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μ -SXRF reveals the role of Arabidopsis Oligopeptide Transporter 3 (AtOPT3) in shoot-to-root copper signaling and copper-iron crosstalk

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Micronutrients copper (Cu) and iron (Fe) are essential for plant growth but can be toxic when over-accumulated in cells. Thus, plants tightly regulate their root uptake systems to prevent deficiency while avoiding toxicity. This includes balancing Cu/Fe accumulation via the Cu-Fe crosstalk and systemic shoot-to-root signaling through the phloem to report the shoots' demand. However, only systemic Fe deficiency response has been documented. It involves *Arabidopsis thaliana* oligopeptide transporter 3 (AtOPT3) that transports Fe into the phloem companion cells to reflect the real-time Fe status of the shoots to the roots. Here, we show that AtOPT3 also plays an important role in delivering Cu into the phloem and is essential for the systemic signaling of Cu deficiency. Loss of OPT3 function restricted the Cu movement from the xylem to the phloem, and the images of confocal SXRF confirmed the altered distribution of Cu in the vascular tissues of the *opt3* mutant. Also, the 2D-SXRF analysis on different tissues shows that Cu remobilization via phloem is impaired in the *opt3* mutant. The mutant retained Cu in the mature leaves and failed to deliver Cu into the fast-growing tissues, e.g., developing seeds. We further found that the low abundance of Cu in the phloem of the *opt3* mutant triggered the shoot-to-root signals of Cu deficiency and caused the constitutive upregulation of the Cu uptake in the roots. Surprisingly, feeding the *opt3* mutant with Cu or Fe via the phloem in leaves suppressed both Cu- and Fe-deficiency responses in roots. These data suggest the existence of shoot-to-root Cu signaling, highlight the complexity of Cu/Fe interactions, and the role of AtOPT3 in fine-tuning root transcriptional responses to the plant Cu and Fe needs.

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