Abstract

In higher plants, it is essential for different organs to interact with one another to ensure that the entire plant body develops and functions properly. The xylem vessels are the main route for transporting materials and information from the roots to the shoot. This study elucidated the mechanism controlling the production of a xylem sap protein, XSP30, in cucumber root, and characterized the lectin activity of XSP30.

In Part I, the expression of the gene encoding XSP30 in roots of cucumber (*Cucumis sativus* L.) was shown to be dependent on exposure of the aboveground tissues to light. Gene expression of *XSP30* was barely detected in the roots of 4-day-old seedlings, but expression increased with subsequent seedling growth. The expression of *XSP30* in root clearly showed a diurnal oscillation; expression began to rise at midday and decline at dusk. In synchrony, the XSP30 protein level in xylem sap increased in the afternoon, reached a maximum at dusk and decreased during the night. When transferred to continuous dark, the diurnal pattern of expression of *XSP30* continued for two diurnal cycles. Exposure to continuous light, however, caused an abnormal oscillation in the third cycle. To demonstrate that expression of *XSP30* relied on aboveground tissues, portions of these were removed. When all aboveground tissues were removed, the expression of *XSP30* fell drastically within 24 hours. When only the first leaf was removed from the seedlings, the expression of *XSP30* declined for three diurnal cycles, but recovered with the growth of the second leaf. When GA was applied to the shoot apex and cotyledons of seedlings when the first leaf was excised, the oscillating pattern of gene expression was clearly restored. When uniconazole-P, an inhibitor of GA biosynthesis, was applied to the aboveground organs of intact plants,

the oscillation of gene expression drastically dropped after two cycles; simultaneous co-application of GA eliminated the effect of uniconazole-P. These results suggest that leaf gibberellins contribute to the pattern of gene expression and the resulting concentration of xylem sap protein, XSP30, in cucumber roots.

In Part II, the expression of *XSP30* in cucumber root was analyzed histologically using the *XSP30* promoter and a GUS (ß-glucuronidase) reporter gene. The *XSP30* promoter directed specific GUS expression in the xylem parenchyma and pericycle cells in the central cylinder of transgenic cucumber hairy roots. The GUS expression was the strongest in the mature part of the root, and gradually decreased in the younger region near the root tip. These results suggest that XSP30 is produced in the xylem parenchyma and pericycle cells in the central cylinder of the mature root in cucumber.

In Part III, the lectin activity of XSP30 was analyzed by lectin blot coupled with immunological detection of XSP30. XSP30 is homologous to galactose-binding lectin, but it had only weak binding activity against asialofetuin, an animal glycoprotein with a terminal galactose. This reaction was not reduced by galactosidase treatement of asialofetuin. XSP30 bound strongly to soybean agglutinin, whose sugar chains consist solely of mannose and *N*-acetylglucosamine (GlcNAc), but bound only weakly to soybean peroxidase and γ-globulin, whose GlcNAc is fucose-modified. The binding activity was inhibited by tri-*N*-acetylchitotriose ($GlcNAc_3$). Leaf parenchyma cells were stained for lectin activity; this staining was reduced by proteinase treatment of the sections, and XSP30 bound to proteins in leaf particulate fraction. These results

suggest that XSP30 transported from root via xylem sap binds to core GlcNAc-GlcNAc in *N*-linked glycans of leaf glycoproteins.

In conclusion, this study provides novel information about the interaction between the roots and the aboveground organs in plant.