How a Mangrove Tree Can Help to Improve the Salt Tolerance of Arabidopsis and Rice

Mangrove trees live and thrive in intertidal zones, where they are regularly inundated with salt water. To survive such harsh environmental conditions, they have evolved several features to improve their salt tolerance. *Avicennia officinalis*, which has increasingly been used in the past couple of years to study salinity tolerance, is a salt-secretor mangrove tree with specialized salt glands in aboveground tissues to secrete excess salt. *A. officinalis* also has evolved enhanced hydrophobic barrier depositions in the endodermis of their roots. These barriers, namely the Casparian strips and suberin lamellae, can reduce salt uptake from the environment into the vasculature by up to 95% (Tan et al., 2013; Krishnamurthy et al., 2017).

In a previous study, Krishnamurthy et al. (2017) identified several members of the *Cytochrome P450* (CYP) gene family that are up-regulated in the root transcriptome of *A. officinalis* in response to salt treatments. Out of those, *AoCYP94B1* is among the family members that showed the strongest up-regulation (Krishnamurthy et al., 2017). CYP94 subfamily members are fatty acid ω-hydroxylases, and *AtCYP94B1* has been implicated in stress-induced jasmonic acid biosynthesis (Koo et al., 2014; Bruckhoff et al., 2016). However, ω-hydroxylation is also required for suberin biosynthesis, leading Krishnamurthy et al. (2017) to investigate a possible role for *AoCYP94B1* in suberin deposition to enhance barrier formation in the root.

In this issue of *Plant Physiology*, Krishnamurthy et al. (2020) functionally characterize CYP94B1 from *A. officinalis*, as well as its ortholog from Arabidopsis (*Arabidopsis thaliana*), and describe its conserved role in root suberin deposition for enhanced salt tolerance.

Having identified *AoCYP94B1* in a transcriptomics screen, Krishnamurthy et al. (2020) analyzed the expression pattern of *AoCYP94B1* and *AtCYP94B1*. Both orthologs are expressed in all tissues tested, with higher expression in the aboveground organs. Upon salt treatment, however, expression is strongly and rapidly induced in the root. As no transformation protocols are available for *A. officinalis*, the authors checked the expression and localization of the GFP-tagged Arabidopsis ortholog and found that *AtCYP94B1-GFP* is expressed in the vasculature and endodermis of Arabidopsis roots. In response to salt treatment, the protein further accumulates at the plasma membrane of the endodermis, the location of apoplastic barrier formation.

The authors analyzed Arabidopsis *cyp94b1* mutants for their salt tolerance and found that these mutants are indeed more sensitive to salt, while overexpression of either the *AoCYP94B1* or *AtCYP94B1* ortholog not only restores but increases their tolerance (Fig. 1A). Similarly, overexpression of *AoCYP94B1* in wild-type rice (*Oryza sativa*) also was sufficient to enhance the salt tolerance of this crop plant (Fig. 1B). Such overexpressing Arabidopsis lines accumulated less Na⁺ ions in leaves compared with the mutant, while root Na⁺ was unchanged, further indicating a role for *AoCYP94B1* in the formation of an apoplastic barrier that limits salt uptake into the root vasculature for subsequent transport to the aboveground organs. To confirm these observations, the authors screened for suberin monomers using mass spectrometry and found that the *atcyp94b1* mutant had decreased amounts of root suberin monomers. Accordingly, staining with Nile Red showed decreased suberin in the endodermis of Arabidopsis mutant roots and enhanced uptake of fluorescein diacetate into the endodermis and pericycle. In rice, while mutants were not available, overexpression of *AoCYP94B1* also resulted in stronger Nile Red staining of the endodermis, indicating enhanced suberin deposition.

To gain insight into the transcriptional regulation of *AtCYP94B1*, the authors then screened the putative regulatory sequences of *AtCYP94B1* for known transcription factor-binding sites. Among the motifs identified, they found several potential WRKY-binding sites, and the authors’ previous transcriptomic data showed an up-regulation of several WRKYS after salt stress, most notably WRKY33 (Krishnamurthy et al., 2017). WRKY33 has previously been implicated as a key regulator of biotic and abiotic stresses in Arabidopsis, and its ortholog in the halophyte *Eutrema salsugineum* has been found to be important for the plant’s enhanced salt tolerance (Jiang and Deyholos, 2009; Birkenbihl et al., 2012; Mucha et al., 2015). Accordingly, the authors focused on WRKY33 as a potential transcriptional regulator of *AtCYP94B1*. Indeed, they found that *atwrky33* mutants show reduced suberin deposition in their roots, are more sensitive to salt stress, and express *AtCYP94B1* at lower levels than the wild type. Overexpression of *AtCYP94B1* in *wrky33* mutants restored these phenotypes, indicating that the role of CYP94B1 in enhancing salt tolerance by increasing suberin deposition is indeed dependent on positive transcriptional regulation by WRKY33. Using chromatin immunoprecipitation-quantitative PCR and yeast one-hybrid assays, they then also confirmed that
the AtCYP94B1 regulatory sequences are bound by AtWRKY33.

The work described in this article presents CYP94B1 as a central regulator of salt tolerance in a WRKY33-dependent pathway that furthermore seems to be conserved among several distantly related plant species, from a mangrove tree to Arabidopsis to rice (Fig. 1C). Previous work has implicated WRKY33 as a key transcriptional regulator not just for salt tolerance but for a whole range of biotic and abiotic stresses, most notably resistance toward the fungal pathogen *Botrytis cinerea* (Jiang and Deyholos, 2009; Birkenbihl et al., 2012). In this regard, WRKY33 has been shown to act by regulating phytohormones such as jasmonic acid and salicylic acid. Since CYP94B1 has also been implicated to function in stress-induced jasmonic acid biosynthesis, and suberin deposition also protects from pathogen invasion, it could be interesting to further investigate how WRKY33 and CYP94B1 integrate different pathways in response to a variety of stresses.

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Figure 1. AoCYP94B1 overexpression increases salt tolerance in Arabidopsis and rice. A, Arabidopsis plants after treatment with 100 nM salt. Left, The wild type (WT); middle, atcyp94b1 mutants; right, atcyp94b1 mutants overexpressing AoCYP94B1. Bar = 10 mm. B, Rice plants after treatment with 100 nM salt. Left, The wild type; right, the wild type overexpressing AoCYP94B1. C, Working model. Under salt stress, AtWRKY33 positively regulates AtCYP94B1 expression, which induces the formation of hydrophobic barriers to confer enhanced salt tolerance. Adapted from Krishnamurthy et al. (2020), figures 3, 4, and 8.
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