General Discussion

The roots are the main source of water and inorganic nutrients. Drought alters the activity of aquaporins that regulate water movement in the roots (Martre *et al.* 2001). It has been reported that information on the water status is transferred, as a hormonal signal, from the roots to the shoot via xylem. Abscisic acid, which causes stomatal closure, is produced with dehydration or salinity stress in the roots and transported to the aboveground organs via the xylem sap (Else *et al.* 1995, Liang *et al.* 1997). Moreover, cytokinin synthesized in root tissues is transferred to the aboveground organs via the xylem and is involved in leaf greening (Kato *et al.* 2002) and adventitious root formation (Kuroha *et al.* 2002). However, there are no reports on information transfer from the aboveground organs to the root.

I analyzed the aboveground organ-dependent production of XSP30 in the roots. *XSP30* expression in roots was drastically increased with development of the aboveground organs (Fig. 1). The *XSP30* promoter directed the reporter gene expression only in the mature region of roots (Fig. 12A). Therefore, *XSP30* expression in roots was correlated with both the development of the aboveground organs and with root maturity (Fig. 12A). It is thought that signals from aboveground organs increase with their development, and that roots strengthen their function with growth. *XSP30* expression in the roots might reflect the tight relationship between the development and growth of the aboveground organs and the roots.

Furthermore, the production of XSP30 in roots was controlled by the photoperiod perceived by the aboveground organs and gibberellin produced in mature young leaves (Figs. 2A and 7A). The photoperiod is perceived by photoreceptors, such

as phytochromes and cryptochromes, and clock component genes, such as *TOC1, LHY*, and *CCA1*, precisely control the 24-hr period of the biological clock (Mouradov *et al.* 2002). The information from the biological clock distributed to each organ defines many aspects of developmental phenomenon, such as flowering, leaf movement, and hypocotyl elongation (McClung 2001). Moreover, gibberellin is one of the most important plant hormones produced in the active part of the aboveground organs; it induces drastic developmental changes, such as flowering and germination (Yamaguchi and Kamiya 2000). These facts suggest that production of XSP30 in roots is very sensitive to the condition of the aboveground organs and is precisely controlled by these signals.

Furthermore, the *XSP30* promoter directed *GUS* expression only in the mature region of transgenic hairy roots (Fig. 12A). The mature region of the roots is the main site for the absorption of water and minerals. The production of XSP30 in the mature part of the roots is thought to be essential for loading XSP30 into the xylem sap, because the flow of water approaches the vascular bundle from the epidermis and root hairs in the mature part of the roots. Therefore, the production of XSP30 is very sensitive to the condition of the roots. The amount of XSP30 in xylem sap is thought to reflect the condition of the whole plant, including the aboveground organs and roots.

Although XSP30 is homologous to the toxic protein ricin, a galactose-specific lectin (Masuda *et al.* 1999), I found that XSP30 has lectin-like activity, and can bind to core GlcNAc-GlcNAc groups in *N*-linked glycans (Fig. 17). It has also been suggested that binding activity is inhibited by fucosil modification of the core GlcNAc structure.

Many lectins have toxic domains and contribute to plant defenses (Peumans *et al.* 1995); however, since XSP30 lacks a toxic domain and its binding site differs from that in the homologous toxic lectin ricin, it is thought that XSP30 does not contribute to plant defense.

I also analyzed the delivery of XSP30 from the roots to the aboveground organs. The XSP30 binding site might be abundant in the membrane proteins of leaf parenchyma cells (Figs. 18C and 19). Although XSP30 was found in xylem sap (Fig. 3A) and can bind to glycoproteins in leaf parenchyma cells, no XSP30 accumulation was found in any aboveground organ (Fig. 18, B and F). These results imply that XSP30 is broken down by a specific system in aboveground organs. The synchronized production in roots and the rapid decomposition in aboveground organs imply that XSP30 acts as a signal molecule from the roots to the shoot.

The xylem is thought to be the main route for transporting water, inorganic substances, and many organic substances from the roots to the shoot (Satoh *et al.* 1992). Since the production of XSP30 in root follows diurnal light cycle perceived by leaves and the main destination of xylem sap from the roots is thought to be leaf parenchyma cells, XSP30 might be involved in the function and development of leaf parenchyma cells by the lectin activity (Oda *et al.* 2003b).

This paper proposes the existence of a novel system for the exchange of information between the roots and the aboveground organs. In the future, the novel relationship between the roots and aboveground organs will be clarified when the physiological function of XSP30 is elucidated. I have discovered more than 40 kinds of

proteins in cucumber xylem sap, but to date I have analyzed only two of these xylem sap proteins. Analysis of all the macromolecules in xylem sap will be required for full understanding of the interactions between organs.