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Analysis of *Arabidopsis* SYT1 mutants and chimeras reveal insights about its dynamic and function on ER-PM MCS

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The endoplasmic reticulum (ER) extends throughout the cell and forms Membrane Contact Sites (MCS) with other organelles. MCS are essential for lipid transport, calcium signaling and organelle communication. In plants, ER-Plasma Membrane (ER-PM) MCS contain some proteins which, while being anchored to the ER, can attach with the PM through electrostatic interactions. *Arabidopsis thaliana* SYT1 is one of the most studied ER-PM tethers in plants, as it contributes to both biotic and abiotic stress tolerance.

To study SYT1 dynamics and function, our group has generated *Arabidopsis* stable lines expressing mutant versions of SYT1, to remove SYT1 Ca^{2+} binding site ($SYT1^{MutC2}$), and to cause a blockage of the SMP hydrophobic channel ($SYT1^{MutSMP}$). Additionally, we generated chimeric versions of SYT1, switching the SMP domain by its analogue of human E-Syt1 and TMEM24 proteins ($SYT1^{ESYT1}$ and $SYT1^{TMEM24}$ respectively).

Our results demonstrate that both $SYT1^{MutC2}$ and $SYT1^{MutSMP}$ lines complement the cold and salt stress phenotypes of the *syt1* mutant. On the other side, $SYT1^{ESYT1}$ and SYT^{TMEM24} chimeras do not complement such phenotypes, which confirms the essential role of SYT1 SMP domain in cold and salt stress tolerance, as well as the functional differences of SYT1 SMP domain compared to E-Syt1 and TMEM24 SMP domains. Moreover, confocal analysis of $SYT1^{MutC2}$ line evidenced an altered dynamic of SYT1 in terms of cortical ER relocalization upon prolonged salt stress treatment. Altogether, these findings indicate that SYT1 Ca^{2+} binding site is not essential for SYT1 salt stress tolerance, but influences SYT1 dynamics on salt-induced ER-PM MCS expansion.