

PHYTOCHROME B INFLUENCES SHOOT MERISTEM ACTIVITY AND ROOT MORPHOPHYSIOLOGY IN *LOTUS JAPONICUS*

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Phytochrome (PHY) is a major light responsive molecule in plants, which regulates various shoot morphological functions throughout the life cycle. Influence of phytochrome on shoot apex functions and root morphophysiology is less reported and the mediation on legume symbiosis is not documented.

We found two EMS mutants of model legume *Lotus japonicus*, 01-0017 and 01-1428, with elongated shoot, pale green foliage and elongated hypocotyls when germinated under red and white light conditions. Subsequent sequencing analysis revealed mutations in the *PHYB* gene of both lines. Plants were grown at Saga, Japan in vermiculite: perlite media (5:1-V/V), 25°C, 70 % RH, 16/8-hr light/dark cycles for morphological observations and under continuous red, blue and white light conditions to investigate differential light responses of mutants. Shoot length, number of nodes, leaf chlorophyll content, root length, shoot and root dry weight, number of nodules per plant and the nodule activity were observed at 28 days. In addition to univariate analysis, data were subjected to MANOVA and subsequent canonical variate (CV) analysis.

In contrast to elongated shoots, both mutants formed short root systems (190-200 mm) and lesser number of nodules (2.6-2.7) per plant compared to the wild type (WT), Miyakojima MG20. However, the nodule activity of *phyB* mutants was similar (30-40 nmol C₂H₄ plant⁻¹ hr⁻¹) for that of WT, as expressed by acetylene reduction assay (ARA). Even though mutants formed elongated shoots, the shoot: root dry weight ratio of mutants was significantly higher. This might be due to the lesser photosynthetic capacity of *phyB* mutants and due to poor dry matter partitioning to the root zone. A continuous red light treatment for 28 days from the time of inoculation resulted non-nodulated seedlings with unopened cotyledons of *phyB* mutants. These plants did not have normal shoot, root phenotypes, while WT plants showed normal phenotypes. Further, *phyB* mutants grown under continuous blue light for 28 days produced short shoots, roots and formed nodules, at a very low frequency (0.12-0.14 nodules/plant) compared to the WT. The differential red light responses of *phyB* mutants provided strong evidence for the regulation of shoot apical activities, root elongation and legume symbiosis by PHYB, apart from the well known shade avoidance response. Further PHYB may interact, at least partly, with cryptochrome signaling in *Lotus japonicus* under blue light. Multivariate analysis revealed the morpho-physiological deviation of *LjphyB* mutants from WT plants.