

Undercovering the molecular mechanisms of lipid signalling at ER-PM contact sites in tomato (*Solanum lycopersicum*) under abiotic stress conditions.

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Highlights

- Abiotic stress is the primary cause of crop loss worldwide, reducing average yields for most major crop plants by more than 50%. This impact is predicted to increase over time in the face of global environmental changes.
- Understanding how plants sense and respond to these environmental changes is imperative in order to generate resistant crops.
- The cultivated tomato (*Solanum lycopersicum*) is a major crop plant and a model system for fruit development.
- In this project, we are using our previous knowledge in lipid signaling pathways from *Arabidopsis thaliana* to enhance tomato tolerance to abiotic stresses.
- We are focusing on the signaling pathway of phospholipase C (PLC) and diacylglycerol kinase (DGK), with the novel involvement of SYT, a contact site tether capable of transporting lipids (Figure 1).

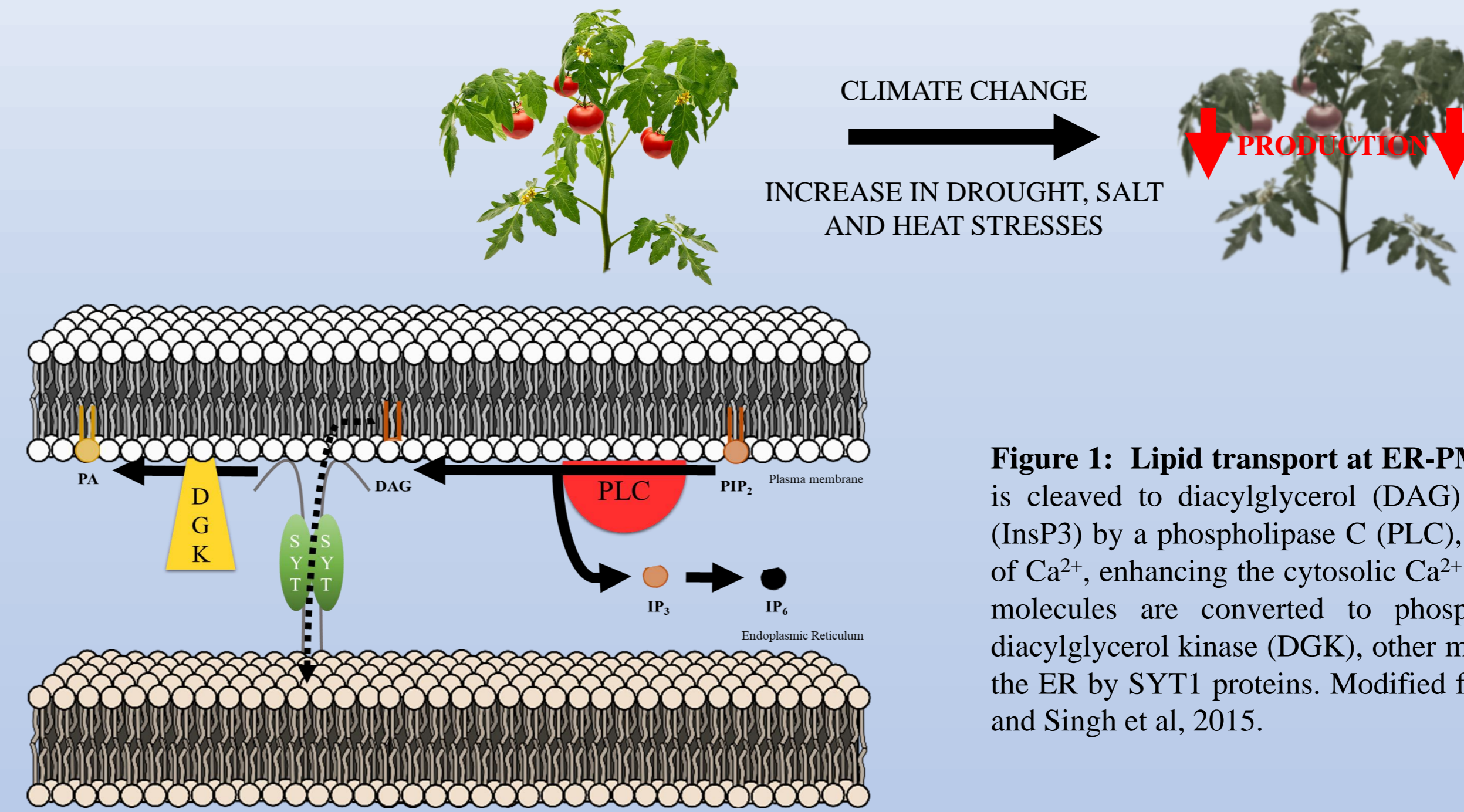


Figure 1: Lipid transport at ER-PM CS. PtdInsP or PtdInsP2 is cleaved to diacylglycerol (DAG) and inositol triphosphate (InsP3) by a phospholipase C (PLC), which leads to the release of Ca²⁺, enhancing the cytosolic Ca²⁺ concentration. Some DAG molecules are converted to phosphatidic acid (PA) by a diacylglycerol kinase (DGK), other molecules are transported to the ER by SYT1 proteins. Modified from Wikimedia Commons and Singh et al, 2015.

Synaptotagmin 1 (SYT1) is involved in stress tolerance

SYT1 is a glycerolipid binding protein and it belongs to the SYT family, which has other four members. This protein is a plant ortholog of the mammal extended synaptotagmin (E-Syts) and yeast tricalbin families. The functional characterization and subcellular localization of SYT1 show a role of plant ER-PM contact site components in the cellular adaptation to environmental stresses such as cold or salinity (Figure 2).

Determination of the lipid transfer properties of the SMP domains by *in vitro* assays.

The defining feature of SYT proteins is the presence of the SMP domain. SMP domains are typically found in intracellular proteins that act in membrane contact sites. Structural and biochemical studies of the SMP domain of E-SYT2 revealed that it forms a 90--long cylinder with a deep hydrophobic groove which contains glycerophospholipids (Figure 3, 4). Our previous studies have demonstrated that AtSYT1 is a protein that transfers saturated DAG molecules *in vivo*. The aim of this task is to identify the lipid molecules transported by the SMP domains of the four selected *Solanum lycopersicum* SYT proteins and their PM anchoring mechanism. The generated knowledge will elucidate the specificity of SMP domains, the chain length or the saturation/unsaturation degree of the fatty acyl chains of the ligands. These details remain elusive and they will provide useful information to decipher the role of each SYT protein in tomato.

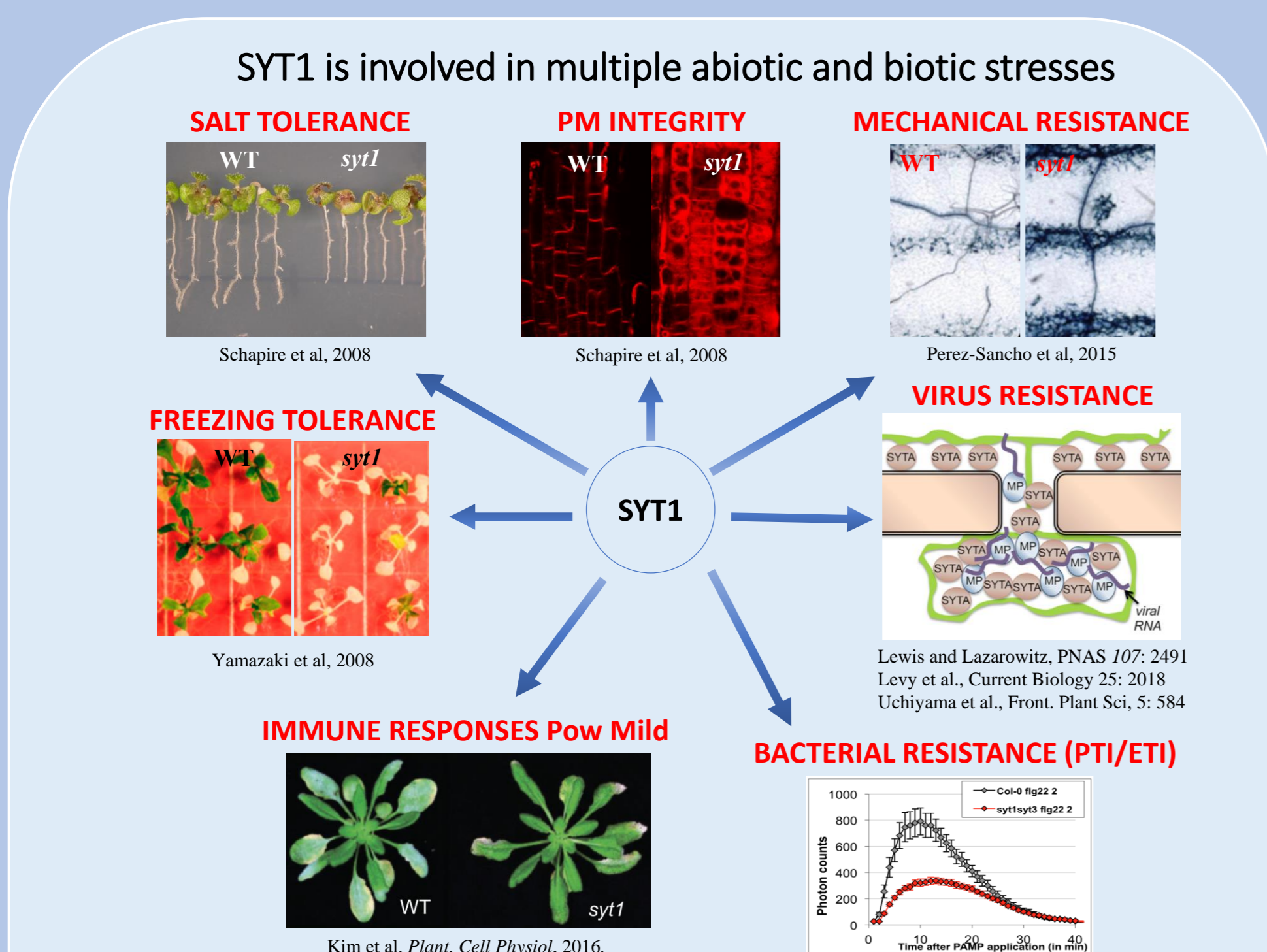


Figure 2: Summary of the roles of AtSYT1 in biotic and abiotic stresses. The *sytl* loss-of-function mutant shows increased sensitivity to several abiotic stresses such as salt, freezing and mechanical damage; and it shows differential resistance to fungal and viral infections

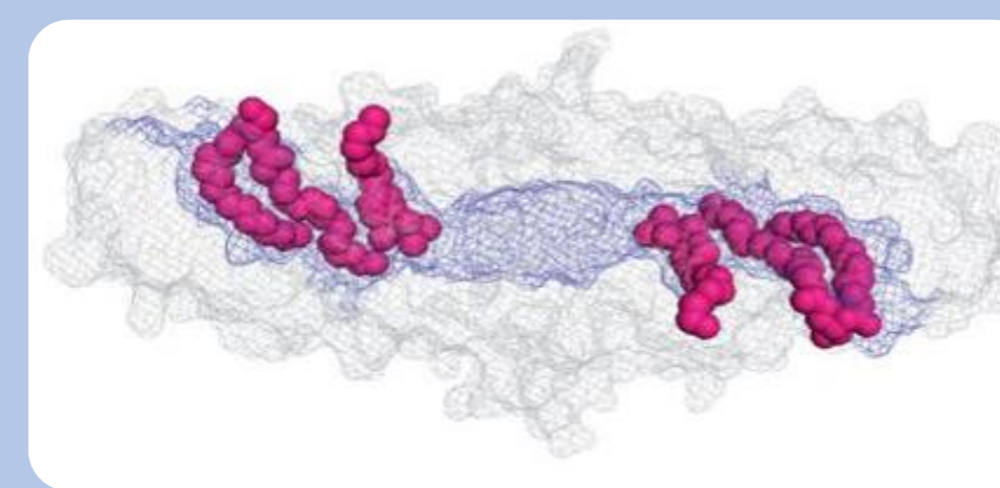


Figure 3: Surface representation of the SMP dimer. Hydrophobic residues (blue) line the channel. Lipid fatty acid moieties are in space-filling representation. De Camilli & Reinisch, 2014.



Figure 4: Crystal structure of the SMP domains of a E-Syt2 dimer with bound hydrophobic molecules. Schauder et al, 2014.

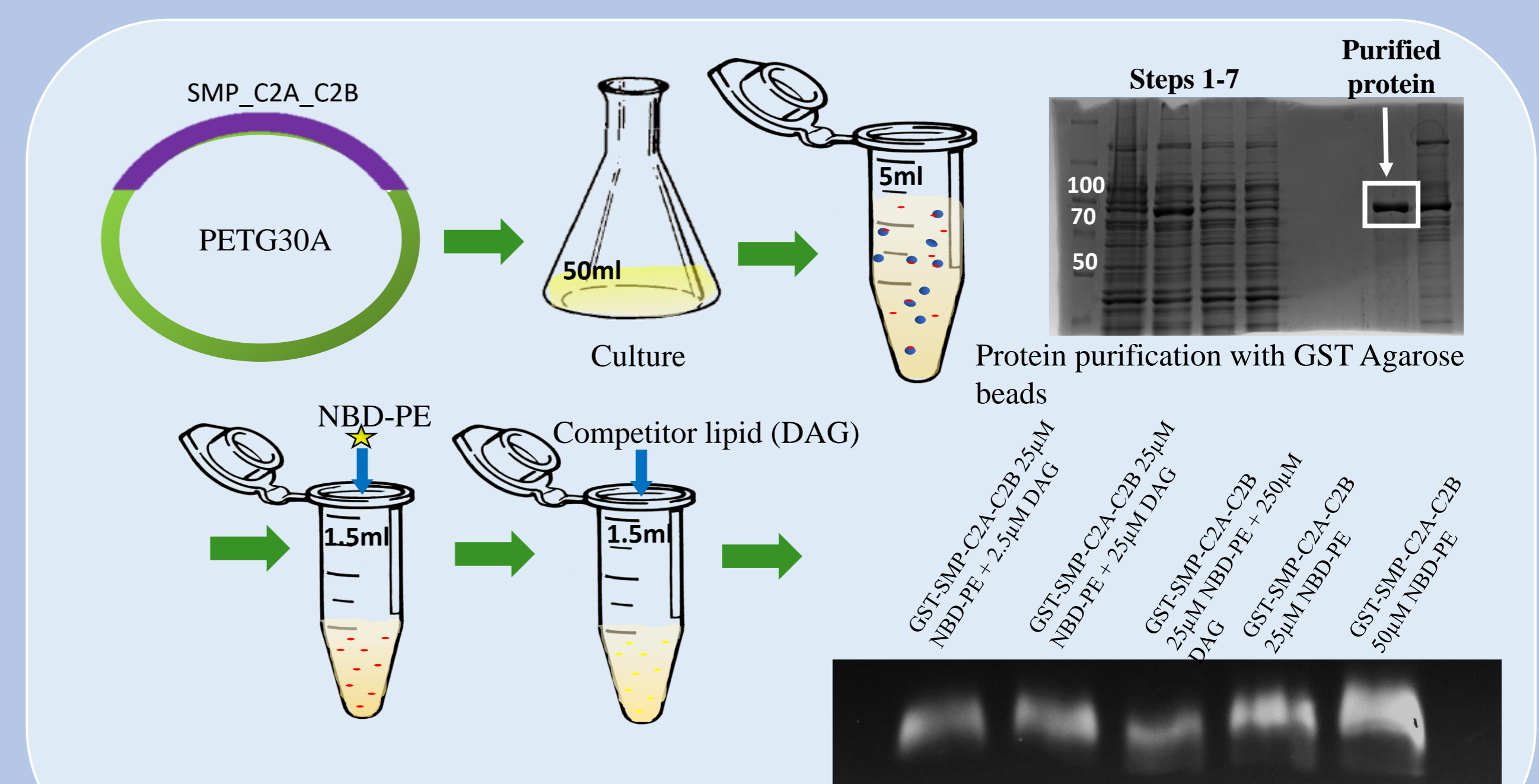


Figure 5: Lipid displacement assay in SYT proteins.

Homologs of *Arabidopsis thaliana* PLCs, DGKs and SYTs in *Solanum lycopersicum*

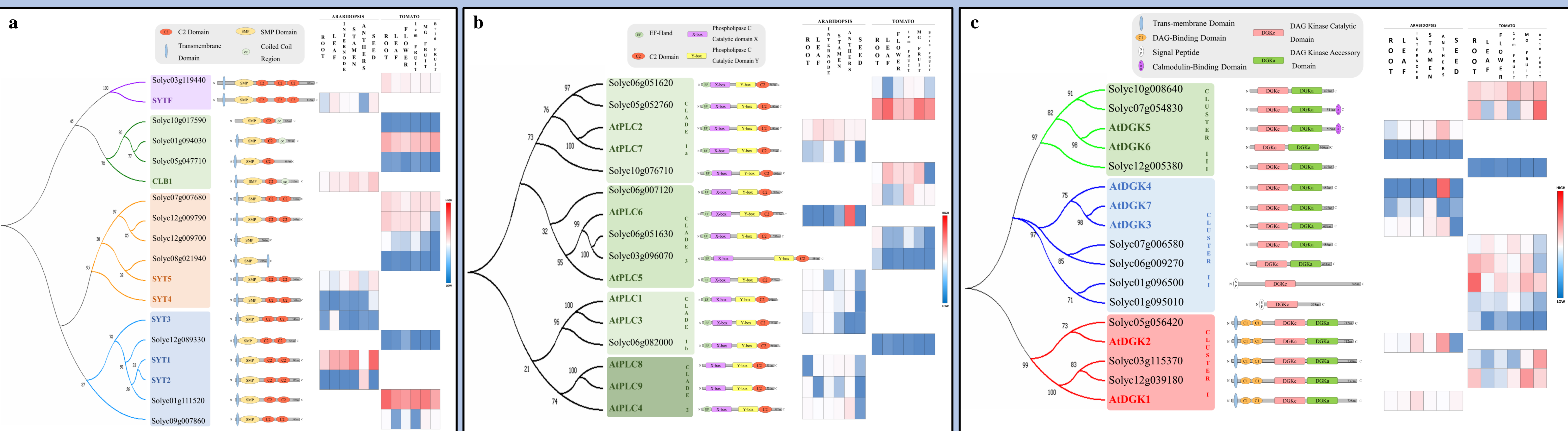


Figure 6: Phylogenetic analysis, protein domain and expression pattern comparison between *Arabidopsis thaliana* and *Solanum lycopersicum* SYT (a), PLC (b) and DGK (c) protein families. Red color indicates high expression values while blue colors indicates low expression values. Expression values are related to the values from the same specie.

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