Involvement of Auxin Distribution on the Nodule Development in a model legume plant, *Lotus japonicus*

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Legume plants establish symbiosis with rhizobia in soils by forming organ called nodules, where symbiotic nitrogen fixation occurs. The initiation of nodule formation by rhizobia is stimulated by a signaling molecule from rhizobia, Nod factor, and nodules are mostly developed from outer cortical cells and form spherical nodules in determinate legumes as *Lotus japonicus*. This nodule formation process is regulated by several phytohormones, such as auxin, cytokinins, gibberellins, and brassinosteroids as positive regulators of nodule formation, while ethylene, jasmonic acid and abscic acid as negative regulators. Auxin regulates plant growth and development, particularly by the regulated polar movement along the vertical axis, forming concentration gradient through-out the plant. Inhibitors of auxin transport such as 1-naphtylphthalamic acid (NPA) and tri-indobenzoic acid (TIBA) have been generally employed to observe the effect of the auxin transport. Another biochemical tool to study auxin functions is α-(phenyl ethyl-2-one)-indole-3-acetic acid (PEO-IAA) as an auxin antagonist. We have investigated the auxin distribution in the determinate nodules of *L. japonicus* using auxin inhibitors and found that the development of lenticels and nodule vascular bundles were was strongly affected by auxin during the nodulation process.

The auxin responsible GH3:GUS transformant of *L. japonicus* showed auxin responses in a nodulation-specific manner during nodule development. Before inoculation of *Mezorhizobium loti*, GH3 expression was observed only in central cylinder, while after inoculation, GH3 expression started to increase in cortical cells in nodule primordial where cell divisions occur. In mature nodules, GUS staining disappeared, whereas strong expression was observed in vascular tissues. These results suggest that an important role of auxin in nodule formation, especially in the development of vascular tissues. By using auxin inhibitors, we have demonstrated the effects of auxin transport inhibitors on nodulation by showing that the treatment of *L. japonicus* with NPA or TIBA altered the nodule number at concentrations 10 μM and 100 μM. We also investigated the effects of PEO-IAA on nodulation in *L. japonicus*. Although PEO-IAA affected the root growth and root hair formation in *L. japonicus*, the number of nodules was not strongly altered. The effect of PEO-IAA was observed in the development of lenticels on nodules. We also found the inhibition of the development of nodule vascular bundle by these inhibitors.

Lenticels, which develop opposite the nodule vascular bundle, are suggested to arise from specific phellogen cells in the nodule cortex. Lenticels appear on the nodule surface, and they regulate gas permeability of nodules. The relation between the lenticels and the nodule vascular system is not yet known; however, it was suggested that the development of lenticel is associated with growth substances supplied from the vascular system, suggesting that auxin is necessary to form nodule vascular bundle and lack of lenticel is an indirect effect of the inhibition, or auxin is a growth substance of lenticels.

We have shown the involvement of auxin in formation of nodule vascular bundle and lenticel. For further understanding of the auxin distribution and the role of auxin in the nodule formation of *L. japonicus*, we need to identify and characterize auxin transporters involved in the nodulation

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References

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