Synaptotagmin 1 regulates essential membrane fusion events for abiotic stress tolerance in plants

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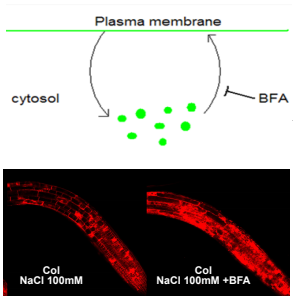
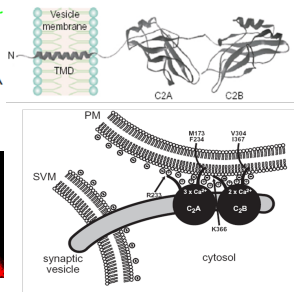
Plants undergo continuous exposure to various abiotic stresses in their natural environment. For this reason they have evolved intricate mechanisms to perceive external signals and trigger the physiological changes necessary to adapt and survive under such conditions. The plasma membrane is a physical barrier that separates the intracellular and extracellular environments and its integrity is essential for stress tolerance. Here we present a role for plant synaptotagmins in plasma membrane integrity maintenance through Vesicle-PM fusions. We also evaluate the physiological consequences of the SYT depletion under several abiotic stress conditions.

Vesicle – PM fusions are required for stress tolerance

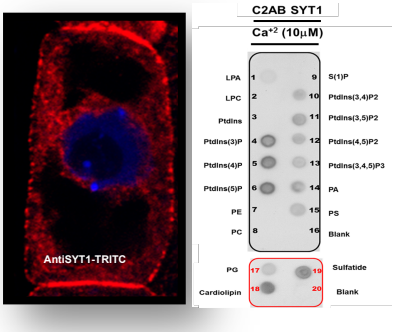




BFA as a marker of Vesicle-PM fusions. BFA is a vesicle fusion inhibitor **(A)** that causes the lost of PM integrity after stress as observed by FM4-64 labelling **(B)**.

Animal SYTs fuse membranes in a Ca^{2+} dependent manner. Structural domains of a canonical SYT (C). Vesicle-PM fusion mediated by SYTs requires phospholipid binding and Ca^{2+}.

Plant SYTs are PM localized and binds membrane lipids

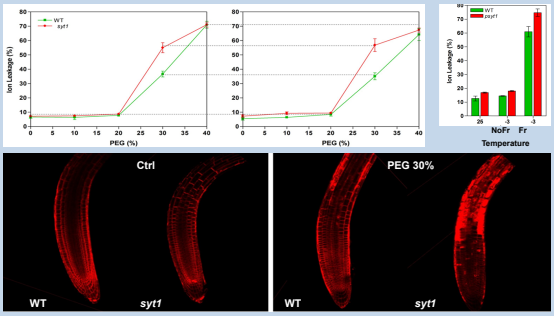


Arabidopsis SYT1 is PM localized. Immunolocalization of SYT1 in root meristematic cells using specific Anti-SYT1 antibodies. SYT1 signal is characterized by a punctated pattern in the PM **(A)**.

SYT1 shows high affinity for negatively charged phospholipids. Protein-Lipid overlay assays were performed with purified SYT1 soluble C2 domains and developed with specific Anti-SYT1 antibodies **(B)**

SYT MUTATIONS MEMBRANE FUSIONS WITH WHOLE PLANT HYPERSENSITIVITY TO ABIOTIC STRESSES

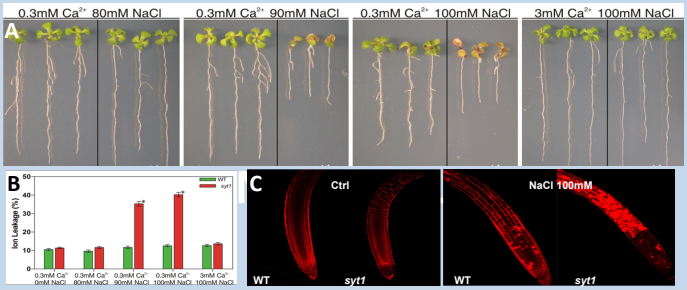
Hydric Stress



***syt1* hydric hypersensitivity correlates with defects in vesicle-PM fusions.**

(Top) Hydric sensitivity in WT and *syt1* backgrounds was assessed by measuring ion leakage after PEG and freezing treatments. **(Bottom)** After 30% PEG treatment *syt1* displays enhanced PM defects resembling those of BFA.

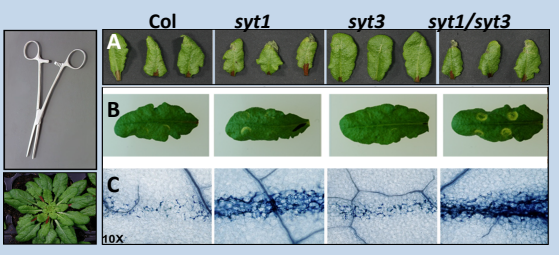
Salt Stress

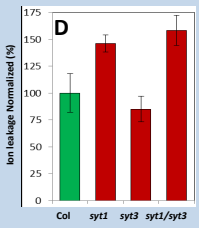


***syt1* hypersensitivity to salt stress is Ca^{2+} dependent**

Low Ca^{2+} concentration (0.3mM) enhances the *syt1* growth hypersensitive phenotype **(A)** and cellular damage **(B)** of NaCl treated seedlings. After 100mM NaCl treatment *syt1* displays enhanced PM defects resembling those of BFA **(C)**.

Mechanical Stress

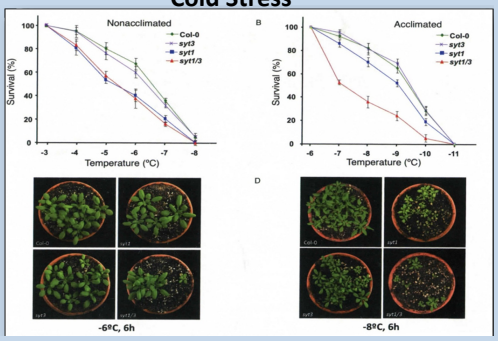




The PM defects in *syt1* and *syt1/syt3* translate into mechanical hypersensitivity at the tissular level

Leaves from adult plants from the different genotypes were mechanically damaged at constant pressure using an hemostat **(A)** or a syringe **(B).** The extend of the damage was assesed using trypan Blue staining **(C)** and ion leakage **(D).**

Cold Stress



Different SYTs have specific roles in cold stress tolerance . While SYT1 is constitutively required for freezing stress tolerance, SYT3 is only required for acclimation processes in the *syt1* background. Nonacclimated plants were directly subjected to the indicated freezing temperatures. Acclimated plants were exposed for 1 week to 4 ºC prior to the freezing stress

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