Multispectral Phloem-Mobile Probes: Properties and Applications

Michael Knoblauch, Marc Vendrell, Erica de Leau, Andrea Paterlini, Kirsten Knox, Tim Ross-Elliot, Anke Reinders, Stephen A. Brockman, John Ward, and Karl Oparka*

Plant Cell Biology Laboratory, School of Biology, Washington State University, Pullman, Washington 99164-4236 (M.K., T.R.-E.); University of Edinburgh/Medical Research Centre Centre for Inflammation Research, Queen’s Medical Research Institute, University of Edinburgh, Edinburgh EH16 4TJ, United Kingdom (M.V.); Institute of Molecular Plant Sciences, University of Edinburgh, Edinburgh EH9 3BF, United Kingdom (E.d.L., A.P., K.K., K.O.); and Plant Biology Department, University of Minnesota, St. Paul, Minnesota 55108 (A.R., S.A.B., J.W.)

Using Arabidopsis (Arabidopsis thaliana) seedlings, we identified a range of small fluorescent probes that entered the translocation stream and were unloaded at the root tip. These probes had absorbance/emission maxima ranging from 367/454 to 546/576 nm and represent a versatile toolbox for studying phloem transport. Of the probes that we tested, naturally occurring fluorescent coumarin glucosides (esculin and fraxin) were phloem loaded and transported in oocytes by the sucrose transporter, AtSUC2. Arabidopsis plants in which AtSUC2 was replaced with barley (Hordeum vulgare) sucrose transporter (HvSUT1), which does not transport esculin in oocytes, failed to load esculin into the phloem. In wild-type plants, the fluorescence of esculin decayed to background levels about 2 h after phloem unloading, making it a suitable tracer for pulse-labeling studies of phloem transport. We identified additional probes, such as carboxytetraethylrhodamine, a red fluorescent probe that, unlike esculin, was stable for several hours after phloem unloading and could be used to study phloem transport in Arabidopsis lines expressing green fluorescent protein.

The phloem of higher plants consists of a series of longitudinally arranged sieve elements (SEs), companion cells (CCs), and associated parenchyma elements (Heo et al., 2014). The SEs translocate a diverse range of solutes, proteins, and RNAs from source to sink organs and perform key roles in solute delivery and signaling (Turgeon and Wolf, 2009; Ham and Lucas, 2014). The phloem is a delicate tissue, and examining its structure and function has proven to be a difficult task (Knoblauch and Oparka, 2012; Truernit, 2014). Arguably, the most reliable way to assess the rate of phloem transport in different organs is by using radiolabeled solutes derived photosynthetically from 14CO2 (Kölling et al., 2013). In parallel, autoradiography provides a valuable means of imaging the distribution of radiolabeled solutes in different tissues (Housley and Fisher, 1975; Kölling et al., 2013). However, both of these methods are time-consuming and limited by resolution. In the last decade, the use of fluorescent tracers has become prominent, allowing phloem transport to be imaged in living SEs with significantly improved resolution above autoradiography (Knoblauch and Oparka, 2012). Importantly, these probes cannot be used as substrates for Suc loading, which in many species, occurs by active, carrier-mediated transport (Turgeon and Wolf, 2009).

Schumacher (1933) was the first plant biologist, to our knowledge, to study phloem transport using the fluorescent molecule fluorescein. Since then, however, only a few additional phloem-mobile probes have been discovered. Two such probes are carboxyfluorescein (CF; Grignon et al., 1989; Oparka et al., 1994) and 8-hydroxypropyrene-1,3,6-trisulphonic acid (HPTS; Wright and Oparka, 1996). When applied in the ester (acetate) form, these probes are phloem mobile, although the exact mechanism by which they enter the phloem is unknown. In the case of CF, it is possible that this probe diffuses into the phloem and is retained in SEs by ion trapping (Wright and Oparka, 1996), a characteristic that it may share with many phloem-mobile herbicides (Hsu and Kleier, 1996). In contrast, HPTS is a highly charged molecule that should not cross membranes (Wright and Oparka, 1996), but it enters the phloem readily. Despite a lack of understanding of how these probes are loaded into the phloem, they have been used extensively in monitoring phloem transport (Knoblauch and van Bel, 1998). They have also found use in imaging sympodial pathways after unloading (Oparka et al., 1994; Roberts et al., 1997; Savage et al., 2013) and identifying sympodial domains...
Table I. Fluorescent coumarin-based glucosides evaluated as phloem-mobile fluorophores

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical Structure</th>
<th>$M_r$</th>
<th>ASUC2 Activity</th>
<th>Phloem Transport</th>
</tr>
</thead>
<tbody>
<tr>
<td>6,7-dihydroxy-coumarin 6-β-D-glucopyranoside (esculin)</td>
<td><img src="image1" alt="Chemical Structure" /></td>
<td>340.3</td>
<td>✓ ✓</td>
<td></td>
</tr>
<tr>
<td>7,8-dihydroxy-6-methoxycoumarin 8-β-D-glucopyranoside (fraxin)</td>
<td><img src="image2" alt="Chemical Structure" /></td>
<td>370.3</td>
<td>✓ ✓</td>
<td></td>
</tr>
<tr>
<td>7-hydroxy-coumarin 7-β-D-glucopyranoside (skimmin)</td>
<td><img src="image3" alt="Chemical Structure" /></td>
<td>324.3</td>
<td>X ✓</td>
<td></td>
</tr>
<tr>
<td>4-methyl-7-hydroxycoumarin 7-β-D-glucopyranoside</td>
<td><img src="image4" alt="Chemical Structure" /></td>
<td>338.3</td>
<td>X X</td>
<td></td>
</tr>
<tr>
<td>3-acetyl-7-hydroxycoumarin 7-β-D-glucopyranoside</td>
<td><img src="image5" alt="Chemical Structure" /></td>
<td>366.3</td>
<td>nd X</td>
<td></td>
</tr>
<tr>
<td>3-phenoxazone- 7-β-D-glucopyranoside (resorufin glucoside)</td>
<td><img src="image6" alt="Chemical Structure" /></td>
<td>375.3</td>
<td>X X</td>
<td></td>
</tr>
</tbody>
</table>
in developing tissues (Gisel et al., 1999; Stadler et al., 2005). However, both CF and HPTS emit in the green spectrum, restricting their use for imaging movement in cells that express GFP as a reporter.

The limited number of existing probes for phloem transport prompted us to explore unique small molecules differing in excitation and emission spectra as potential tracers. Using an Arabidopsis (Arabidopsis thaliana) seedling screen, we tested the phloem mobility of several small-molecule probes. In addition, we explored the use of esculin as a phloem-mobile tracer. Esculin is a fluorescent coumarin glucoside that is transported in oocytes by AtSUC2 (Sivitz et al., 2007), the major Suc transporter that loads the phloem in Arabidopsis (Gottwald et al., 2000). Here, we describe the development and application of a range of probes for monitoring phloem transport. These small probes cover absorbance/emission maxima ranging from 367/454 to 546/576 nm, allowing them to be used on plant material expressing different fluorescent reporter proteins. We describe the properties of these probes and show how they can be used in both pulse- and dual-labeling studies of phloem transport.

RESULTS

Esculin

In our initial studies, we examined the use of esculin as a potential phloem-mobile probe. Esculin (Table I) is a coumarin glucoside that is transported in oocytes by the Arabidopsis Suc transporters AtSUC2 and AtSUC9 (Sivitz et al., 2007; Reinders et al., 2012). It is also transported into transgenic yeast (Saccharomyces cerevisiae) cells expressing AtSUC2 (Gora et al., 2012). However, its phloem-transport properties have not been determined in planta. When we applied esculin to the leaf surface, the probe entered the phloem rapidly (Fig. 1A) and was unloaded subsequently from the terminal protophloem (Fig. 1B) as described previously for both CF and HPTS (Oparka et al., 1994; Wright and Oparka, 1996). We noted that esculin entered the vacuoles of sink cells after unloading (Fig. 1C) and that its fluorescence did not persist in the root, declining to background levels in the phloem about 2 h after loading the leaf. There was no difference in the pattern of esculin unloading between plants grown in agar and those grown in microscopy rhizosphere chambers (micro-ROCs). We took advantage of the natural quenching of esculin fluorescence to apply a second pulse of label to the same root. To do this, we blotted the application site after esculin treatment, replaced the probe with distilled water, and then, applied a second esculin treatment to the same leaf. The arrival of this second pulse was clearly visible in the root phloem (Supplemental Movie S1).

Esculin Is Loaded into the Phloem by AtSUC2

The barley (Hordeum vulgare) transporter HvSUT1, when expressed in oocytes, transports Suc (Sivitz et al., 2005) but does not transport esculin (Sivitz et al., 2007; Reinders et al., 2012). To test whether esculin uptake was dependent on AtSUC2, we expressed the HvSUT1 complementary DNA, driven by the AtSUC2 promoter, in an atsuc2-5 mutant background. This line showed no visible phenotype (Supplemental Fig. S1) and failed to transport esculin (n = 32 plants), consistent with the view that AtSUC2 is essential for loading esculin into the phloem. However, the Arabidopsis line expressing HvSUT1 readily transported HPTS, a probe that does not depend on AtSUC2 activity for its transport (n = 21 plants; Fig. 1D).

A Range of Fluorescent Coumarin Glucosides Is Loaded into the Phloem

The ability of AtSUC2 to load esculin offers an opportunity using different fluorescent glucosides to study the specificity of AtSUC2 for its substrates. We studied a range of coumarin-based glucosides for their ability to
be transported by AtSUC2 expressed in oocytes and in parallel, whether they were transported into the Arabidopsis leaf phloem. In initial experiments, we concentrated on three naturally occurring coumarin glucosides: esculetin, fraxetin, and umbelliferone (Table I). Of these, esculetin and fraxetin were phloem loaded and translocated to the root. However, umbelliferone was not transported by AtSUC2 in oocytes (Fig. 2; Table I). The fluorescent precursors of these glucosides (esculin, fraxin, and umbelliferone) were not loaded into the phloem (data not shown).

Next, we tested whether alterations to the coumarin molecule affected glucoside loading. We tested additional coumarin glucosides, in which the coumarin was methylated (4-methyl-7-hydroxycoumarin-β-D-glucopyranoside; Table I) or acetylated (3-acetyl-7-hydroxycoumarin-β-D-glucopyranoside; Table I) or in which the coumarin core was extended with an aromatic ring to form the compound resoruflavin (β-D-glucopyranoside). None of these probes were loaded into the phloem (data not shown).

The glucosides that showed phloem transport were tested further for their ability to be transported by AtSUC2 and/or enhanced phloem transport. Specifically, we searched for small molecules that had a low Mr and covered a range of fluorescence emission spectra (Fig. 3). These included glucosides of bimane, dansyl, nitrobenzoxadiazole (NBD), rhodamine, and malachite green (Fig. 3). For each of these compounds, we assayed both the fluorescent glucoside and also, its fluorescent precursor for phloem mobility on Arabidopsis seedlings. Those glucosides that showed phloem transport were tested further for their ability to be transported by AtSUC2.

**Additional Phloem-Mobile Probes**

We tested whether additional small fluorophores could be linked to Glc to facilitate transport by AtSUC2 and/or enhanced phloem transport. Specifically, we searched for small molecules that had a low Mr and covered a range of fluorescence emission spectra (Fig. 3). These included glucosides of bimane, dansyl, nitrobenzoxadiazole (NBD), rhodamine, and malachite green (Fig. 3). For each of these compounds, we assayed both the fluorescent glucoside and also, its fluorescent precursor for phloem mobility on Arabidopsis seedlings. Those glucosides that showed phloem transport were tested further for their ability to be transported by AtSUC2.

**Figure 2.** Transport of coumarin glucosides by AtSUC2 expressed in oocytes. Currents were recorded using two-electrode voltage clamping. A, Representative data from one oocyte at a holding potential of −40 mV showing inward currents induced by Suc and esculin. Synthetic glucosides (4-methyl-7-hydroxycoumarin-β-D-glucoside and resoruflavin-β-D-glucoside) did not produce inward currents. B, Substrate-dependent currents recorded at −100 mV. Data are presented as mean ± se (n = 3). Mean Suc-dependent (5 mM) currents were 59.6 ± 11.1 nA at −100 mV (n = 7).
AtSUC2 in oocytes. Of the glucosides tested, we found two (2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose [2-NBDG] and carboxytetraethylrhodamine [CTER] glucoside) that were phloem mobile (Figs. 3 and 4). However, these probes were not transported by AtSUC2 in oocytes (data not shown). We then compared the phloem mobility of these glucosides with esculin (Fig. 4). 2-NBDG was phloem mobile, which has been reported previously (Hofmann et al., 2009), but its precursor (NBD) showed limited mobility. In contrast, we found both CTER and CTER glucoside to be highly phloem mobile, making them excellent tracers for phloem transport studies (Fig. 4).

**Dual-Labeling Studies**

Of the probes that we identified, esculin and CTER proved to be excellent phloem transport tracers. These probes are commercially available and can be imaged with conventional UV (405 nm) and red (561 nm) laser lines, respectively. Together with 5(6)-carboxyfluorescein diacetate (CFDA) and HPTS acetate (488 nm), these probes provide a versatile toolbox for studying translocation in a range of species. The properties of a range of phloem-mobile probes are shown in Table II. Most of these probes have high extinction coefficients and quantum yields. Ultimate probe brightness is a function of these parameters (Lavis and Raines, 2008; Table II). The strong spectral separation of the probes means that they can be used in dual- or triple-labeling studies of phloem transport. To test this, we applied different probes (e.g. esculin and CTER) to separate cotyledons of Arabidopsis seedlings. The probes were transported simultaneously, resulting in dual labeling of the phloem strands (Fig. 5A) and unloading at the root tip (Fig. 5B). In a line expressing GFP under the SUC2 promoter (AtSUC2.GFP), GFP is unloaded into sink tissues (Imlau et al., 1999). The unloading of CTER showed a strong correlation with GFP distribution in the root tip (Fig. 5D). However, CTER was sequestered by the vacuoles, whereas GFP remained in the cytoplasm (Fig. 5E). In a line expressing a sieve element occlusion-related (SEOR)-yellow fluorescent protein (YFP) fusion (Froelich et al., 2011), CTER was unloading from the terminal SEs of the protophloem and showed a strong tropism through the cortex toward the root tip (Fig. 5E; compare with Oparka et al., 1994). We next examined the phloem of the above reporter lines at higher magnification. In the AtSUC2.GFP line, we identified double-labeled SEs and CCs in the phloem after CTER labeling (Fig. 6A). In the line expressing SEOR-YFP, CTER and esculin exchanged freely between SEs and CCs as expected, whereas SEOR-YFP was restricted to the SEs (Fig. 6, B and C; Froelich et al., 2011).

**DISCUSSION**

This study has identified unique fluorescent probes that should facilitate studies of phloem transport, allowing pulse- and dual-labeling studies of the same plants. It is unlikely that all of the probes described here enter the phloem by the same mechanism, and the exact mode of phloem loading of some probes remains unknown. In the case of the coumarin glucosides esculin and fraxin, it seems that these fluorescent compounds are phloem loaded by AtSUC2. Plants in which AtSUC2 was replaced with HvSUT1 did not load esculin into the phloem. This is consistent with the inability of HvSUT1 to transport esculin when expressed in oocytes (Sivitz et al., 2007; Reinders et al., 2012). These results also indicate that no other transport activity loads esculin into the phloem in the absence of AtSUC2. Coumarin glucosides are transported by other Suc transporters (e.g. AtSUC9; Sivitz et al., 2007), and it remains to be shown whether additional Suc transporters (for example, located in sink tissues) influence its final distribution. The inability of AtSUC2 to transport skimmin when expressed in oocytes suggests that another transporter...
may load this compound into the phloem. Coumarin glucosides seem to be a unique class of phloem-mobile fluorophores that enter the phloem by carrier-mediated transport. Our results for synthetic coumarin glucosides suggest that relatively small changes to the coumarin core may disrupt transport by AtSUC2 and subsequent phloem loading. In the future, additional selective modifications of coumarin glucosides may provide additional clues as to how Suc carriers recognize and transport their diverse substrates.

Other than coumarin glucosides, we were unable to detect additional fluorescent glucosides transported by AtSUC2, although some of these substrates were phloem mobile (e.g. 2-NBDG and CTER glucoside). Additional glucoside carriers may have transported these compounds, or they may have simply been able to diffuse more readily into the phloem. The addition of Glc to fluorescent compounds has been shown previously to facilitate phloem transport (Wang et al., 2014) and may provide a generic means of enhancing the transport of these substrates.

Table II. Spectral properties of optimal phloem-mobile fluorophores

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical Structure</th>
<th>$\lambda_{\text{abs}}$ (nm)</th>
<th>$\lambda_{\text{em}}$ (nm)</th>
<th>$\varepsilon$ (M$^{-1}$ cm$^{-1}$)</th>
<th>Quantum Yield ($\phi$)</th>
<th>Brightness ($\phi \times \varepsilon$) (M$^{-1}$ cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6,7-dihydroxy-coumarin 6-β-D-glucopyranoside (esculin)</td>
<td><img src="https://example.com/esculin.png" alt="Chemical structure" /></td>
<td>367</td>
<td>454</td>
<td>24,000</td>
<td>0.47$^b$</td>
<td>11,280</td>
</tr>
<tr>
<td>2-NBDG</td>
<td><img src="https://example.com/2-NBDG.png" alt="Chemical structure" /></td>
<td>494</td>
<td>551</td>
<td>23,900</td>
<td>0.55$^c$</td>
<td>13,145</td>
</tr>
<tr>
<td>HPTS acetate</td>
<td><img src="https://example.com/HPTS.png" alt="Chemical structure" /></td>
<td>455</td>
<td>511</td>
<td>20,000</td>
<td>0.90$^d$</td>
<td>18,000</td>
</tr>
<tr>
<td>CFDA</td>
<td><img src="https://example.com/CFDA.png" alt="Chemical structure" /></td>
<td>492</td>
<td>517</td>
<td>75,000</td>
<td>0.91$^e$</td>
<td>68,250</td>
</tr>
<tr>
<td>CTER</td>
<td><img src="https://example.com/CTER.png" alt="Chemical structure" /></td>
<td>546</td>
<td>576</td>
<td>90,000</td>
<td>0.73$^f$</td>
<td>65,700</td>
</tr>
</tbody>
</table>

Figure 5. Dual labeling of the phloem in Arabidopsis roots. A, Phloem strands dual labeled with esculin and CTER. i, Bright field. ii, Esclulin (405 nm). iii, CTER (561 nm). B, Corresponding root tips to images shown in A. C, Unloading of CTER in a root expressing an SEOR-YFP fusion (Froelich et al., 2011). *, SIEVE ELEMENT OCCLUSION (SEO) aggregates delimit the terminal protophloem elements. The probe has moved laterally from the phloem and apically through the cortex to the meristem. D, Root system of an AtSUC2::GFP plant labeled with CTER. Yellow fluorescence represents overlay between GFP and CTER signals. E, Single root tip from D showing separation of GFP (cytoplasm) and vacuolar (CTER) signals. Bars = 0.1 mm.
properties of phloem-recalcitrant compounds. It remains to be shown if the glucoside-based probes that we examined here are transported by the recently described SWEET transporters (Chen, 2014).

Significantly, all but one of the fluorescent precursors that we tested was unable to enter the phloem. The exception was the red fluorescent probe CTER. This molecule has a high extinction coefficient and quantum yield (Table II) and showed excellent phloem-transport properties, making it a good choice for studying translocation in plant material expressing GFP. CTER has a similar structure to CF, a probe that is also phloem mobile. It has been suggested that CF possesses physicochemical characteristics that permit its retention in the SE by ion trapping (Hsu and Kleier, 1996), but this has not been shown unequivocally (Wright and Oparka, 1994). The ability of HPTS to enter the phloem remains enigmatic. This probe is a trisulphonated compound that is highly charged, and its physicochemical properties predict that it would be membrane impermeant across a wide pH range (Wright and Oparka, 1994). One possibility is that it is recognized by a sulfate transporter localized in the phloem. Sulfate transporters have been described in the Arabidopsis SE-CC complex (Yoshimoto et al., 2003), but it remains to be shown if such transporters recognize HPTS.

Pros and Cons of Phloem-Mobile Fluorescent Probes

For all but one of the probes that we studied, cell-to-cell movement after unloading was restricted by vacuolar sequestration (Tucker et al., 1989). This phenomenon also applies to the movement of herbicides, where enhanced vacuolar sequestration is a major mechanism of herbicide resistance in many plants (Hawkes, 2014). Thus, the final distribution of probes in sink tissues is likely to be a dynamic balance between symplastic movement and vacuolar sequestration. The exception in this case is also HPTS, which after unloaded from the phloem, remains in the symplast for significantly longer periods than any of the other probes that we studied. This property is probably related to its membrane-impermeant nature (Wright and Oparka, 1996). It would seem that the putative transporters that load HPTS into the phloem are lacking on the vacuolar membranes of sink tissues.

Probe Quenching

Most of the probes that we studied were stable after unloading for several hours. The exception was esculin, which was degraded in root cells after its sequestration by the vacuoles. This means that esculin should only be used in short-term studies of phloem unloading (1–2 h), after
which time the fluorescence signal will diminish substantially. The mechanism of esculin degradation is not known but may involve previously described routes for xenobiotic detoxification in plants (Etxeberria et al., 2012). The loss of esculin fluorescence allows the same plant to be pulse labeled a number of times. This approach may be useful when studying the effects of altering source-sink relationships in plants or when studying changes in solute partitioning during development.

MATERIALS AND METHODS

Plant Material

Arabidopsis (Arabidopsis thaliana) seeds were sterilized with 10% (v/v) bleach, rinsed one time in 70% (v/v) ethanol, and then rinsed four times in distilled, deionized water. Unless otherwise stated, seeds were plated in petri dishes on 0.5× Murashige and Skoog medium (without Suc), solidified with 1.2% (w/v) phytoagar, and grown for 10 d in 16-h photoperiods with 200 μE m–2 s–1 at 18°C to 22°C.

Probes

The following probes were used in this study: esculin (CAS: 66778-17-4; Alfa Aesar), fraxin (CAS: 524-30-1; Sigma), skimmia (CAS: 93-39-0; Sigma), esculent (CAS: 305-01-1; Sigma), fraxatin (CAS: 574-84-5; Sigma), umbelliferone (CAS: 93-35-6; Sigma), NBD chloride (CAS: 10199-89-0; Sigma), 2-NBDG (CAS: 186689-07-6; Life Technologies), HPTS acetate (CAS: 115787-83-2; Life Technologies), CFDA (CAS: 305-01-1; Sigma), fraxetin (CAS: 574-84-5; Sigma), umbelliferone (CAS: 93-35-6; Sigma), NBD chloride (CAS: 10199-89-0; Sigma), 2-NBDG (CAS: 186689-07-6; Life Technologies), HPTS acetate (CAS: 115787-83-2; Life Technologies), CFDA (CAS: 124387-19-9; Sigma), and CTER (CAS: 37299-86-8; Acros).

Imaging in Petri Dishes

For screening purposes, plants were imaged in situ inside plastic petri dishes (Opara et al., 1994). Fluorescent probes were applied to cotyledons when the seedlings were 10 d old. Approximately 30 seedlings were labeled with each fluorescent compound depending on the experiment. Screening was conducted through the petri dish lid using a 10× Nikon Long-Working Distance microscope. To obtain higher resolution images, some seedlings were removed from the agar, placed in water under a coverslip, and imaged under a 20× water-dipping lens with a Leica SP2 Confocal Laser-Scanning Microscope operating at variable emission wavelengths (405–561 nm) depending on the probe.

Adjuvant Loading

Previous studies of phloem-mobile probes have required some degree of damage to the leaf surface (Opara et al., 1994) or stem (Gisel et al., 1999; Stadler et al., 2005) to facilitate phloem uptake. This approach introduces the possibility that the probe may enter the xylem stream. To avoid tissue damage and enable uniform labeling of leaves, we applied the fluorescent probes to seedlings grown on agar in a solution of Adigor, an adjuvant used extensively in the agrochemical industry to facilitate the uptake of pesticides by leaves (Hammani et al., 2014). This vegetable oil-based compound facilitates penetration of cuticular leaf surface waxes (Hammani et al., 2014) and allows xenobiotics to enter the phloem without surface wounding. All probes were applied to the leaf at a final concentration of 10 mM except esculin (1.47 mM), HPTS (8.8 mM), and CFDA (13 mM). Probes were applied in a solution of 0.5% (v/v) Adigor and 80% (v/v) acetonitrile.

Esculin Loading and Imaging in Micro-ROCs

Plants were grown in micro-ROCs (Advanced Sciences Tools) in growth chambers for a 16-h photoperiod at 300 to 400 μE m–2 s–1 at 22°C for day and 18°C for night as described in Froelich et al., 2011. Probes were loaded through the cotyledons 7 to 10 d after germination by clipping one side halfway to the midrib and applying 10 μL of esculin (5 mg mL–1 of distilled, deionized water) to encompass the entire cut site. Images were obtained with a Leica TCS SP8 Confocal Microscope. Excitation and emission for esculin were 405 diode/420 to 470. Processing of images was done with Leica LAS AF Lite software.

Pulse-Loading Esulin

Plants were grown and loaded in micro-ROCs as described above. Esulin was applied to the leaf for 15 min and replaced with water for 5 min, constituting one pulse cycle. The pulse cycle was repeated up to three times over a period of 3 h. Time-lapse imaging was done at 20-s intervals.

Expression of AtSUC2 in Xenopus spp. Oocytes and Electrophysiology

Cloning AtSUC2 complementary DNA in the oocyte expression vector pPO2 and preparation of Xenopus laevis oocytes have been previously described (Chandran et al., 2003). Oocytes were injected with 55 ng of complementary DNA and incubated in Barth’s solution at 15°C for 4 d. Two-electrode voltage clamping was used to measure substrate-induced currents. Oocytes were bathed in modified sodium ringer solution (115 mM NaCl, 2 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2, and 5 mM MES, pH 5.6 with Tris). Substrates were applied in the same solution at a concentration of 5 mM or less depending on solubility of the substrate. Oocytes were held at ~40 mV, and substrate-dependent currents (background subtracted) were recorded at membrane potentials of +40 to ~120 mV.

Arabidopsis atsuc2-5 Complementation with HvSUT1

Construction of the vector for plant transformation that included the AtSUC2 (At1g22710) promoter, the barley (Hordeum vulgare) Suc transporter HvSUT1 coding sequence (Weschke et al., 2000; Sivitz et al., 2005), and the 3′ untranslated region of AtSUC2 in the pB7m34/GW binary vector (Karimi et al., 2005) was described previously (Reinders et al., 2012). Agrobacterium tumefaciens strain C58C1 was used to transform Arabidopsis atsuc2-5 heterozygous plants (SALK_087046; Wippel and Sauer, 2012). Basta-resistant transformants were selected on soil, and homozygous atsuc2-5 lines were identified by PCR. Progeny that were homozygous for both the HvSUT1 transgene and the atsuc2-5 allele were used in this study.

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession numbers NM_102118 (AtSUC2) and AM058812 (HvSUT1).

Supplemental Data

The following supplemental materials are available.

Supplemental Figure S1. HvSUT1 rescues the growth defect of the atsuc2-5 mutant.

Supplemental Movie S1. Phloem pulse labelling with esculin in Arabidopsis root.

ACKNOWLEDGMENTS

We thank Dr. Tim Hawkes and Dr. Torquil Fraser (Syngenta) for numerous helpful discussions and supplying Adigor.

Received December 12, 2014; accepted February 1, 2015; published February 4, 2015.

LITERATURE CITED


Phloem-Mobile Probes

---


Copyright © 2015 American Society of Plant Biologists. All rights reserved.


