Chapter 44

Lotus japonicus Nodulates When It Sees Red

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44.1 INTRODUCTION

In nature, many legumes develop nodules on their roots in which rhizobia fix atmospheric nitrogen into ammonia by means of the enzyme nitrogenase. In return, the rhizobia obtain photoassimilates from the host that had been fixed within plant leaves, demonstrating that light quantity is critical for the effectiveness of this symbiosis. However, light quality also has a tremendous influence on the success of the legume–rhizobial symbiosis. In this chapter, we review the literature on the effects of light quality on the establishment of the legume–rhizobial symbiosis and describe our own work to update our current knowledge concerning nodule development and light.

44.2 RESULTS AND DISCUSSION

Numerous reports exist in the literature about sufficient light quantity being critical for effective root nodule formation (Fred and Wilson, 1934; Fred et al., 1938). In addition, adding glucose and sucrose to the nutrient medium when light was limiting enhanced root nodule formation in some legumes (Van Schreven, 1959). These observations support the idea that the accumulation of photoassimilates provides energy and compounds important not only for root nodule formation but also for nodule function to maintain a successful mutualism between the two symbiotic partners.

If we consider the fact that chlorophyll absorbs both red (R; 600–700 nm) and blue light (400–500 nm), we can conclude that the difference in light quality (wavelength) also affects the photosynthetic activity leading to the establishment of the symbiosis. The fact that the effects of light quality on symbiosis are independent of photosynthetic activity has been reported by a number of investigators. Lie (1964) studied the effects of short-time irradiation of R or far-red (FR) light at the end of the photoperiod of the light cycle of the day (end-of-day; EOD) on pea and kidney bean root nodule formation, and reported that root nodule formation following irradiation with FR light was suppressed compared to R light treatment (Lie, 1964). This phenomenon—that root nodule formation is suppressed by irradiating FR light at the EOD—was reported not only for pea (Lie, 1969) but also for broad beans (Lie, 1971), soybean (Kasperbauer et al., 1984; Hunt et al., 1987), and southern pea (cowpea) (Kasperbauer and Hunt, 1994). In contrast, Balatti and Montaldi (1986) showed that the number and biomass of soybean root nodules
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20 days after inoculation with *Bradyrhizobium japonicum* were significantly increased following FR irradiation in EOD compared to R irradiation. The reasons for these contradictory results are unclear, but may depend on light quantity or the exact timing of the irradiation. Nevertheless, root nodule formation was restored by the irradiation of R after FR treatment in EOD (FR + R) and repressed once again following a light treatment of FR + R + FR in EOD. Because these results illustrate the classic photoreversibility of FR with R, the involvement of phytochrome in root nodule formation was strongly indicated (Lie, 1964; Hunt et al., 1987; Kasperbauer and Hunt, 1994).

That phytochrome participates in the perception of the R/FR ratio and the shade avoidance syndrome (SAS) is well known. Plants have photoreceptors that sense the presence of their neighbors by monitoring the ratio of R, mainly absorbed by chlorophyll, to FR light, which is not. A low R/FR ratio indicates the presence of neighbors that may be competitors for photosynthetically active radiation (PAR), thereby initiating the SAS, such that plant crowding causes plants to grow taller or to bend to the light to avoid being shaded (Smith and Whitelam, 1997; Neff et al., 2000; Franklin, 2008; Franklin and Quail, 2010). However, these low R/FR light conditions are suboptimal for root nodule formation. Kasperbauer and Hunt (1994) investigated the effect of the difference of R/FR ratio on southern pea root nodule formation by covering the surface of the pots with either gray-white or brick-red soil. Because the spectral distribution of light reflected from different soils is not the same, the R/FR ratio radiated to each pot through its covering differed. The final results showed that the number of root nodules 18 days after inoculation was higher when plants were grown under high R/FR (gray-white soil) than under low R/FR (brick-red) conditions (Kasperbauer and Hunt, 1994). In another experiment, this time in the field where multiple plants were planted together, the R/FR light ratios that these plants perceived depended not only on the degree of shading but also on row orientation because chlorophyll absorbs R light more efficiently than FR light. Root nodule formation for soybean and southern peas planted in either a north–south (low R/FR) or east–west (high R/FR) orientation was investigated. For both species, plants positioned in an east–west row orientation produced more nodules than those orientated north–south. In other words, plants grown under high R/FR ratios produced more nodules (Hunt et al., 1990; Kasperbauer and Hunt, 1994). However, the photosynthetic photon flux density (PPFD) for plants exposed to high R/FR conditions was higher than that for low R/FR exposure in both sets of experiments. Therefore, these investigators did not eliminate the possibility that root nodule number was affected by photosynthetic activity and the greater allocation of photoassimilates. Based on these results, we reasoned that root nodule formation was linked to the perception of the R/FR ratio and used *Lotus japonicus* (Suzuki et al., 2011; Shigeyama et al., 2012) to test this hypothesis.

To verify that root nodule formation is under the control of the perception of the R/FR ratio, we examined nodule formation in *L. japonicus* MG20 wild-type and *phyB* mutant plants grown under different R/FR light conditions. In our study, the PPFD of the R light-emitting diode (LED) remained constant to eliminate differences in the amount of photosynthate produced under low R/FR and high R/FR treatments. Signaling via phyB, the major red light photoreceptor used during seedling development, regulates responses to photoperiodism, end-of-day FR, and R/FR ratios in white light-grown plants (Neff et al., 2000; Franklin et al., 2003). From our experiments, we determined that the shoot and root fresh weights of *phyB* mutants were significantly reduced compared to those of MG20 plants grown under white light conditions. In addition, the number of root nodules of the *phyB* mutants was lowered compared to that of MG20 plants. To better understand the effect of the R/FR ratio on plant growth, we measured the fresh weights of the aerial plant parts, root length, and root nodule number under different R/FR light conditions. We found no statistically significant difference for either root length or shoot fresh weight for *L. japonicus phyB* mutants or MG20 plants, respectively, when the plants were grown under high R/FR and low R/FR light conditions. However, very few nodules developed on the *phyB* mutant roots under different R/FR light conditions. In addition, although nodules developed on MG20 plants grown in high R/FR light, very few nodules were detected on the roots of the low R/FR-grown plants.

Sucrose, a highly soluble disaccharide that provides energy sources for plant cells (Huber, 1989), is synthesized from the primary products of photosynthesis. To investigate whether a difference in photosynthetic activity was present while PPFD is constant, the sucrose content of the roots was measured after the plants were grown under different R/FR light conditions. We found that the sucrose content of roots in low R/FR-grown plants increased compared to the high R/FR-grown plants, suggesting FR light irradiation may be responsible for the increase in sucrose content. Nevertheless, the fact that root nodule number was reduced in the low R/FR-grown plants in spite of the higher sucrose content strongly suggests that phyB sensing of the R/FR ratio has a significant influence on nodulation. However, we cannot completely eliminate the possibility that photosynthetic products contribute to root nodule formation when the plants are grown under different R/FR light conditions.

Because the number of root nodules for both low R/FR-grown MG20 plants and white light-grown *phyB* mutants decreased, we hypothesized that phyB-mediated signaling in plants controlled by root nodule formation. Robson et al. (2010) suggested that phyA and JA signaling cooperate to regulate the balance between shade avoidance responses in FR-enriched light and defense responses
44.2 Results and Discussion

Shoot-applied methyl jasmonate (MeJA) strongly suppressed JA production and/or sensitivity would be enhanced in low R/FR-grown MG20 plants and white light-grown phyB mutants. We analyzed the expression of JA-responsive genes (PDF1.2, JAR1, and MYC2) in L. japonicus grown under different R/FR light conditions. The expression of PDF1.2, JAR1, and MYC2 genes decreased in low R/FR-grown MG20 plants compared with plants grown under high R/FR light conditions. Moreover, their expression levels declined in phyB mutants under white light conditions in contrast to MG20 plants. These results strongly suggested that JA production and/or sensitivity decreased in low R/FR-grown MG20 plants and white light-grown phyB mutants, which was contrary to our expectations. To check whether JA concentration correlated with the expression of JA-responsive genes, we measured the endogenous concentrations of JA and JA-Ile in roots grown under white light condition. We found no significant difference in endogenous JA concentration between MG20 wild-type plants and phyB mutant plants. However, the endogenous concentration of JA-Ile significantly decreased in roots of phyB mutants. JA-Ile, the isoleucine conjugate of JA, is a biologically active hormonal signal (Staswick and Tiryaki, 2004) and is generated by a JAR1-encoding JA-aminoo acid synthetase. The JAR1 gene product is involved in pathogen defense, sensitivity to ozone, and wound responses (Guranowski et al., 2007; Staswick, 2008; Koo et al., 2009). Our results might be explained by the fact that the conversion of JA to JA-Ile was suppressed due to decreased JAR1 expression in the phyB mutants grown under white light conditions, which would give rise to a reduced concentration of JA-Ile in the phyB mutants. Thus, we concluded that root nodule formation was suppressed because the JA-Ile concentration was low in plants grown under low R/FR light conditions.

To examine the effect of JA directly on root nodule formation under different R/FR light conditions, JA was added to the plant growth medium. Both shoot and root growth of MG20 plants were decreased as the concentration of JA increased. Although the root nodule number per plant was reduced after adding a high concentration (10 μM) of JA, nodule number per plant for plants grown in low (0.1 μM) JA significantly increased when compared to control plants that received no exogenous JA. Furthermore, nodule number per root length increased for plants treated with 0.1, 1, and 10 μM JA over the untreated controls. Our results differed from those reported for M. truncatula (Sun et al., 2006), in that in our study, we found that L. japonicus nodule number was increased at low concentrations of JA.

If reduced nodule formation in low R/FR-grown MG20 plants and white light-grown phyB mutants is due to a low concentration of endogenous JA-Ile, we predicted that nodule formation would be enhanced by JA application. To test this possibility, the effect of JA treatment on root nodule development was analyzed. We found that the number of root nodules and nodule primordia in 0.1 μM JA-treated plants was slightly increased compared to untreated plants. We also examined the expression of JA-responsive genes and the NIN gene in 0.1 μM JA-treated or untreated plants. NIN is required for infection thread formation and nodule primordium initiation in L. japonicus (Schauser et al., 1999). The expression of NIN gene was significantly increased in 0.1 μM JA-treated plants. Although JA addition did not affect the total root length, the number of infection threads per root length was significantly increased in MG20 plants compared to the untreated plants. These results strongly suggest that low concentrations of JA function as a positive regulator for root nodule formation in L. japonicus.

In addition, we analyzed the effect ofJA treatment on root nodule formation in phyB mutants. Following 0.1 μM JA treatment of phyB mutants, shoot length was unaffected and root length was decreased compared to the untreated plants. However, the number of root nodules per plant significantly increased compared to the number in the untreated plants. Moreover, nodule number per root length significantly increased in response to 0.1 and 1 μM JA treatments. Based on the responses of JA treatments to shoot length, total root length and root nodule number, the sensitivity of MG20 plants and phyB mutants to JA is not likely to be significantly different. To summarize, the cause of reduced root nodule formation in low R/FR-grown MG20 plants and white light-grown phyB mutants is due to the inhibition of JA-Ile production.

The literature also reports about the effects of R/FR ratios on the interaction between plants and microbes. For example, low R/FR ratio reduced Arabidopsis resistance to Botrytis cinerea (Cerrudo et al., 2012), and fluorescent illumination with a high R/FR ratio improved the resistance of cucumber seedlings to powdery mildew (Sphaerotheca cucurbitae) (Shibuya et al., 2011). Moreover, in the case of the interaction between plants and pests, wild tobacco and Arabidopsis grown under high R/FR conditions were less susceptible to feeding damage by insect herbivory compared to low R/FR-grown plants (Izaguirre et al., 2006; Moreno...
et al., 2009). JA signaling is involved in these plant defense mechanisms. Plants grown under high R/FR light produced more JA and protected themselves from pathogens, whereas plants grown under low R/FR light were more susceptible to infection or feeding damage by pathogens and predators due to the reduced production of or decreased sensitivity to JA (Cerrudo et al., 2012; Moreno et al., 2009; Ballaré, 2011). In root nodule symbiosis, the rhizobial infection process is enhanced by this mechanism.

For plants, photosynthesis is the most important biochemical reaction for survival. Plants grow taller or bend to light, but at the same time they assume the risk that they are more likely to be invaded by pathogens. In low R/FR light, phyB is inactivated by photoconverting the active Pfr form into Pr, and as a result, phytochrome-interacting factor 4 (PIF4) and 5 (PIF5) accumulate, leading to the synthesis of auxin (IAA) and bioactive gibberellins (Ballaré, 2011), phytohormones required for plant growth. However, the synthesis of phytohormones is incompatible with the production of JA, making it very difficult for plants to maintain active growth and high-level defense responses at the same time. Legumes show the same energy constraints by limiting the initiation of energy-costly nodules under low R/FR conditions. However, by obtaining sufficient nitrogen through the establishment of a symbiosis with rhizobia for maintaining effective growth, legumes may ultimately be better able to balance growth and defense than nonlegumes.

A model representing the proposed mechanism of JA and phyB signaling for shade perception and root nodule formation is depicted in Figure 44.1.

In high R/FR light conditions, phyB suppresses SAS and enhances root nodule formation through an increased concentration of JA-Ile. In contrast, in low R/FR light conditions, SAS is restored by the inactivation of phyB and root nodule formation is suppressed due to the reduced concentration of JA-Ile. Our data show that root nodule formation involves the perception of the R/FR light ratio and requires signaling through phyB and JA. Although root nodule development is initiated in the soil under low light, the R/FR light conditions in soil are suboptimal for sustaining root nodule function. Thus, host legumes shaded by other plants initiate a shade avoidance response and modify their growth to obtain sufficient light for maximizing photosynthesis. Nevertheless, under such low R/FR light conditions, the host plants suppress root nodule development to conserve energy. We conclude that this SAS for root nodule formation is required for L. japonicus nodule development. In conclusion, sensing both light quality and quantity is essential for establishing and maintaining a successful nitrogen-fixation symbiosis. How common this interaction is among the symbiotic Fabaceae is not known at this time.

REFERENCES


References