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Rapid Report

Detection of Several mRNA Species in Rice Phloem Sap

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Evidence is reported for the presence of the mRNAs of thioredoxin h, oryzacystatin-I, and actin in the rice phloem sap collected by the insect laser method. As the sieve element, the core component of the phloem, is enucleated, these macromolecules are probably transported from the companion cells.

Key words: Actin — Cystatin — mRNA — Phloem — Rice — Thioredoxin.

Phloem is the most important route of long-distance transport of signals and photoassimilates in vascular plants, developed in the evolution of terrestrial plants. The sieve element, the core component of the phloem, is enucleated, although its exudates contain a number of proteins including thioredoxin h, actin, and cystatin (Ishiwatari et al. 1995, Schobert et al. 1995, 1998). Some of these proteins have been shown to dilate and traffic through plasmodesmata upon microinjection into tobacco mesophyll cells (Ishiwatari et al. 1998, Barachandran et al. 1997), suggesting that these proteins are transported from the companion cells to the sieve element through plasmodesmata (Dannenhoffer et al. 1997). Kühn et al. (1997) reported that SUT1 mRNA was found on the sieve element side of the plasmodesmata connecting the companion cells and the sieve elements by in situ hybridization. However, mRNA detection in phloem sap has not been reported. Here we report evidence for the presence of several mRNA species in the rice phloem sap collected by the insect laser technique (Kawabe et al. 1980). To our knowledge, this is the first report of the detection of endogenous mRNA in phloem sap.

Phloem sap was collected from three- to four-week-old rice plants (*Oryza sativa* L. var. Kantou) by the insect laser technique (Kawabe et al. 1980) and its RNase activity was measured using an RNase Detection Kit (MO BIO Laboratories, Inc., Solana Beach, CA). Phloem sap collected just after excision of stylets was discarded and samples collected after more than half an hour from stylectomy were used for subsequent studies. One micro liter of phloem sap was incubated with yeast ribosomal RNA at 37°C for 1 h, as instructed by the manufacturer. This incubation did not result in visible degradation of RNA (Fig. 1), indicating that rice phloem sap contains little or no RNase activity. Earlier reports suggested the presence of RNase activity in phloem exudate collected by the incision method (Kollar and Seemüller 1990). In our case, phloem sap was collected by stylectomy of rice brown planthoppers and was likely to contain little or no contaminants from the cells other than phloem.

Next, the phloem sap was subjected to reverse transcription (RT) reaction using oligo dT primers. The products were then analyzed by polymerase chain reactions (PCR) using three different sets of primers corresponding to sequences of thioredoxin h (Ishiwatari et al. 1995), oryzacystatin-I (Abe et al. 1987) and actin (Genbank accession No. X16280) mRNAs. Sequences of oligonucleotides are shown in Table 1.

These proteins are chosen as they are present in rice phloem sap. Thioredoxin h is one of the major proteins in the rice phloem sap and this mRNA is localized in the companion cells of the leaf sheath of rice plants (Ishiwatari et al. 1998). Actin (Schorbert et at. 1998) and oryzacystatin-I (Hayashi et al., unpublished results) are also detected in the rice phloem sap by western analysis, although cell-type

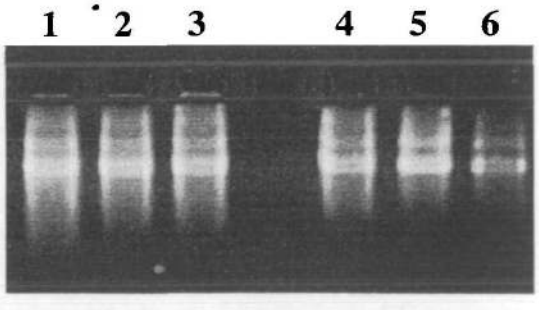

Fig. 1 Rice phloem sap contains no detectable RNase activity. Standard RNA of the RNase Detection Kit (MO BIO Laboratories, Inc., Solana Beach, CA, U.S.A.) was incubated 1 h at 37°C with 1 μl of rice phloem sap (lanes 1 and 2), 1 μl of water (lanes 3 and 4) and RNase A (25 and 37.5 unit for lanes 5 and 6, respectively). Reaction mixture was analyzed by agarose gel electrophoresis.

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Table 1 Oligonucleotide sequences used for PCR reaction

Genes	Oligonucleotide sequences (5′ to 3′)	References
Thioredoxin h	TGCCACAACAAGGACGAGTTCG TTAGGCAGAAGCAGATGCAGCA	Ishiwatari et al. 1997
Oryzacystatin	GGCCGAGGCGCATCG CGTGTGAGATCCTTATATCCAAGCC	Abe et al. 1987
Actin	TTACTCATTCACCACAACGGC TGGATCCTCCAATCCAGACAC	McElroy et al. 1990

specific expression patterns of these genes have not been reported.

Figure 2 illustrates an example of the reaction products, in this case thioredoxin h. The size of the amplified fragment from the phloem sap corresponded to the band amplified from cDNA but not genomic DNA (Fig. 2). Restriction enzyme digest patterns were identical to the band from the cDNA (Fig. 2). Moreover, nucleotide sequence analysis of the products (data not shown) proved that the amplified bands were derived from correctly spliced rice thioredoxin h mRNA.

The other primers also detected corresponding mRNAs by RT-PCR (data not shown), although the frequency of detection varied with the mRNA. It was about

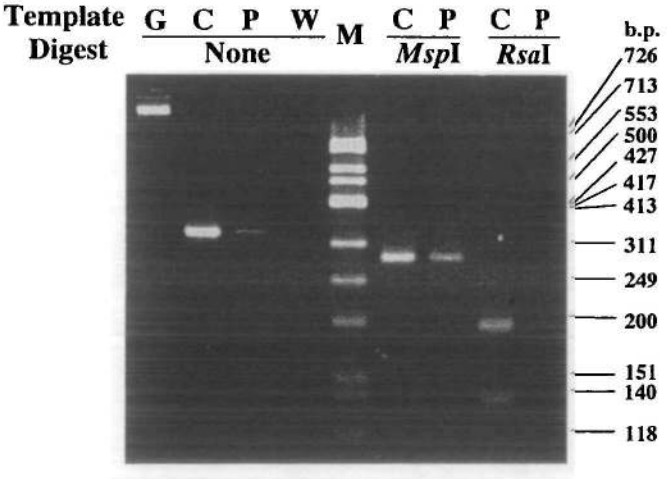

Fig. 2 Spliced thioredoxin h mRNA is present in rice phloem sap. One microliter of rice phloem sap was subjected to reverse transcription reaction using oligo dT as a primer, followed by PCR using primers corresponding to thioredoxin h mRNA. As controls, genomic (Ishiwatari et al. 1997) and cDNA (Ishiwatari et al. 1995) clones of rice thioredoxin h were used as templates. The PCR products were subjected to agarose gel electrophoresis (4%) prior to or after the restriction digests with the enzymes shown. These primer sets amplify the fragments with mRNA splice sites allowing the differentiation of genomic DNA contamination. G, genomic clone of rice thioredoxin h; C, cDNA clone of rice thioredoxin h; P, rice phloem sap; W, water; M, ϕX174 *Hin*fI digest.

66% (18 positives out of 27 trials), 50% (3 out of 6), and 20% (2 out of 10) for thioredoxin h, actin and oryzacystatin-I, respectively. In our hands, the detection limit of the mRNA was within the range of a few hundred molecules per micro liter of sample, suggesting that the actual mRNA concentrations in phloem sap are higher. The difference in the frequency of detection may reflect the fluctuation in the occurrence or concentration of each mRNA in rice phloem sap. The possibility of occasional contamination of fluids other than phloem sap has been reported (Sogawa 1970), but we found that the frequency of collecting fluids other than phloem sap by the insect laser method is less than a few %, because more than 99% of the sap collected by this method in our lab contained a high concentration (5–30%) of sucrose (Hayashi, unpublished results). Such a high concentration of sucrose can only be found in the phloem sap in rice plants. By our methods, the rate of phloem sap exudation was about one microliter per hour and usually lasted for more than a few hours, suggesting that most of the exudation occurs from the sieve element but not from the companion cells. All samples were collected at least half an hour after the excision to avoid possible contamination from the exudates from cells other than the sieve elements. Our finding that the rice phloem sap lacks RNase activity (Fig. 1) also indicates that our rice phloem sap contains little or no contaminants.

Our findings revealed the presence of several mRNA species in the rice phloem sap. As the mature sieve elements are enucleated, these mRNAs are likely be transported from neighboring cells, most presumably from the companion cells, through plasmodesmata, similar to the case of the SUT1 mRNA (Kühn et al. 1997). Our findings also demonstrated that the abundance of mRNA varies depending on the mRNA species, suggesting that the process of mRNA transfer through plasmodesmata is selective. The nature of the transport process remains to be elucidated. Several plant viral movement proteins and plant homeoproteins have been shown to selectively transport nucleic acids through plasmodesmata (Fujiwara et al. 1993, Noueiry et al. 1994, Lucas et al. 1997). Phloem sap also contains nearly a hundred proteins (Ishiwatari et al. 1995, Schobert et al.

1995) and recently, many of the phloem proteins were shown capable of dilating plasmodesmata in tobacco mesophyll cells upon microinjection (Balachandran et al. 1997, Ishiwatari et al. 1998). Such proteins may be involved in mRNA transport to the sieve elements through plasmodesmata.

The rate of sucrose transport through phloem has been estimated to be about 50 cm per hour in rice plants (Nakamura 1994). Provided that these mRNAs have a substantially long half life, our findings suggest that mRNAs in rice phloem sap travel a long distance within the plant body. These mRNAs may act as long-distance signal molecules in higher plants (Jorgensen et al. 1998). This process may also be employed by plant viruses for systemic infection through the phloem.

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