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Novel Roles for Phospholipase C in Plant Stress Signalling and Development
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Summary

For many years, efforts have been made to explore PLC signaling in plants. Compared to the classical eukaryotic, mammalian PLC signaling pathway, a different picture is emerging for plants. Several roles for PLC in plant development and stress responses have been claimed but genetic evidence for this is mostly missing.

In **chapter 2**, we functionally characterized the role of *PLC3* and found it mainly expressed in vascular tissue, but was also present in guard cells and trichomes. Loss-of-function mutants showed different phenotypes, including delayed seed germination, slightly less-elongated primary roots (~5%), fewer lateral roots (15%), and decreased sensitivity to ABA in terms seed germination inhibiting and stomatal-closure induction. ABA triggered an increase in PIP_2 in various Arabidopsis tissues, however, no difference in PLC substrate- or product levels in two independent *plc3* KD mutants were found. Overexpression of *PLC3* increased the drought tolerance of plants and decreased their stomatal aperture.

In **chapter 3**, similar analyses were processed for *PLC5*, which was also predominantly expressed in the vasculature, but also in the root apical meristem, guard cells and trichomes. Knock-down mutant, *plc5-1* displayed shorter primary roots (~10%) and exhibited fewer lateral roots (~20%), which could be restored by the expression of the endogenous *PLC5*-wt gene, driven by its own promoter. Double-*plc3plc5* mutant did not intensify the root phenotype, indicating the involvement of (a)other *PLC*(s). Overexpression of *PLC5* enhanced drought tolerance and reduced stomatal aperture, like PLC3, but in this case also led to a new phenotype, i.e. a stunted root-hair growth. ^{32}P-phospholipid labeling analyses revealed no differences between wt and *plc5-1* seedlings, however, decreased levels of PIP and PIP were found with increased levels of PA in *PLC5* over-expressor lines. Inducible overexpression of *PIP5K3* in *PLC5-OE* line rescued the root hair phenotype and restored the level of PIP_2, providing independent evidence for PIP_2's crucial role in tip growth.

In chapter4, we characterized the role of *PLC7*, another phloem-expressed *PLC*. Expression was throughout the plant vasculature, including roots, leaves and flowers, and also appeared in trichomes and hydatodes. We obtained a *plc7-3* KO- and *plc7-4* KD mutant but found no affects in root development, which is different from the *plc3*- and *plc5* mutants. However, like *plc3* mutants, *plc7* mutants exhibited a reduced sensitivity to ABA-dependent stomatal closure. Double-knockout mutants of *plc3plc7* were lethal, whereas *plc5plc7* (*plc5/7*) mutants were viable, and revelead several new phenotypes not observed earlier. These include a defect in seed coat mucilage, enhanced leaf serration, and an increased tolerance to drought. The latter phenotype was previously found when *PLCs* were overexpressed. Overexpression of *PLC7* also led to an enhancd drought tolerant phenotype. *In vivo* $^{32}P_i$-labeling of seedlings

treated with sorbitol to mimick drought stress revealed increased PIP_2 responses in both drought tolerant *plc5/7* and *PLC7-OE* mutants. Together, these results reveal several novel functions for PLC in plant stress and development.