations of synthesized JA and SLs, but also by controlling the amounts secreted from roots into the rhizosphere. Gutjahr and colleagues reported that JA is not required for AM colonization of rice and that high levels of JA in the roots suppress AM development through the induction of defense responses (Gutjahr et al. 2015). In support, we found that AM colonization in *S. lycopersicum* and *L. japonicus* grown under high R/FR light conditions was not influenced by the application of JA (**Supplementary Fig. S5A**, **B**). However, under low R/FR light conditions, the level of AM colonization was rescued by 0.5 μM JA treatment for both *S. lycopersicum* and *L. japonicus* (**Fig. 3A, B**). Based on these results, we propose that JA as well as SLs is one of several important factors for the AM symbiosis in low R/FR light-grown plants.

Although the AM symbiosis changes both plant hormonal and transcriptional profiles in tomato roots, especially for the oxylipin pathway (López-Ráez et al. 2010), host plants have to regulate SL levels once the AM symbiosis has been established, and in 8-week-old AM-colonized plants, SL production is significantly reduced (López-Ráez et al. 2011). We focused on the effect of light quality and the plant hormones JA and SL at an early stage of the development of the AM symbiosis in this study. Whether light, JA and SLs exhibit additional interactions in the later stages of the AM symbiosis will require further investigation.

In conclusion, monitoring light quality is essential for establishing and maintaining a successful mycorrhizal symbiosis. Moreover, the interactions described herein are ancient. All of the players—AM fungi, phytochrome, SL and JA—date from the early Devonian (see references in Foo and Reid 2013), making it highly probable that the signaling pathways described evolved long before the appearance of higher land plants. We have shown that by studying present-day plant–microbe interactions, we can gain insights towards understanding the evolution of these fundamental signaling pathways.

Materials and Methods

Plant material, growth conditions and mycorrhizal inoculation

Lotus japonicus MG20, its *phyB* mutant, *S. lycopersicum* cv. Micro-Tom, the host plants, and the AM fungus, *R. irregularis* (*Glomus intraradices*, Premier Tech Biotechnologies), were the subjects of study. *Lotus japonicus phyB* mutants 01-0017 were derived from *L. japonicus* MG20 by mutagenesis with ethylmethane sulfonate (Suzuki et al. 2011). Seeds of *L. japonicus* MG20 and the *phyB* mutant 01-0017 were scarified with concentrated sulfuric acid for 10 min and then rinsed with sterilized distilled water. Seeds were then surface-sterilized with a 0.2% sodium hypochlorite solution containing Tween-20 and, after thorough rinsing with sterilized distilled water, were germinated on 0.8% agar plates at 24°C in the dark. Seeds of *S. lycopersicum* cv. Micro-Tom were surface-sterilized by soaking them in 70% ethanol and subsequently in a 0.5% sodium hypochlorite solution. The tomato seeds were then copiously rinsed with sterilized distilled water and germinated on 0.8% agar plates at 24°C in the dark. Surface-sterilized seeds of *L. japonicus* MG20, the *phyB* mutant and *S. lycopersicum* cv. Micro-Tom (Ariizumi et al. 2011) were germinated on 0.8% agar plates at 24°C in the dark. Three days after germination, the seedlings were transplanted into pots (6 cm width × 6 cm length × 10 cm depth) containing a soil mixture [vermiculite : river sand : sod soil (Green Sangyo) = 1 : 1 : 1, v/v/v] supplemented with 1/2 strength Hoagland's liquid medium (Phyto Technology Laboratories) containing 100 μM KH_2PO_4. To assess AM colonization under continuous white light (light intensity = 100 μmol $m^{-2} s^{-1}$), the plants were inoculated with *R. irregularis* (3,000 spores per pot) and grown at 24°C for 28 d. To measure AM colonization under low or high R/FR light conditions, the plants to be transplanted were cultivated as described above at 24°C for 14 d, and then inoculated with *R. irregularis* (**Fig. 1A**). The inoculated plants were transferred to continuous low or high R/FR light for an additional 14 d at the same temperature. For the experiments on measuring the level of AM colonization in response to added JA in *L. japonicus*, the seedlings were grown in 1/2 strength Hoagland's agar medium for an additional 7 d under white light, after which the plants were transplanted into pots and inoculated with AM fungi. They were grown for 28 d under low or high R/FR light conditions. Light-emitting diodes (LEDs; Sanyo) were used for R and FR light irradiation. In the low R/FR light conditions, the R intensity was 10 μmol $m^{-2} s^{-1}$ and the FR intensity was 100 μmol $m^{-2} s^{-1}$ (R/FR ratio = 0.1). In the high R/FR conditions, the R intensity was 10 μmol $m^{-2} s^{-1}$ and the FR intensity was 0 μmol $m^{-2} s^{-1}$ (R/FR ratio = ∞). We confirmed that the LED intensity was sufficient for plant growth in chambers.

Expression analysis by quantitative real-time PCR

To quantify the relative amount of transcripts derived from JA-related genes, real-time RT–PCR was performed using the one-step SYBR Primescript RT-PCR Kit (TAKARA). For the quantitative RT–PCR experiments, total RNA was isolated from roots, which had been stored at −80°C, using the RNeasy Plant Mini Kit (Qiagen). A DNase I treatment was performed with DNase RT-Grade (Wako). RNA was precipitated by ethanol and resuspended in RNase-free water. The enzymatic reactions were performed with a LightCycler 1.5 system (Roche). The nucleotide sequences of the primers used are indicated in **Supplementary Table S1**.

Evaluation of AM colonization in higher plants

Rhizophagus irregularis-inoculated root samples were placed in a 2% KOH solution warmed to 90°C for 20 min, and then transferred to a 3% acetic acid solution for 10 min. The roots were then stained with 0.05% trypan blue/lactic acid at 90°C for 10 min. Trypan blue-stained mycorrhizal colonization intensity was observed under bright-field microscopy according to the grid intersect method (Giovanetti and Mosse 1980). Images were captured by a Leica DM2500 microscope equipped with a Leica DFC490 digital camera (Leica Microsystems). Two hundred random fields per plant were used to assess the level of mycorrhizal colonization. The percentage of hyphae, arbuscules and vesicles in 200 random fields per plant were determined.

Quantification of strigolactones in root exudates from the two different R/FR light-grown plants

Fifty plant seedlings were grown and the medium upon which they grew was collected as described above. Root exudate samples were extracted with chloroform and then subjected to ultrafiltration. Quantification of SLs was conducted by capillary electrophoresis time-of-flight mass spectrometry (CE/TOF MS).

All CE-TOF MS experiments were performed using an Agilent 7100 CE capillary electrophoresis system (Agilent Technologies), an Agilent 6224 LC/MS TOF system (Agilent Technologies) and an Agilent1260 series ISO pump as in Ooga et al. (2011) and Kami et al. (2012) with modifications. For system control and data acquisition, we used the Agilent MassHunter workstation version B.06.00 for Agilent CE and Agilent TOF MS software.

Separations were carried out in a fused silica capillary (50 μm internal diameter × 80 cm total length) pre-conditioned with a commercial buffer [H3301-1001, Human Metabolome Technologies Inc. (HMT)] as electrolyte, and a commercial sheath liquid (H3301-1020, HMT) was delivered at a rate of 10 μl min^{-1}. The sample solution was injected at a pressure of 50 mbar for 3 s. The applied voltage was set at 27 kV. Electrospray ionization-mass spectrometry (ESI-MS) was conducted in the positive-ion mode, and the capillary and fragment voltages were set at 4,000 and 110 V, respectively. Nebulizer pressure was configured at 5 psig, and N_2 was delivered as a drying gas at a rate of 7 l min^{-1} at 300 °C. Exact mass data were acquired at the rate of 1.5 cycles/s over a 50–1,000 *m*/z range. The data obtained by CE-TOF MS analysis were processed using Agilent MassHunter software. Each metabolite was identified based on the peak information including *m*/z, migration time and peak area.

Supplementary data

Supplementary data are available at PCP online.

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Disclosures

The authors have no conflicts of interest to declare.

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