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Phospholipases are involved in hypoxia-induced intracellular calcium and ROS signaling in *Arabidopsis thaliana*

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In order to investigate the involvement of the different isoforms of phospholipase D, pld (α1, α2, α3, β1, β2, γ1, γ2, γ3, δ, ε, ζ1 and ζ2), in hypoxia signaling, all the isoforms lacking the respective phospholipases were used. The hypoxia-induced changes in cytoplasmic free Ca^{2+} concentration, $[Ca^{2+}]_{cyt}$ and reactive oxygen species (ROS) were detected by epi-fluorescence and confocal microscopy in mesophyll protoplasts of Arabidopsis thaliana, (Col-0), labeled with calcium-binding benzofuran, Fura-2, AM and a ROS sensitive dye, chloromethyl dichlorodihydrofluorescein diacetate acetyl, CM-H_2DCFDA, respectively. Hypoxia treatment of the protoplast medium induced an immediate increase in ROS and a gradual elevation of $[Ca^{2+}]$ cyt for more than half an hour in the wild type. The increase in hypoxia-induced $[Ca^{2+}]_{cyt}$ was reduced or almost abolished in all isoforms except for pld ε and ζ1. Different inhibitors of calcium channels and of PLD activity were used to study if PLD was involved in the reactions and if the $[Ca^{2+}]_{cyt}$ elevations were from both external and internal stores. Also the hypoxia-induced ROS elevation was much lower in mutants except for and pld γ3 and pld ζ2. By an enzymatic method we could confirm that phosphatidic acid, PA, a byproduct of phospholipases was diminished in all mutants except for γ3, ζ1 and ζ2. Thus, this study demonstrates the importance of PLD in hypoxia-mediated calcium and ROS elevations in *Arabidopsis*.

Biography

Sylvia Lindberg is a Professor Emeritus at EMB, Stockholm University (SU), where she has received his PhD in 1981 at Department of Botany. She was the Professor and Associate Head of Department at Plant Biology, SLU, Ultuna, Sweden, during 1982-2006. She has completed her Post-doctorate at Scoula Superiore in Pisa, Italy. She has published more than 50 peer-reviewed papers and was a Reviewer of applications to European Science Foundation, German and Italian Science Foundations. She has been Associate Editor of *Journal of Plant Physiology* and Guest Editor of *Plants*.

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