



Plant movement and LAC of it: How copper facilitates explosive seed dispersal

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Plants enrich our lives in many ways, and we often think of them as sessile organisms, confined permanently to their growing site. However, plants do move at different scales and speeds. Some of the best-known rapid plant movements are opening and closing of the stomata on the leaf surface and the quick closure of Venus flytrap (*Dionaea muscipula*) leaves and leaflets of mimosa (*Mimosa pudica*) in response to mechanical stimulation; in contrast, a phototropic solar tracking in sunflower or reorientation of tree trunks in response to gravity are examples of slower plant motions (1). The ability of plants to disperse seeds away from the parent plant is another way plants move and colonize new habitats. It turns out that copper is involved in this ability to “move” in *Cardamine hirsuta*, also known as popping cress, an invasive weed species from the Brassicaceae family (2).

Copper is a micronutrient required for nearly every aspect of the growth and development of all organisms, including plants. In addition to respiration and scavenging of oxidative stress, copper is needed for photosynthesis and reproduction (3, 4). The essential nature of copper is attributed to its ability to cycle between reduced and oxidized states. Thus, copper is known for its catalytic cofactor role in essential cellular enzymes that harness copper redox potential (4). The redox properties of copper that make it nutritious also cause toxicity if copper ions accumulate in cells in excess. The dual nature of copper, its essentiality and toxicity, prompted organisms to tightly regulate cellular copper uptake and utilization strategies. In a model plant, *Arabidopsis thaliana*, a conserved transcription factor, SPL7 (SQUAMOSA PROMOTER-BINDING PROTEIN-LIKE 7), and CITF1 (Copper Deficiency-induced Transcription Factor 1) regulate copper absorption by plant roots and internal transport for the delivery to essential copper enzymes (5). Among the nearly 260 copper-dependent proteins that have been identified in *A. thaliana* are LAC genes-coded multicopper oxidases, known as laccases (3, 6). Plant laccases use copper redox properties for the oxidative polymerization of monolignols to form lignin, a naturally occurring woody polymer deposited in specific cell types of plants (6, 7). For example, guided lignin synthesis in specific cell types is associated with the secondary thickening of plant cell walls and their stiffness, and so lignin plays critical roles in mechanical support, water transport, and pathogen defense (8). Copper is also needed for lignin accumulation in anthers, the flower structures where pollen develops. Thus, copper deficiency leads to decreased lignification and a failure of anthers to split open (dehisce) and disperse pollen to fertilize the egg in the flower structure called the pistil (3).

Past studies by the Hay group have shown that the asymmetric lignin deposition in a cell wall of a single cell layer, called the endocarpaceal (endb), of the fruit (a seed pod)

of *C. hirsuta* is responsible for explosive seed dispersal (9). Explosive seed dispersal is one of the ways *C. hirsuta* moves and expands its habitats, providing an advantage over its relative *A. thaliana* that uses a nonexplosive seed dispersal mechanism (10). Interestingly, both *C. hirsuta* and *A. thaliana* disperse seeds via a process known as pod shatter, and the fruits of both species look very similar: they are composed of two valves that enclose the seeds, attached to the replum (10). The fruit dehisces along a thin line of specialized tissues at the valve margins to allow pod shatter. In both species this process depends on the lignification of the discrete regions of the fruit (10). So, why is it that despite a similar pod structure and seed dispersal process, the pod of *C. hirsuta*, unlike *A. thaliana*, explodes, firing seeds far away from the parent plant? Well, there are differences, among which the delivery of copper to specific cell types in the seed pod and a localized synthesis of lignin play a key role.

The Hay group has noted that while in *A. thaliana* the pod shatter occurs as the fruit dries out, in *C. hirsuta* the explosive pod shatter occurs while the fruit still contains water (is turgid), suggesting that the *C. hirsuta* fruit uses a previously undescribed mechanism to generate tension actively (9). Through an elegant combination of genetic and biophysical studies, quantitative imaging, and multi-scale mathematical modeling, the Hay group showed several years ago that this unusual mechanism relies on a combination of three-dimensional cellular geometry and the asymmetric, the U-shaped localization of lignin in the cell wall of the endb of the valve (Fig. 1 and ref. 9). In contrast, the endb cells are uniformly lignified in *A. thaliana* (11). The polarized lignification pattern and the associated polarized stiffness in the endb layer of *C. hirsuta* lead to the differential contraction and expansion in response to hydrostatic pressure and result in tissue tension. The stored elastic potential energy from the tissue tension is released explosively via the coiling of the fruit valves. The generated kinetic power is used to fire seeds away like a firework, reaching as much as a 2-m radius around the parent plant (9, 12).

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Author contributions: O.K.V. wrote the paper.

The author declares no competing interest.

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See companion article, “Explosive seed dispersal depends on SPL7 to ensure sufficient copper for localized lignin deposition via laccases,” [10.1073/pnas.2202287119](https://doi.org/10.1073/pnas.2202287119).

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Published June 29, 2022.

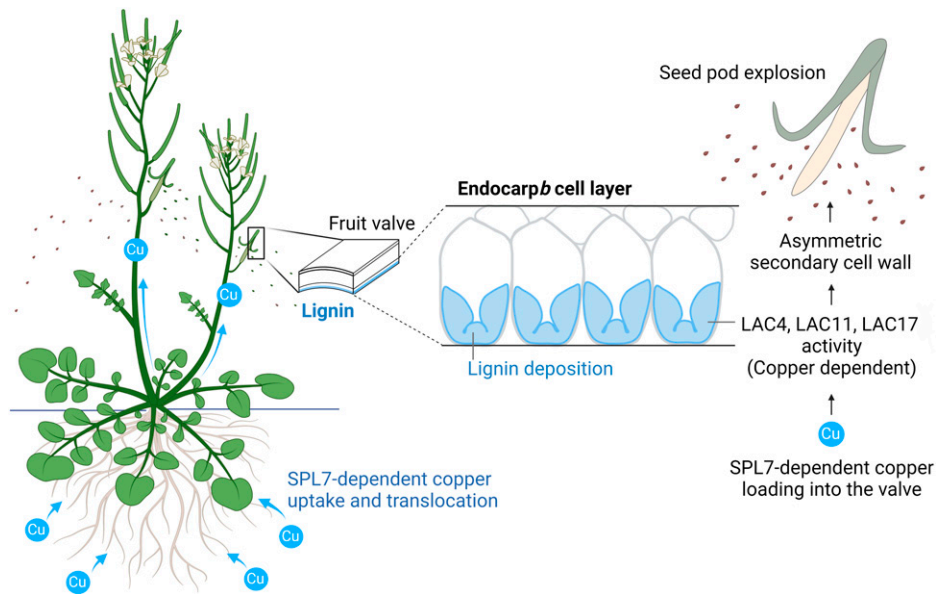


Fig. 1. SPL7-mediated copper uptake and delivery to valves are important for laccase-mediated asymmetric lignification of the endocarp cell (endb) wall and the explosive seed dispersal in *C. hirsuta*. A transcription factor, SPL7, controls the absorption of copper by plant roots and delivery to fruits. LAC4, LAC11, and LAC17 obey U-shaped localization in endb cells and use copper to produce lignin. The resulting U-shaped lignin deposition in endb cells is essential for the explosive seed dispersal. Figure created in BioRender.com.

In the follow-up work published in PNAS, the Hay group provide a genetic basis for this phenomenon that brings about the role of copper and laccases in this process (2). In a genetic screen for mutants with less-lignified fruit valves, the authors identified *less lignin 1* (*lig1*) that contained less lignin in endb cell walls compared with other lignified cell types in the fruit. As a result, *lig1* fruit buckled along its edge compared with the straight edge of wild type, and the seed dispersal range of *lig1* was significantly reduced compared with wild type. It turned out that *lig1* has a mutation in the *C. hirsuta* analog of SPL7. Introducing *lig1* mutation to SPL7 using CRISPR-Cas9 resulted in *lig1* phenotype: a reduced lignin accumulation in fruit valves and less-lignified endb cells. These defects were associated with the decreased copper accumulation in fruits of the *spl7* (alias *lig1*) mutant compared with wild type and were rescued by supplemental copper. Thus, the localized lignin deposition in endb cell walls is contingent on SPL7-mediated copper delivery to fruits of *C. hirsuta*. In this view, the SPL7 function is conserved in *Brassicaceae*.

A question remained: which genes that require SPL7-dependent function in copper transport processes mediate endb lignification? The synthesis of monolignols, the lignin building blocks, was not affected in the *spl7* mutant, and so the authors turned to monolignol-polymerizing and copper-requiring enzymes, laccases. The authors focused on LAC4, LAC11, and LAC17, as these laccases, similar to SPL7, were expressed in endb cells. Interestingly, LAC4, LAC11, and LAC17 protein accumulation patterns resembled precisely the U-shape lignin deposition in endb cell walls of *C. hirsuta*. In contrast, U-shape localization was not maintained in endb cells of *A. thaliana* expressing *C. hirsuta* LAC4, LAC11, and LAC17. Elegant analysis of LAC4, LAC11, and LAC17 activity in wild type and the *spl7* mutant showed that copper delivery to the *C. hirsuta* endb cells via SPL7 function is a prerequisite for the localized lignin

deposition (Fig. 1). Based on studies in *A. thaliana*, SPL7 regulates the expression (abundance) of many genes, including those that code for proteins that facilitate the movement of solutes, including copper (13). The SPL7-dependent copper transport pathways responsible for copper delivery to fruits of *A. thaliana* and *C. hirsuta* merit further investigation.

A fascinating and distinguishing feature of *C. hirsuta* that underlies its explosive seed dispersal is the asymmetry of LAC4, LAC11, and LAC17 localization in endb cells. How and why did this evolutionary novelty evolve in *C. hirsuta*? The asymmetry in *C. hirsuta* LAC4, LAC11, and LAC17 protein localization is not maintained when these laccases are transferred to *A. thaliana*, and native *A. thaliana* LAC4, LAC11, and LAC17 are localized uniformly in its endb cells. Interestingly, the same laccases of *A. thaliana* localize to discrete locations in the cell wall of water-conducting vascular tissue, the xylem; this discrete location is associated with a distinct lignification pattern of the xylem and is important for the long-distance water and dissolved solute transport in plants (14). It is noteworthy that laccases are glycoproteins that are synthesized inside cells and are secreted to precise locations in the plasma membrane domains below the developing secondary cell wall (14, 15). Molecular determinants that are responsible for the directed delivery of laccases in one tissue but not another and in the same cell type of one species but not another are unknown and await further investigation. All in all, this work integrates a mineral nutrient, copper, with polarized lignin synthesis and explosive seed dispersal mechanism, allowing plants to effectively conquer new habitats.

ACKNOWLEDGMENTS. O.K.V.'s research is supported by the NSF (NSF-IOS-1656321) award and Agriculture and Food Research Initiative Awards 2018-67013-27418 and 2021-67013-33798 from the US Department of Agriculture National Institute of Food and Agriculture. I thank laboratory members for their comments and Dr. Chia for preparing Fig. 1.

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