Summary

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Title	Study on the control of root nodulation by light in leguminous plants

Key word（ **root nodule** ）　（ **blue light** ）　（ **light exposure** ）

Chapter 1: General introduction

Leguminous plants and rhizobia establish a symbiosis in which root nodules develop on the host root. Chemical compounds, flavonoids, secreted from the roots of host plant activate nodulation (*nod*) gene expression, whose compounds are involved in the synthesis and secretion of specific Nod factor triggering nodule morphogenesis. These specific Nod factor structures determine the strict specificity between rhizobia and host legume species, and elicit both the rhizobial infection process and the initiation of nodule primordia in the roots of the compatible host legumes. Rhizobia penetrate the root tissues from curled root hair cells and progress toward the root cortex through an intracellular channel called the infection thread. The rhizobia are released from infection threads into plant cells, enclosed within the nodules, rhizobia fix atmospheric nitrogen into ammonia, which eventually results in the synthesis of amino acids that are utilized by the host. In return, the plants provide photosynthetic products to the rhizobia as an energy source that drives the nitrogen fixation process. Light perceived by the aboveground parts of the plant is essential for the establishment of this legume-rhizobia symbiosis.

Because the plants do not have ability of movement, the plants rely various environmental cues for their survival. Light is one such cue. Because plants assimilate from photosynthesis to energy by photosynthesis, plants necessary to perceive exact light quality, intensity, and direction. These lights are perceived by photoreceptors. As the photoreceptors, phototropins (PHOTs) and cryptochromes (CRYs) are well-known blue light and UV-A photoreceptors, and phytochromes (PHYs) are red and far-red light receptors.

It was reported that in many legumes, roots that are exposed to light do not form nodules. However, the detailed mechanism of inhibition of nodulation was unclear. Because of their belowground location, detailed molecular studies on the effect of light on root development have not been as numerous as studies with aboveground organs. It is necessary to understand the effects of light irradiation to the root on the establishment of root nodule symbiosis.

Chapter 2: Light perception by *Lotus japonicus* roots inhibits root nodule formation.

Plants rely on environmental cues for survival. Light is one such cue, and plants perceive its quality, intensity, and direction. It was known that light perceived by the aboveground parts of the plant is essential for the establishment of legume-rhizobia symbiosis. In addition, light triggers the suppression of nodulation in many legume roots. In this chapter, it was investigated the effects of light on the roots of *Lotus japonicus* Miyakojima MG20 and on the rhizobial strain (*Mesorhizobium loti* MAFF303099) to clarify the mechanisms of light perception required for nodulation in this symbiosis.

First, the effect of root exposure to light on nodulation in *L. japonicus* was investigated. Plants were inoculated with *M. loti*, and the roots of some plants were shaded. Although root lengths were not significantly different between unshaded and shaded plants 21 days after inoculation (dai), the shoots from the unshaded plants were significantly shorter than those of the shaded plants. Also, unshaded roots had significantly fewer root nodules per plant than shaded roots.

Next, to determine which wavelength of light is critical for the inhibition of nodulation, roots were irradiated with blue, green or red light supplied from above for 21 days and analyzed nodulation thereafter. Under red or green light, no large differences in the number of root nodules between unshaded and shaded plants were observed. However, under blue light, nodule number was significantly reduced in the unshaded plants.

Finally, to investigate whether the inhibition of nodulation could be caused by a reduced population of rhizobia in response to light, rhizobia were exposed to blue, green or red light. Growth, measured by absorbance at 610 nm of *M. loti* under red or green light, was similar to that of the dark. On the other hand, blue light had a strong inhibitory effect on *M. loti* growth compared to the dark control. These results indicated that rhizobial growth is inhibited by blue light and that the lack of rhizobial proliferation may be an explanation for the reduction in nodule number.

In this chapter, it was found that the inhibition of nodulation in white light was caused by its blue component, and that root nodule number reduction under blue light was not related to the lack of a carbon source. In addition, *L. japonicus* inhibition of nodulation by light is caused by blue light perception by both the host plant roots and rhizobia.

Chapter 3: Inhibition of root nodulation is caused by both plants CRYs and rhizobial blue light receptors.

In the chapter 2, it was showed that root nodulation of *L. japonicus*, a model legume, was inhibited by white light irradiation to root, and that the inhibition of nodulation is caused by blue light perception, but not green or red light. It is well known that blue light is perceived by PHOTs and CRYs. In addition, like higher plants, many bacteria synthesize photoreceptors such as phytochrome, and the analysis of numerous bacterial genomes has shown that photoreceptor proteins are present in many prokaryotes. In this chapter, it was investigated which photoreceptor regulate the root nodulation after blue light perception by root.

Using RNA interference (RNAi), it was silenced the *phototropin* (*phot1A*, *1B*, and *2*) and *cryptochrome* (*cry1A*, *1B*, *2A*, *2B*, and *3*) genes in *Agrobacterium rhizogenes*–induced hairy roots of *L. japonicus*. When plants with hairy roots carrying an empty vector (EV) control were illuminated with blue light, root nodules did not form on the unshaded plants. In contrast, root nodule number was significantly higher in *cry1A* and *2B*-targeted and unshaded roots and was correlated with a reduced expression of several *Ljcry* genes (except *Ljcry3*). No root nodules were observed in *phot1B*, *2*-silenced plants, and reduced nodule numbers were measured in the *phot1A* RNAi plants, which is similar to the results for *cry1B* and *cry2A* plants. These results support a role for cryptochromes in blue light–induced inhibition of root nodulation.

Rhizobial growth is inhibited by blue light. Then the growth of two signature-tagged mutagenesis strains in *M. loti* (STM) with disrupted genes homologous to LOV-HK/PAS protein (*mllpp1*) and photolyase (*mlphl*) was investigated. In the dark, the STM cell densities were similar to that of WT *M. loti*. However, high intensity blue light prevented the growth of WT *M. loti*, the growth of the STM strains was partially restored. This result was suggested that the lack of rhizobial proliferation may be an explanation for the reduction in nodule number. The growth parameters in plants inoculated with the STM strains and exposed to blue light was also measured. For the shaded roots, no significant difference between the WT *M. loti* and STM strains was observed in nodule number. For the unshaded plants, inoculation with the STM strains significantly increased nodulation under blue light in comparison with inoculation with *M. loti*. Taken together, these results suggested that both sets of rhizobial blue light receptors are required to inhibit nodulation.

Because blue light perception by both the root and rhizobia leads to an inhibition of nodulation, it was inoculated RNAi plants with the STM mutants and assessed nodulation status 21 dai. When the *Ljcry1A* and *2B*-targeted RNAi plants were inoculated with the STM strains, an additive increase in nodule number was observed. These results thus demonstrate that the inhibition of nodulation by light is caused by blue light perception by both host plant roots and rhizobia.

Chapter 4: *Sesbania rostrata-Azorhizobium caulinodans* symbiosis is not inhibited by light perception on root.

In the chapter 2 and 3, it was showed that root nodulation of *L. japonicus*, a model legume, was inhibited by white light irradiation, and that the inhibition of nodulation is caused by blue light perception by both the host plant roots and the rhizobia. *Sesbania rostrata*, a tropical legume, develops nodules on both root and stems in response to *Azorhizobium caulinodans* ORS571. Although root nodulation of *L. japonicus* is inhibited by white light, stem nodulation of *S. rostrata* is not inhibited under high intensity sunlight, which includes the blue component of the spectrum. Thus, it was hypothesized that stem nodulation would not be inhibited by blue light, but that the subterranean roots might be affected. Because inhibition of root nodulation in *L. japonicus* by white light is actually caused by blue light perception, the effect of blue light irradiation on nodulation on *S. rostrata* underground roots in response to inoculation with *A. caulinodans* was investigated.

To study the effect of root exposure to light on root nodulation in *S. rostrata*, roots were irradiated with blue light supplied from above for 14 days and analyzed the nodulation process thereafter. Following the root nodulation tests, no significant differences in shoot and root lengths were observed when unshaded and shaded plants were compared. However, although the root nodule number of *L. japonicus* unshaded roots was drastically decreased compared to that of the shaded roots, the number of nodules developed on *S. rostrata* unshaded roots was slightly, but significantly increased, compared to the shaded roots. This result suggested that in the *S. rostrata-A. caulinodans* symbiosis, a significant inhibitory effect by blue light irradiation on root nodule formation was not observed.

Because *S. rostrata* develops stem nodules under high intensity sunlight and root nodulation was not inhibited by irradiating the roots with blue light, we hypothesized that the sensitivity of *A. caulinodans* to blue light might be different from that exhibited by *M. loti*. *A. caulinodans* growth under blue light as measured by absorbance was not inhibited during the logarithmic growth phase but a slight, but significant, absorbance difference was observed between the blue light and dark treatments at the stationary phase. Cell numbers were checked at the end of logarithmic phase and stationary phase using dilution plate methods. However, no difference in viability was observed at logarithmic phase based on cell number counts between dark and blue light treatments. In contrast, cell numbers under blue light-illuminated conditions at stationary phase were drastically decreased compared to the dark treatment. From these data, it is concluded that blue light does not affect rhizobial growth based on absorbance measurements, but rhizobial survival is reduced by long-term blue light exposure.

Chapter 5: General discussion

It was well known root nodulation was suppressed by root exposed to light. Therefore, in this thesis, the effect on root nodulation by light exposure to leguminous plants root was investigated. First, it was studied this in the symbiosis of *L. japonicus* and *M. loti*. As a result, even in symbiosis between *L. japonicus* and *M. loti*, root nodulation is inhibited by light exposure to root. Moreover, it was revealed that this inhibition is caused by blue component, and by light perception of both plants CRYs and rhizobial blue light receptors. Next, it was checked the effect of inhibition of nodulation by blue light on symbiosis of *S. rostrtata*, which forms nodules on both root and stem, and *A. caulinodans*. As a result, root nodules developed under root exposed by blue light.

Higher plants developed avoidance mechanisms to survive under conditions of biotic or abiotic stress. It is well recognized that plants utilize light as the trigger for these responses (e.g.; anthogenesis, light-induced germination, root negative phototropism, and shade avoidance syndrome). Therefore, it can be considered inhibition of nodulation by light as one of several avoidance responses plants use to conserve energy in response to environmental stress.

The mechanism of inhibition of nodulation by blue light for *L. japonicus* is not conserved in the symbiosis between *S. rostrata* and *A. caulinodans*. It is speculated that plants evolved for the acquisition of an avoidance response plants to conserve energy in response to environmental stress. In contrast, it can be considered that because *S. rostata* and *L. japonicus* have different nodulation system and life style, e.g., perennial or annual, *S. rostarta* got different mechanism of avoidance response between *L. japonicus*. In the future, it need to study about blue light effect of other plants and rhizobial symbiosis.