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Abiotic stress upregulates the expression of genes involved in PSV and autophagy routes

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Abstract: Adverse conditions caused by abiotic stress modulate the plant development and growth by altering morphological and cellular mechanisms. To face this problem, plants, along with physiological adaptations, developed intracellular mechanisms, including changes in protein production and trafficking or modifications of the endomembrane system. It is known that stress situations can alter protein sorting to the vacuole, changing their routes via a Golgi-independent pathway. Our goal is to evaluate the expression levels of different aspartic proteinases and well-characterized genes involved in the vacuolar pathway, in plants submitted to different abiotic stresses (osmotic, oxidative, saline and heavy metals). The results obtained point to a different response of the three aspartic proteinases under study, indicating that different, yet related, genes respond differently to different types of stress, resulting in a fine-tuned regulation. Furthermore, our results regarding the endomembrane system effectors show that EXO70, RMR1, SYP51, SYP121 and VTI12 are up regulated, while VAMP, SYP23 and BP80 are downregulated in the same situations. This demonstrates that adverse conditions caused by abiotic stress can alter the expression of key proteins involved in the protein trafficking machinery, which can be related with the activation/deactivation of certain pathways.

IECPS

Keywords: abiotic stress; aspartic proteinases; endomembrane trafficking

Introduction

Climate change



Crop failures



Food insecurity

Adverse environmental conditions affect growth and development of the plant, so they developed intracellular mechanisms to face this conditions, such as:



Alteration of the intracelular trafficking/sorting



Diferencial expression of genes related to protein sorting



Morphologic changes in the endomembrane system

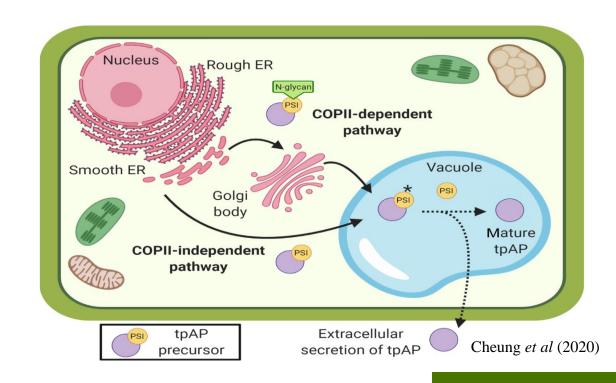
Introduction

Plant Specific Insert (PSI)

Independent domain, with approximately 100 aminoacidic residues

Highly conserved in innumerous species

May mediate a Golgi-bypass route to the vacuole



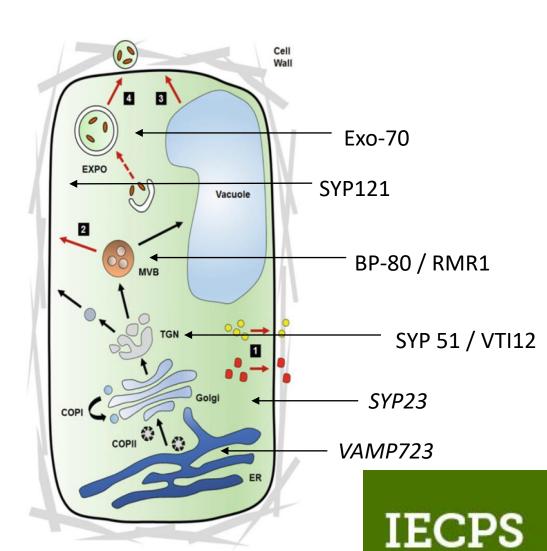
Enzyme inside an enzyme

Introduction

Endomembrane system effectors

Several classes of proteins can control specific events of membrane transport

Adverse environmental conditions can lead to cell reorganization events



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Goal

Evaluate the expression levels of different aspartic proteinases and well-characterized genes involved in the vacuolar pathway, in plants submitted to different abiotic stresses

S1	- 50	mM	NaCl
\mathcal{I}	20	111171	IVACI

S2 - 100 mM NaCl

H1 - 50 mM Manitol

H2 – 100 mM Manitol

Ox- 0,5 mM H2O2

 $Zn - 150 \mu M ZNSO4$

Germination of Arabidopsis thaliana seeds in different stress conditions

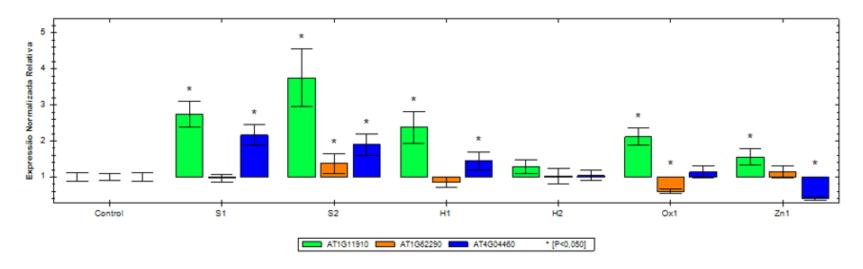


RNA extraction and cDNA preparation



Results

Expression of Aspartic proteinases (AP)



- The 3 APs tested respond differently to the stress conditions applied
 - AP1 (green) is upregulated for all stress conditions
 - AP2 (orange) is downregulated in oxidative stress condition
 - AP3 (blue) is upregulated in salt stress condition and downregulated in heavy metal induced stress



Discussion Expression of Aspartic proteinases (AP)



AP1 is more expressed because the other two have a more restricted pattern



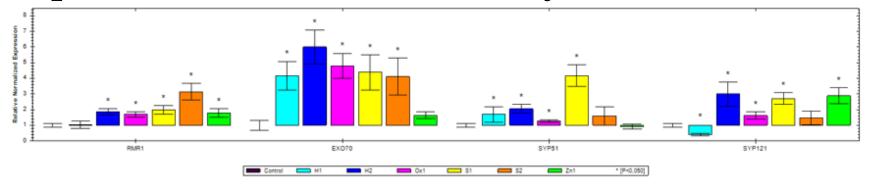
Can also point to a more important role of AP1 in response to abiotic stress

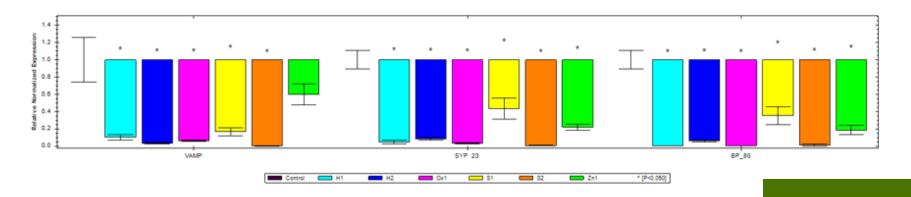


APs can have an antagonistic response

Results

Expression of endomembrane system effectors

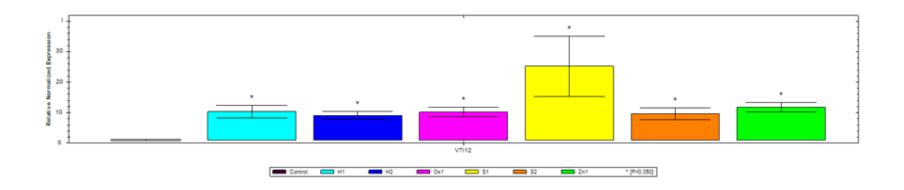




- RMR1, EXO70, SYP51 and SYP121 are upregulated in most of the stress conditions under study
- VAMP, SYP23, and BP80 are downregulated in all the stress conditions tested

Results

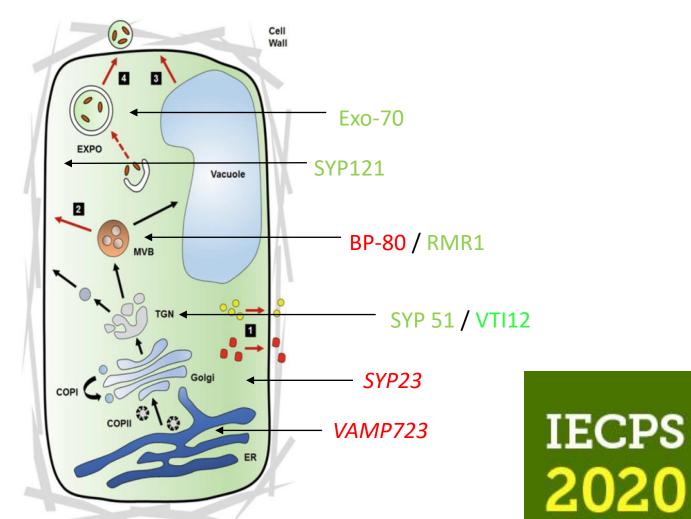
Expression of endomembrane system effectors



• VTI12 is upregulated in all the stress conditions but the increase in this gene expression is much higher when compared to the other genes tested, particularly in the **salt stress** situation.

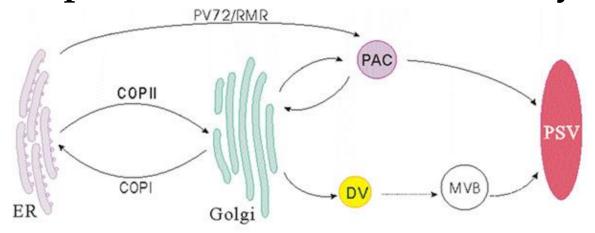


Expression of endomembrane system effectors



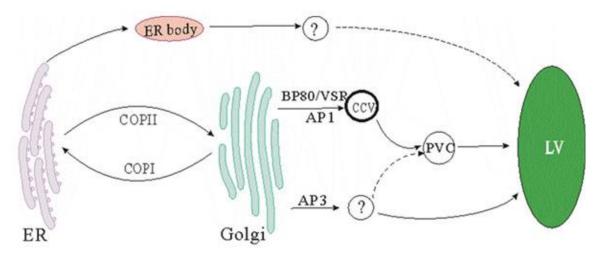
Chung and Zeng (2017)

Expression of endomembrane system effectors



RMR1 is implicated in the sorting of storage proteins to the PSV

BP80 is responsible for protein transport to the lytic vacuole



This can indicate that plant cells are able to shift their sorting mechanisms towards a more restrained state

Discussion Expression of endomembrane system effectors

Hypothesis: Plant cells can shift their sorting mechanisms towards a more restrained state

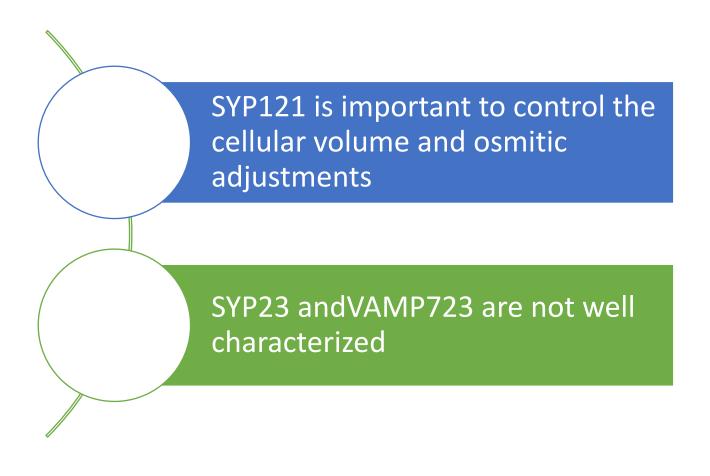
SYP51 and VTI12 are positive regulators of proteins with a CtVSD in their sorting to PSVs

Expression of endomembrane system effectors

Stress response induce autophagy pathaway

VTI12 have a role in this process, such as EXO70

Expression of endomembrane system effectors





Conclusion

Environmental conditions

Physiological processes relative to survival and growth

Membrane reorganization and gene expression

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