Ciências ULisboa
Faculdade de Ciências da Universidade de Lisboa

BioISI
Biosystems and Integrative Sciences Institute

ICGBG
XII International Conference on GRAPEVINE BREEDING and GENETICS
July 15-20, 2018
Bordeaux FRANCE

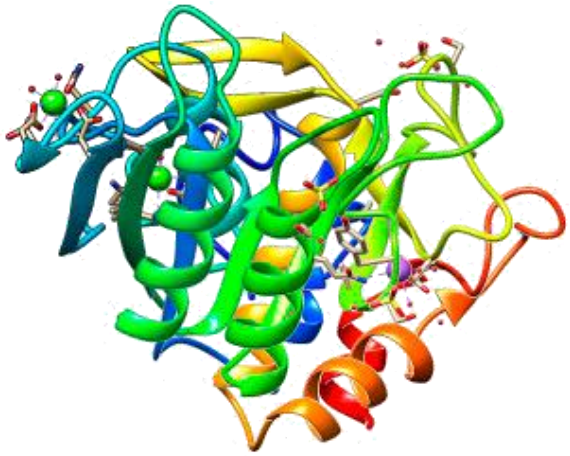
Subtilisin-like proteins and lipid signaling events: the missing links in grapevine resistance to *P. viticola*

Andreia Figueiredo, Joana Figueiredo, Gonçalo Laureano, Ana Rita Cavaco, Marisa Maia, Ana Rita Matos, Marta Sousa Silva

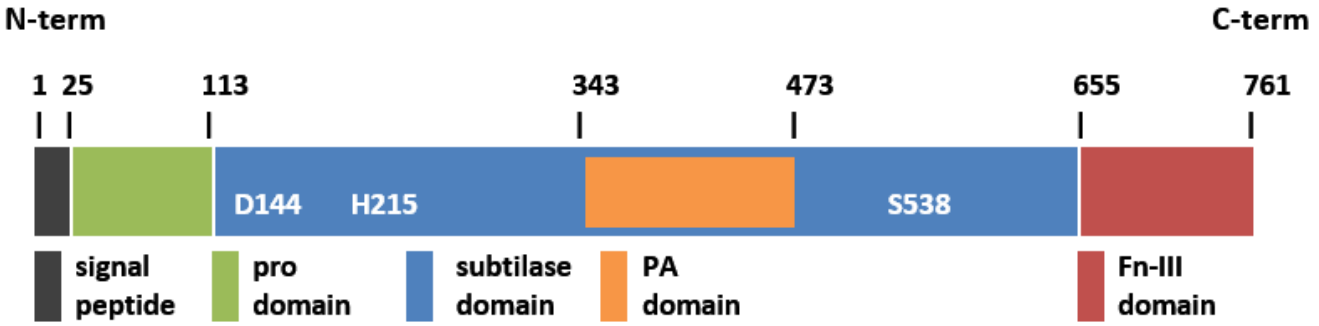
Andreia Figueiredo ***aafigueiredo@fc.ul.pt***

Subtilisin-like proteases

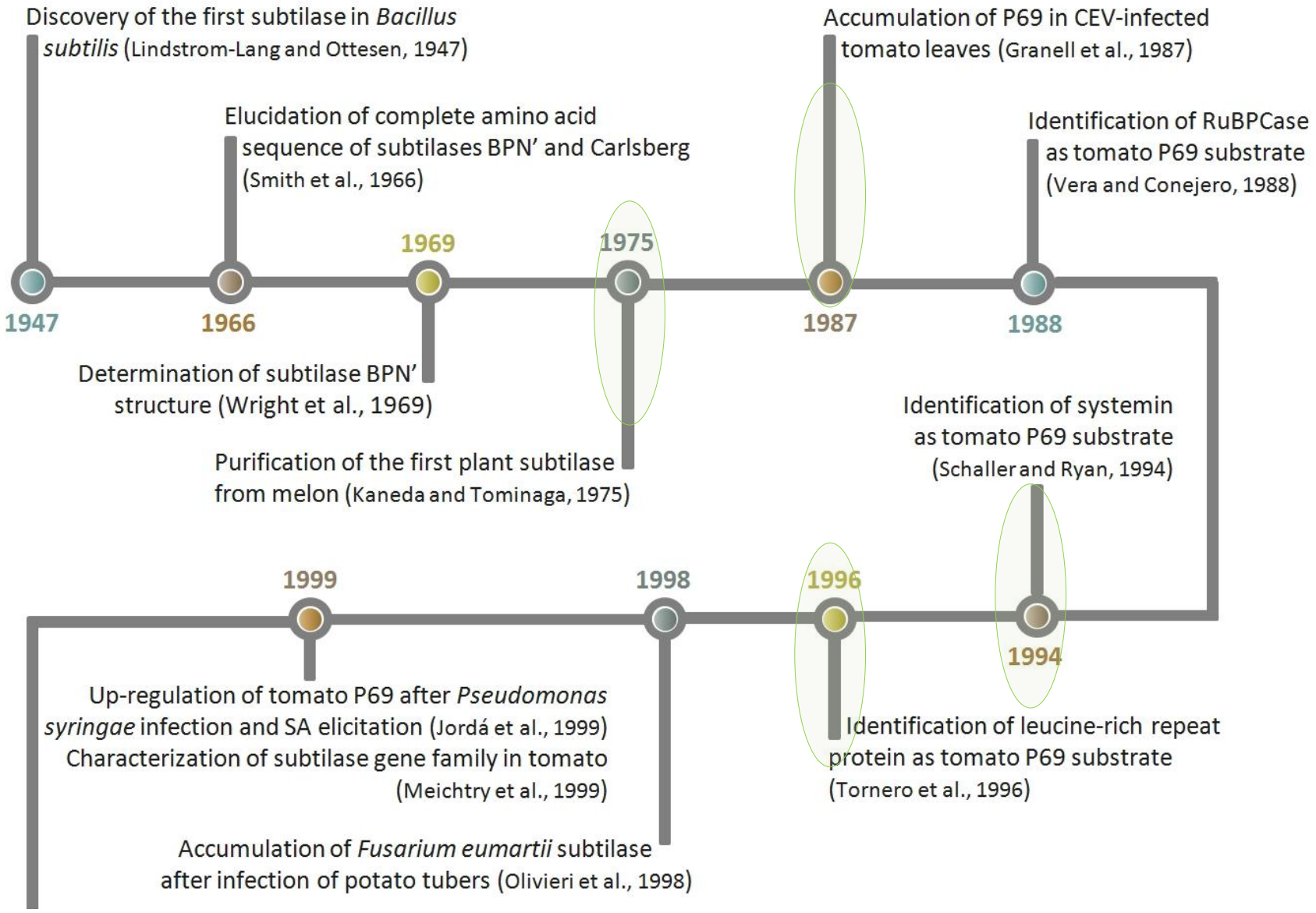
- Large group of serine peptidases
- S8 peptidase family (MEROPES)
- Broad spectrum of biological functions
- Pre-pro-proteins
  - **Catalytic triad** (Asp/His/Ser)
  - **Pro-domain (I9 inhibitor domain)**
  - **Subtilase domain (S8 peptidase domain)**
  - **Protease-associated domain (PA domain)**
  - **C-terminal FnIII-like domain**



Structure prediction of the SlSBt3 monomer (PDB code:1THM)



Plant subtilisin-like proteases



Plant subtilisin-like proteases

2004

Up-regulation of tomato P69 after *Phytophthora infestans* infection (Tian et al., 2004)

Participation of oat saspases in PCD induced by victorin or heat shock (Coffeen and Wolpert, 2004)

2005

Up-regulation of sorghum subtilases after MeJA and SA elicitation (Salzman et al., 2005)

Characterization of subtilase gene family in *Arabidopsis* (Rautengarten et al., 2005)

2006

Characterization of subtilase gene family in rice (Tripathi and Sowdhamini, 2006)

2008

Constitutive expression of *Vitis vinifera* subtilase in pathogen resistant genotypes (Figueiredo et al., 2008)

2009

Activation of *Vitis vinifera* subtilase after methylated cyclodextrins elicitation (Martinez-Esteso et al., 2009)

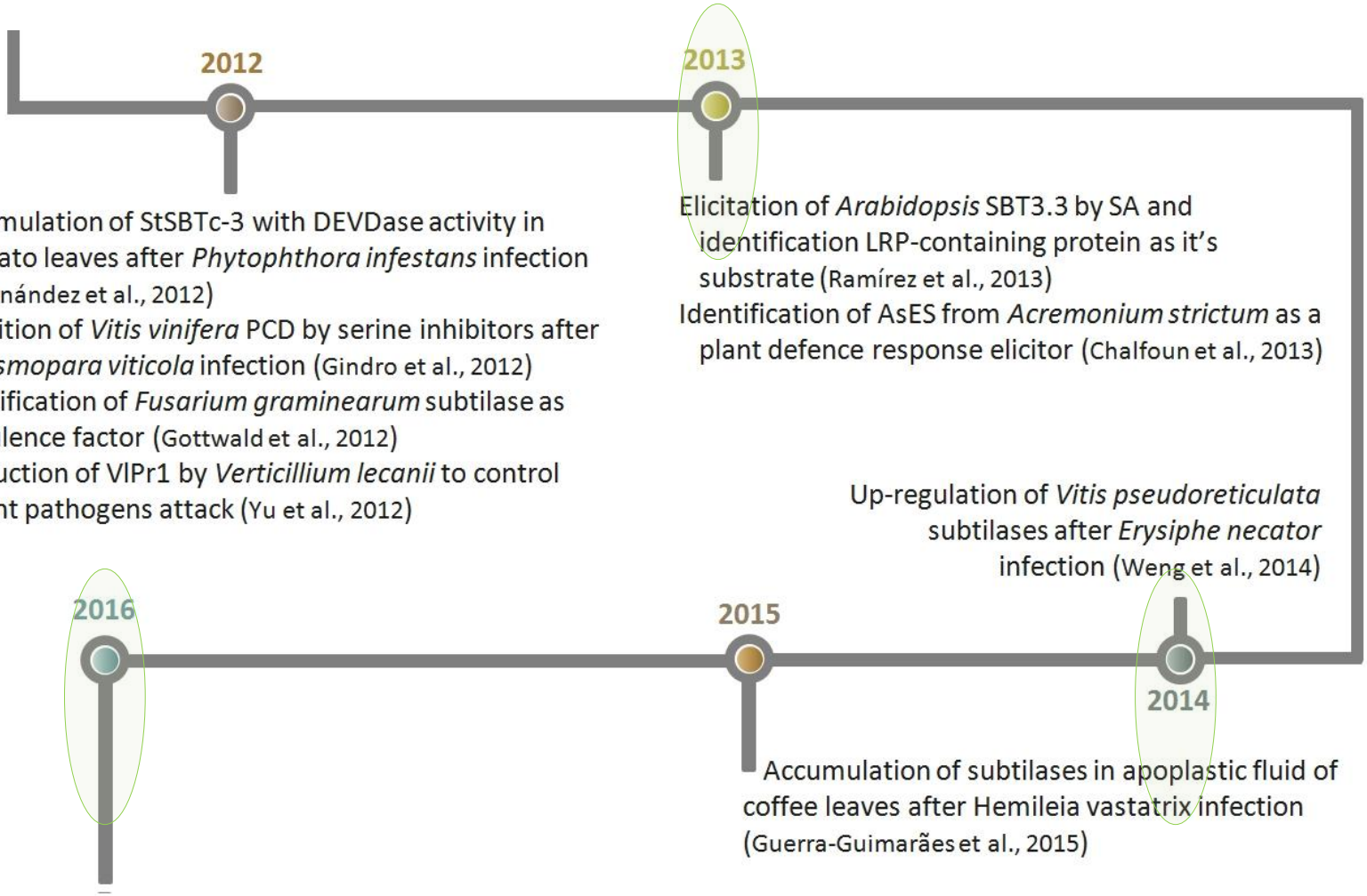
2010

Accumulation of tomato stem cell wall subtilase after *Ralstopnis solanacearum* infection (Dahal et al., 2010)

Identification of GmSubPep from soybean as a plant endogenous elicitor (Pearce et al., 2010)

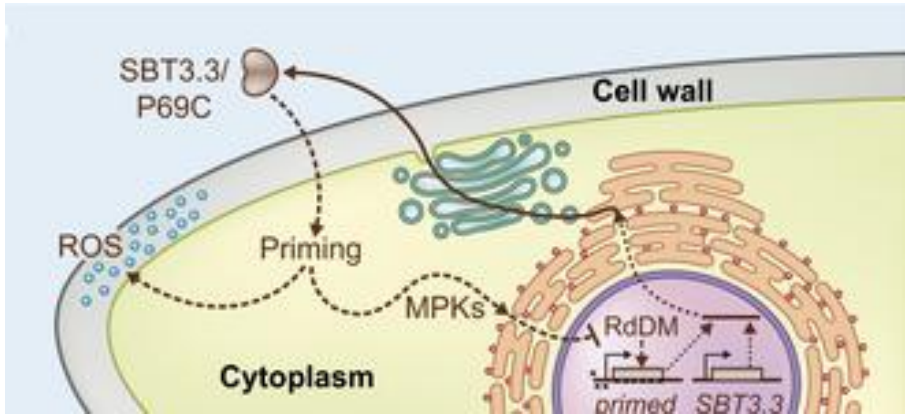
Participation of tobacco phytaspases in PCD induced by TMV (Chichkova et al., 2010)

Plant subtilisin-like proteases



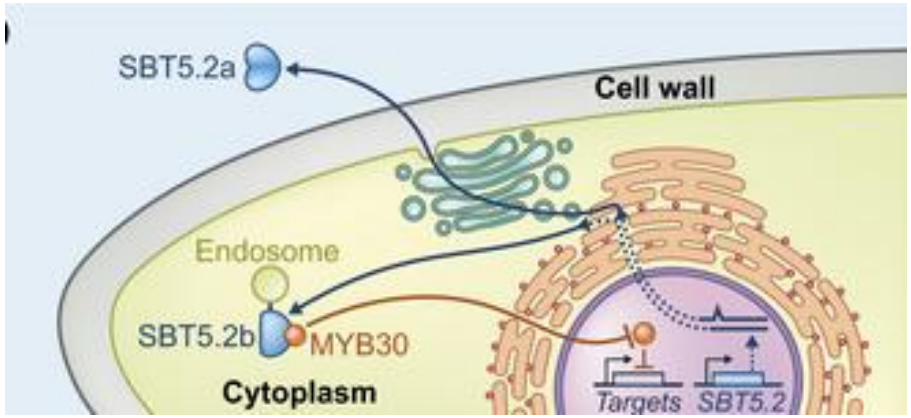
Figueiredo et al. (2018). MPP, 19(4) , 1017–1028

Subtilisin-like proteases in biotic interactions



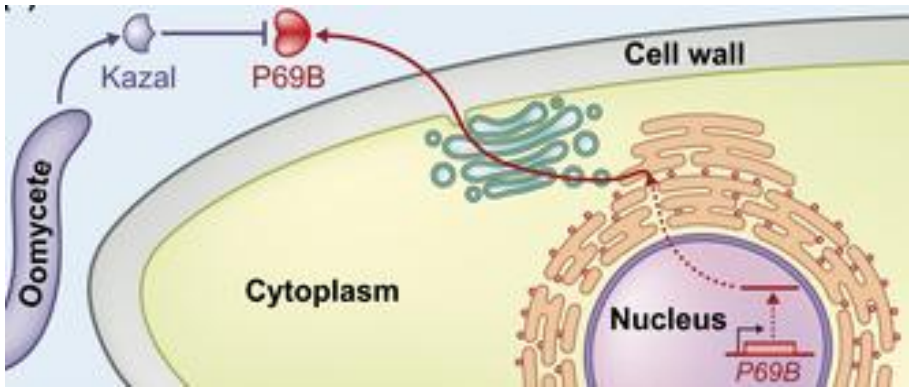
SBT3.3/P69B and immune priming

Pathogen triggers the expression of SBT3.3/P69 – negatively regulates RdDM – priming Increase of SBT3.3 expression – enhances the expression of OXI1 kinase, ROS and MAP kinase.



SBT5.2(b)

Interacts with MYB30 – nuclear exclusion – defense gene expression impairment – HR attenuation enhances the expression of OXI1 kinase, MAP kinase and ROS.

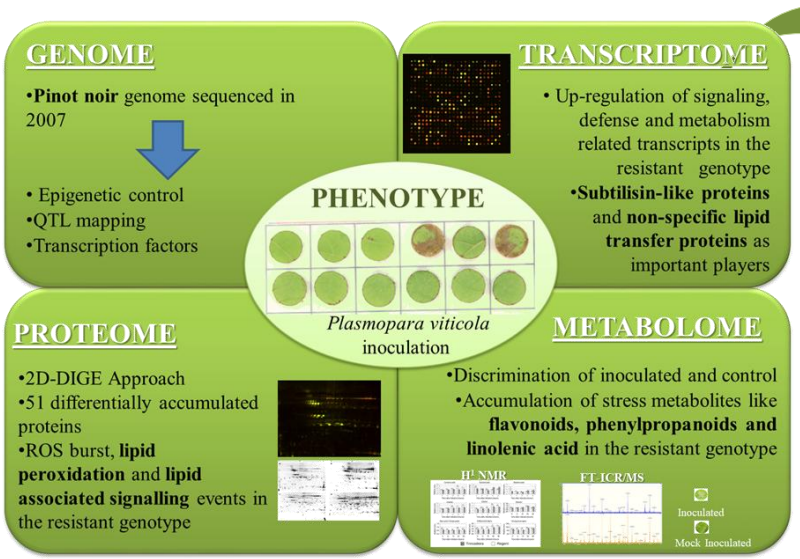


P69B(-like)

Inhibition by Kazal inhibitors from oomycetes.

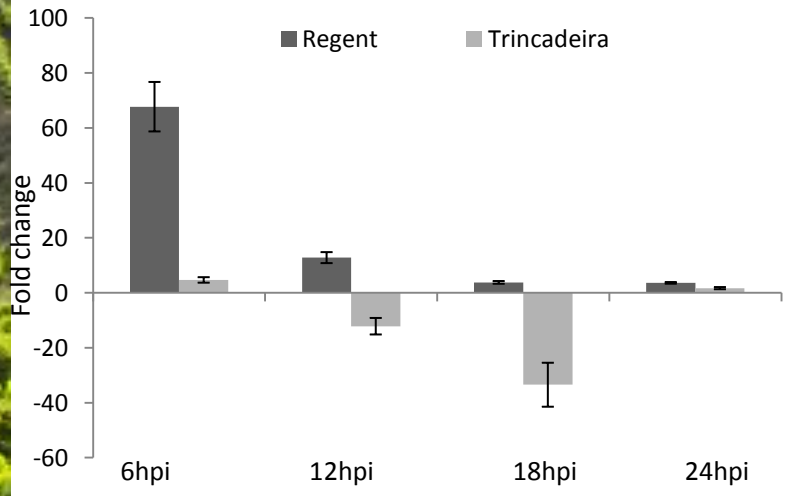
Schaller et al. (2018). New Phytologist, 218:901-915

Grapevine SBTs and P. viticola resistance



Grapevine SBT gene family

- Composed by 87 genes encoding 109 predicted proteins
- Divided into 5 groups
- Unevenly distributed among 15 of the 19 grapevine chromosomes
- 80% secreted, 10% mitochondrion, 9% chloroplast
- Glycosylated



Selection of 14 grapevine subtilases:

-Previous microarray and expression data

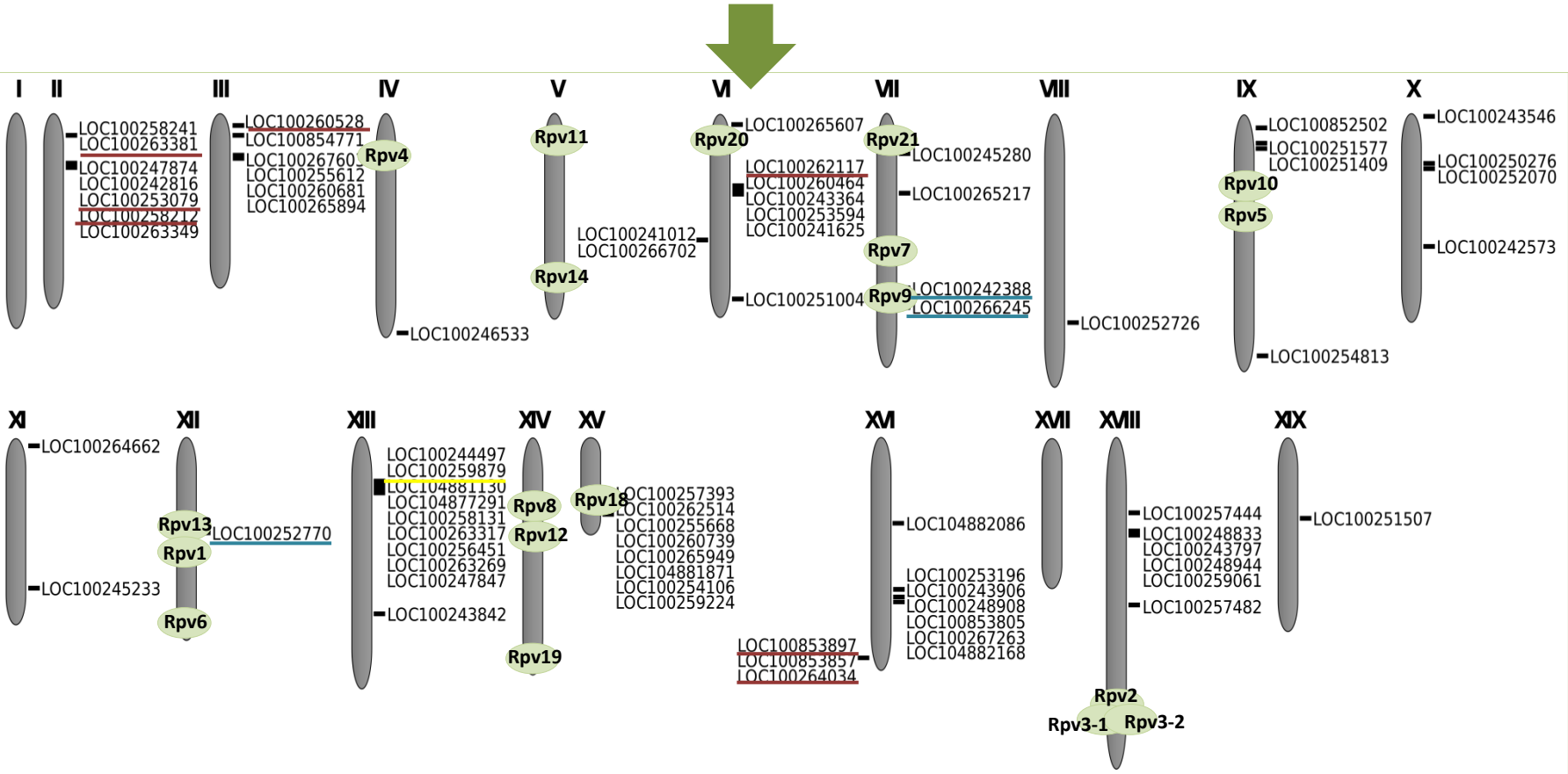
- Sequence similarity with AtSBT3.3 and P69C

-Location near RPV loci (RPV9 in Chr7 and between RPV1 and RPV13, Chr12)

Figueiredo et al. (2016). FPS, 7:1783
Figueiredo et al. (2017), FPS, 8:716

Grapevine SBTs

New RPV identified - revision of candidates

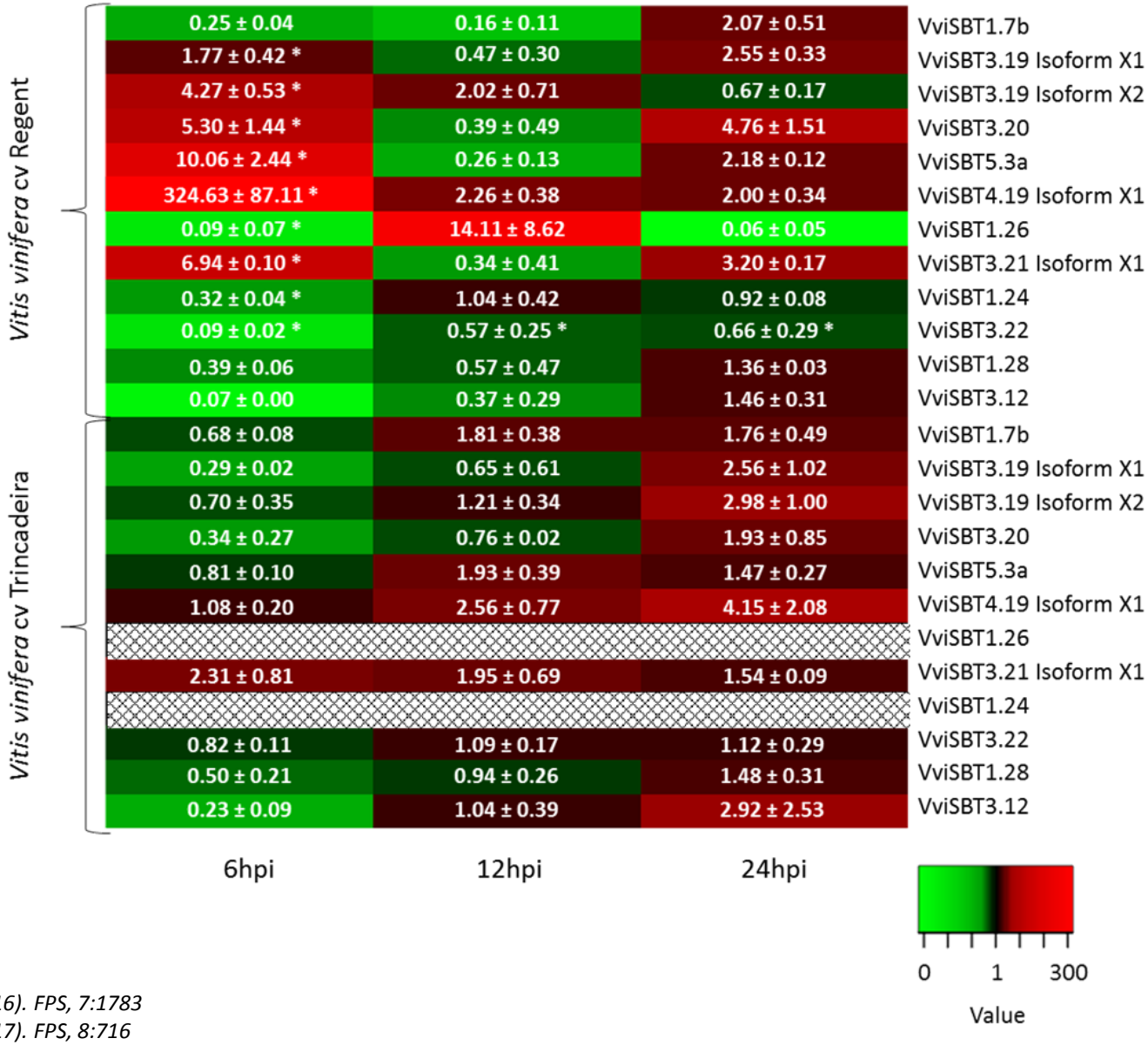


Homology with SBT3.3/P69B

Location near/whitin Rpv

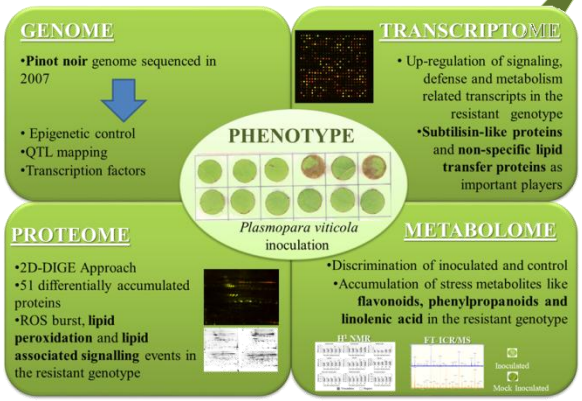
Our previous data

Grapevine SBTs



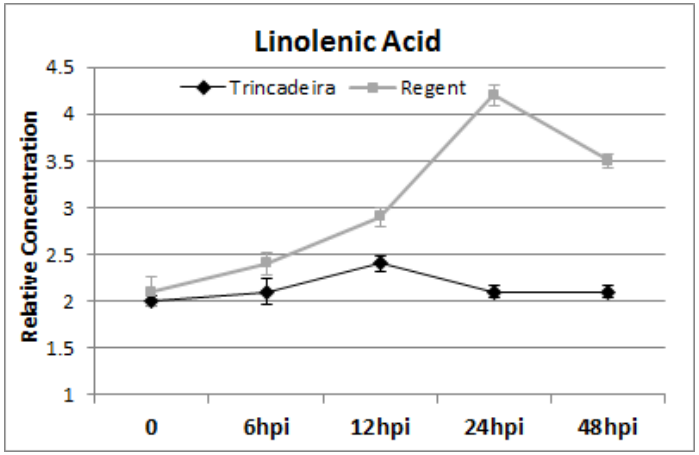
Figueiredo et al. (2016). FPS, 7:1783
Figueiredo et al. (2017). FPS, 8:716

Lipid signaling in grapevine defense

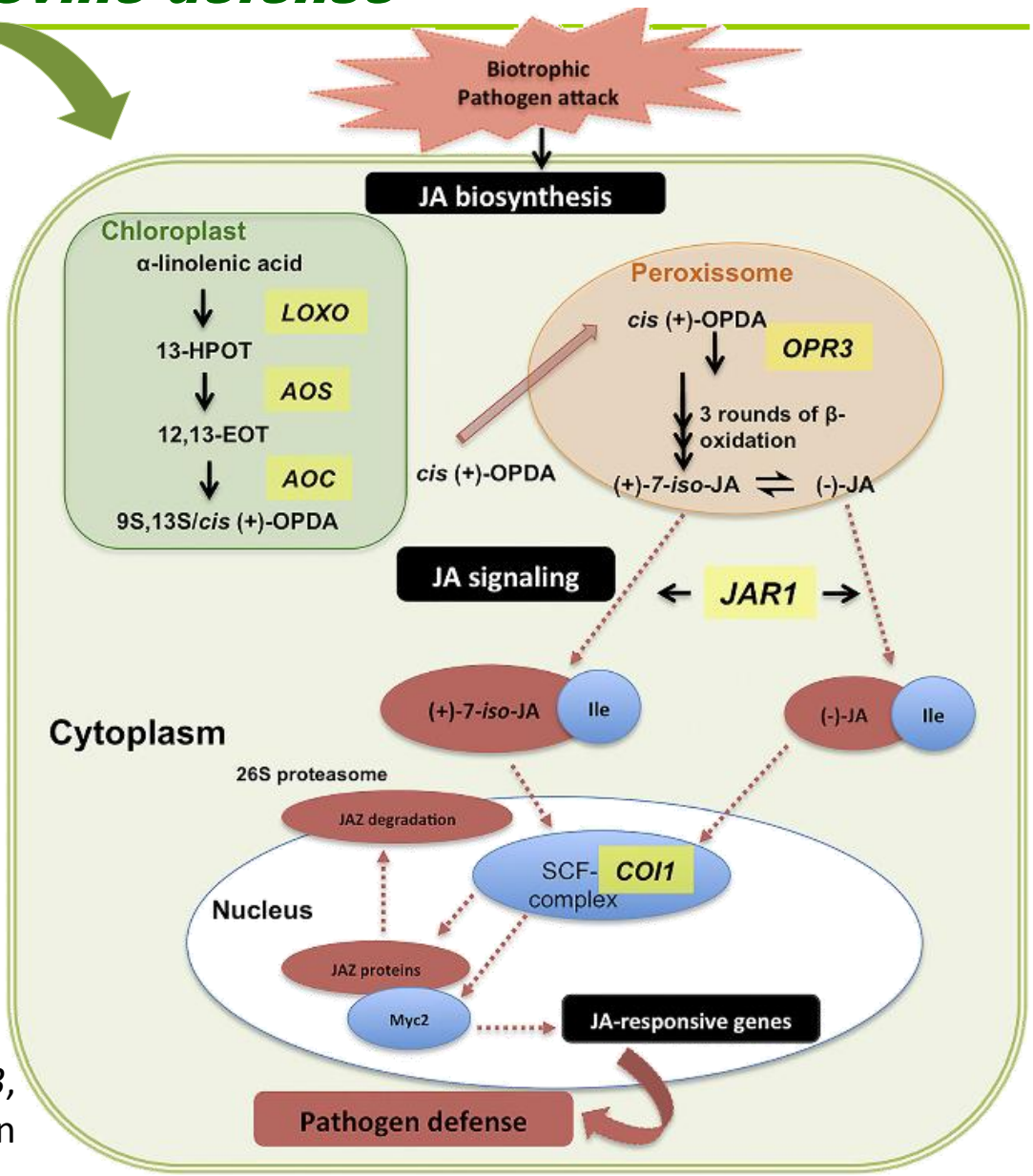


JA synthesis:

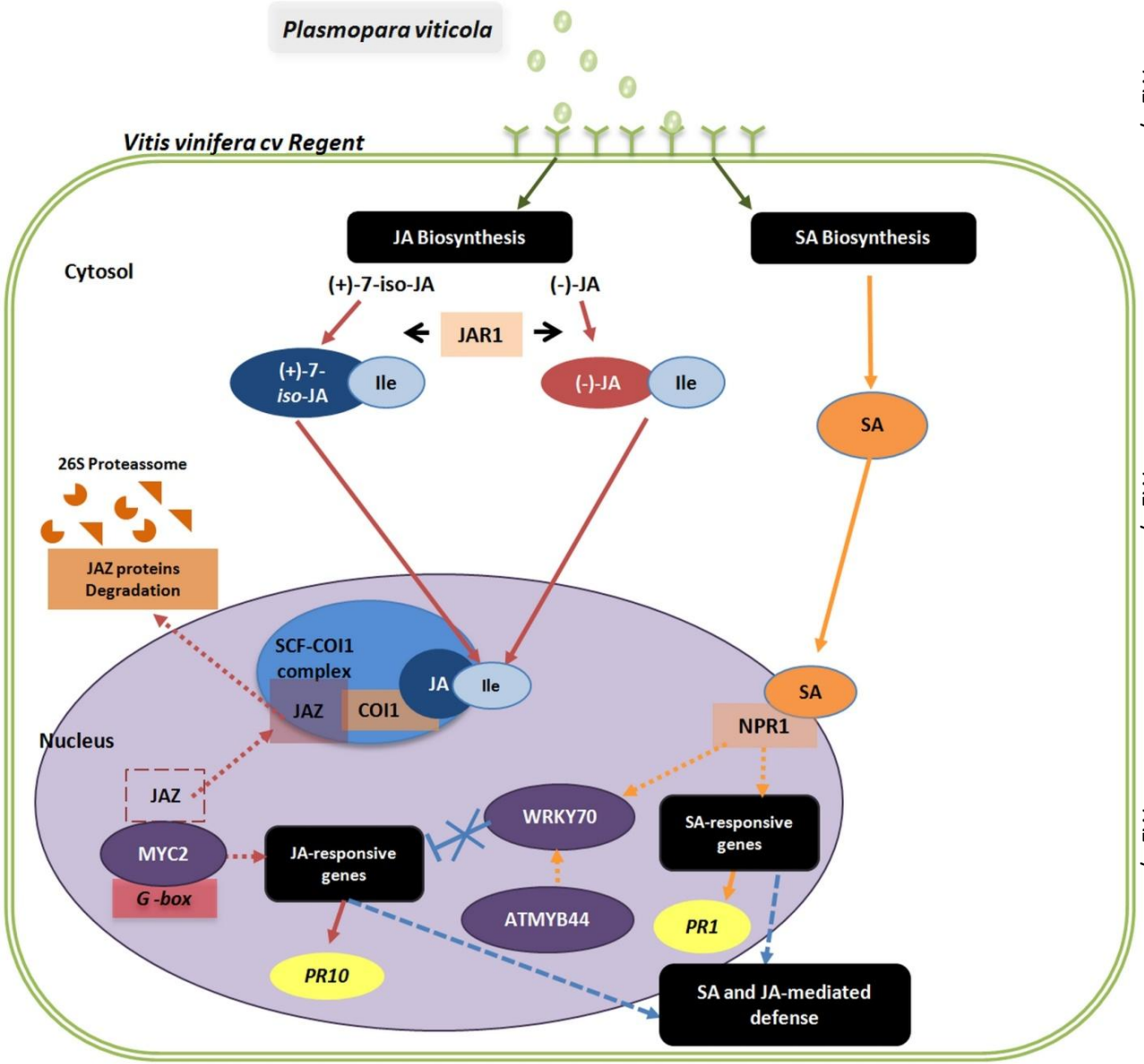
-C18:3 accumulation

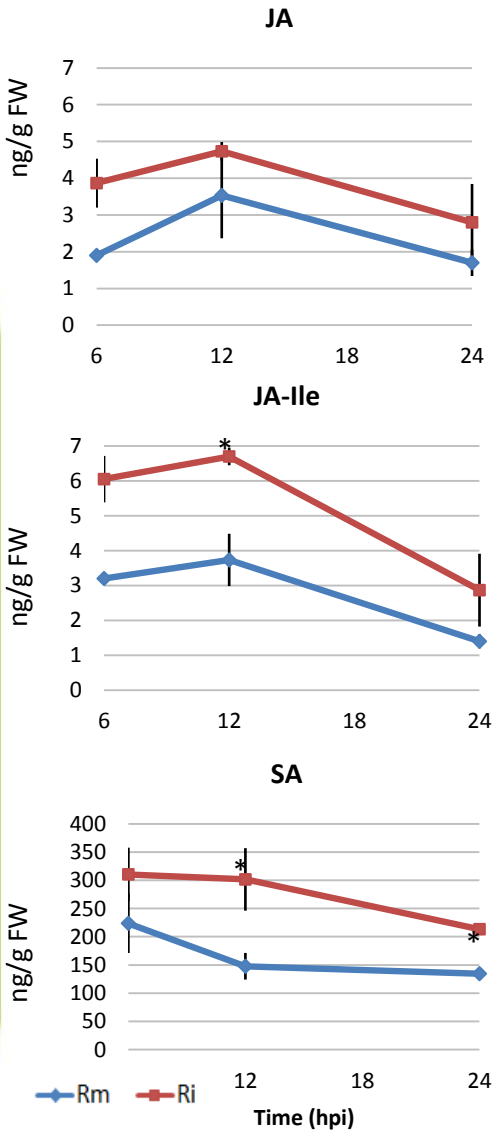


-Increase of *LOX2*, *AOC*, *AOS*, *OPR3*, *JAR1*, *COI1* after *P. viticola* inoculation – incompatible interaction



Lipid signaling in grapevine defense





Guerreiro et al. (2016). FPS, 7:565

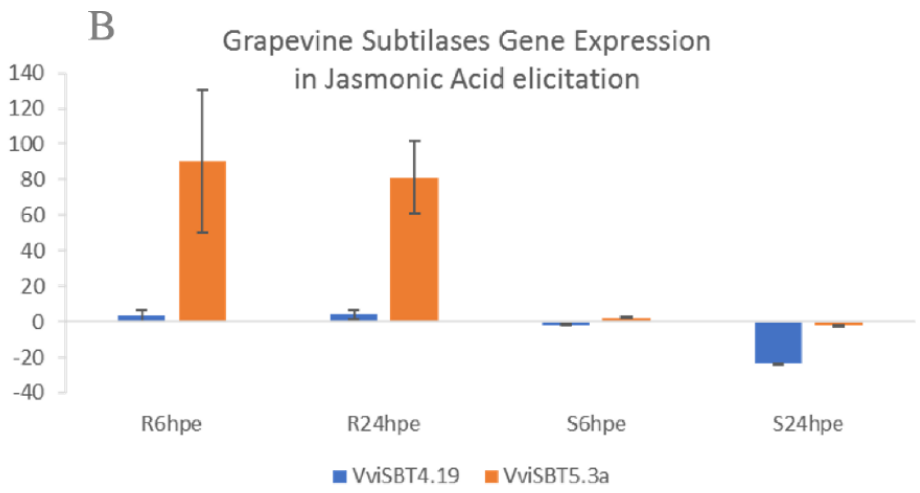
SBTs and lipid signaling

•Systemin is derived from a precursor called prosystemin, similar to polypeptide hormones

•Systemin activates a lipid-based signaling cascade

•Wounding of tomato plants triggers the release of systemin from its precursor prosystemin by SBT3

•Systemin is perceived at cell surface by a LRR receptor-like kinase - JA biosynthesis induction – JA accumulation



SBTs and lipid signaling in P. viticola resistance

- Some grapevine subtilases are linked to defense mechanisms towards *P. viticola*
- Lipid signalling and jasmonic acid are also important players
- Subtilases, particularly SBT3.3 and P69C are believed to be associated to immune priming events and activation of signalling cascades
- A link between JA and SBT3.3 has been shown for other model

Are grapevine subtilisin-like proteins linked to lipid signaling events – grapevine immune priming?

Apoplast proteomics and lipidomics

Recombinant protein production (structure and substrates)

Interactome analysis

Acknowledgments

BioISI
Andreia Figueiredo
Ana Matos

CQB
Marta Silva
Vukosava Millic

PhD students
Joana Figueiredo
Marisa Maia
Ana Rita Cavaco
Vanessa Azevedo
Catarina Gouveia

ITQB
Rita Abranches

CIFC
Leonor Guerra-Guimarães

Master students
Gonçalo Laureano; Rui Nascimento

Julius Kuhn Institute (JKI)

RLP AgroScience GmbH/ AlPlanta - Institute for Plant Research

Universitat Hohenhein

Funding:
PTDC/BIA-BQM/28539/2017 – " GRAVITAS - Grapevine immunity: the innovative role of subtilisin-like proteases
FCT PTDC/AGR-PRO/2438/2014 - Characterization of grapevine subtilisin-like proteases and their role in pathogen recognition and immune priming


Thank you for
your attention!