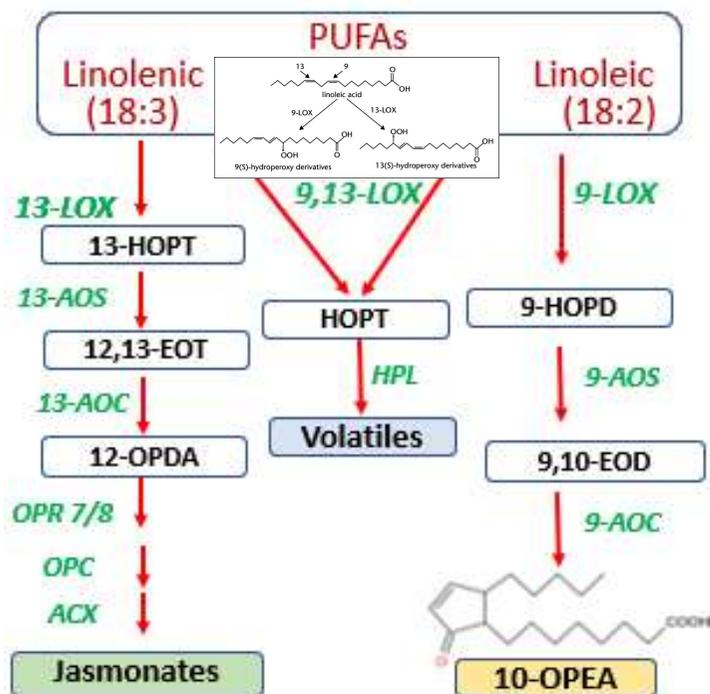


Functional Study of Lipxygenase-mediated Resistance against *Erysiphe necator* in Grapevine

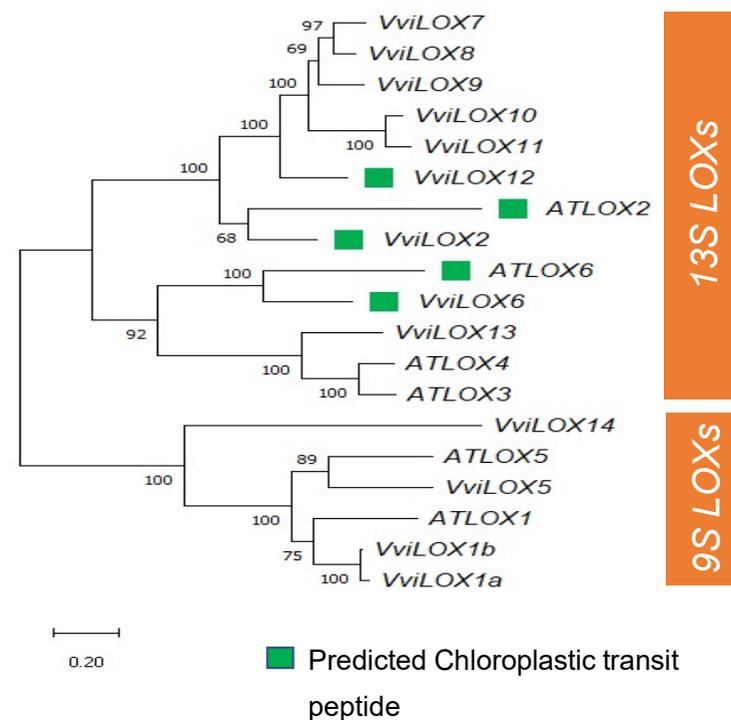
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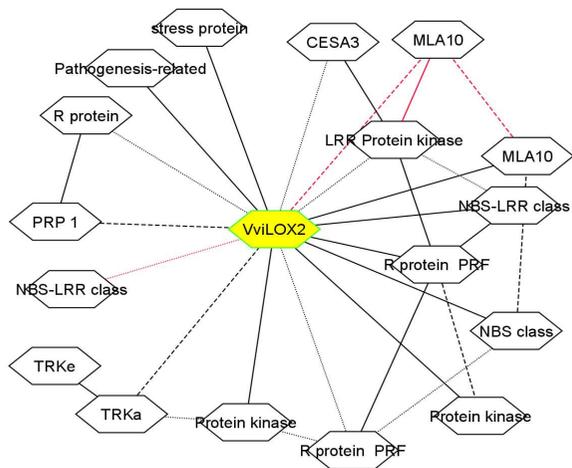
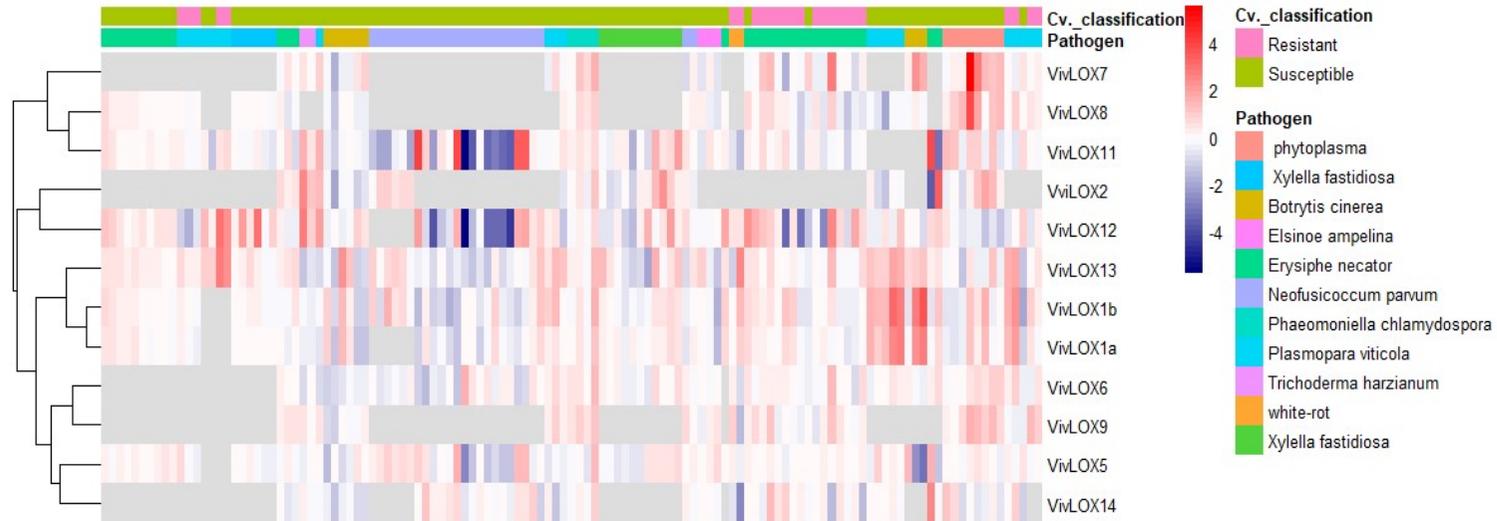
The Lipxygenase (LOX) Pathway and Key compounds



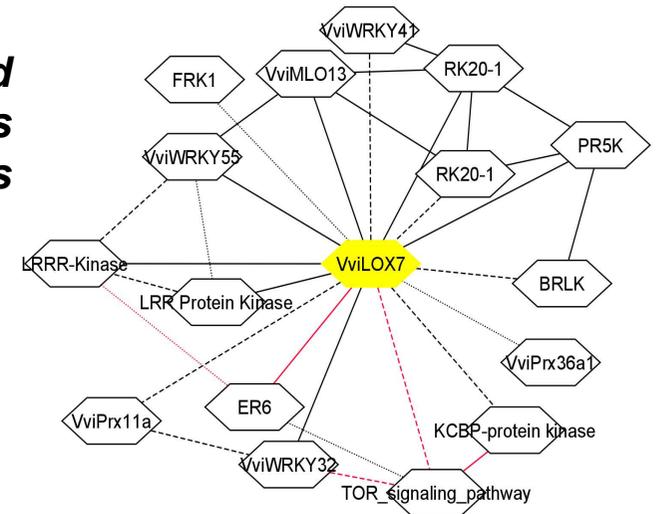
Phylogenetic Analysis of the Grapevine *VviLOX* family



VviLOXs Gene Expression Profiles in published experiments on Grapevine response to pathogens. Data taken from VESPUCCI new release



OneGenE network analysis showed association of some VviLOX isoforms with numerous putative pathogenesis related genes

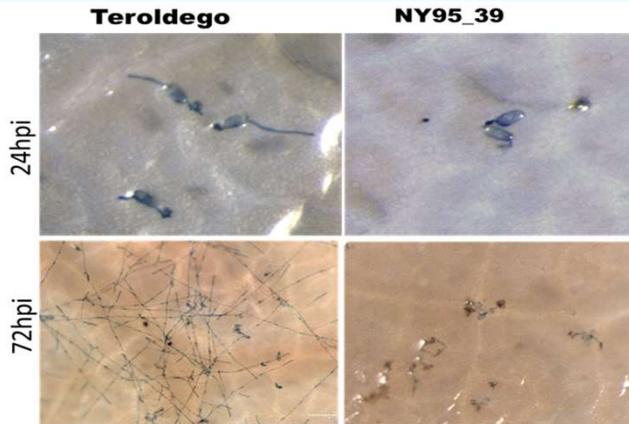


E. necator infection of Grapevine leaves under controlled conditions and *VviLOXs* expression analysis

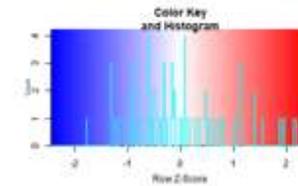
E. necator infection on *V. vinifera* cv. Teroldego (S) and a *Vitis* spp. hybrid NY95-39 (R)



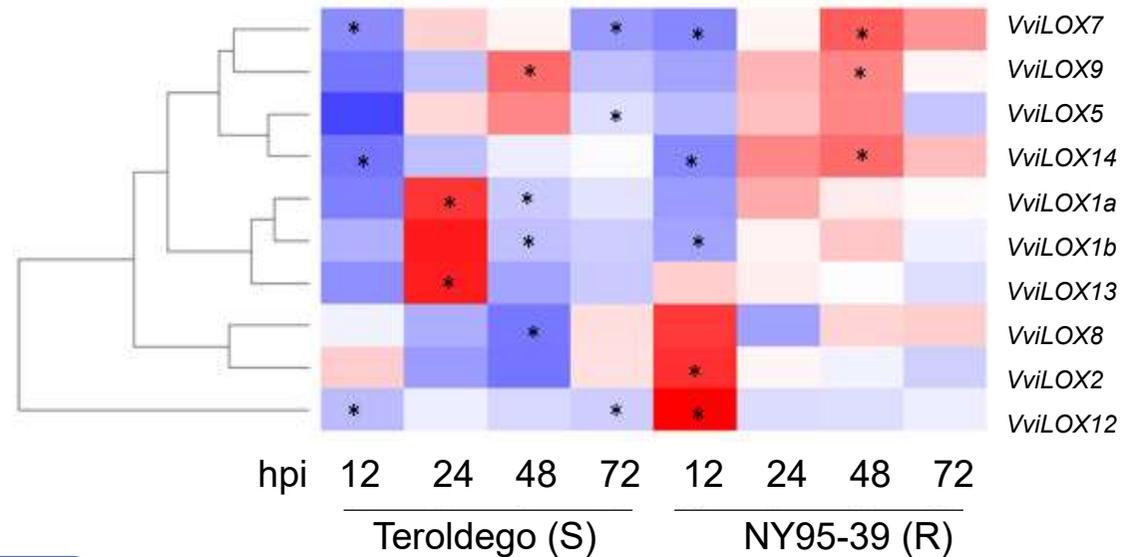
Microscopic analysis of aniline blue stained infected leaves under stereomicroscope



Several *VviLOXs* were significantly modulated in the infected leaves collected at different time points post inoculation in the (R) and (S) genotypes



VviLOX genes expression profiles upon *E. necator* infection



E. necator penetrated at 24 hpi in the (S) with a secondary hyphal growth at 72 hpi. In contrast establishment and growth was hampered in NY95_39 (R) with necrotic spots at the infection site.

Functional *in planta* study of Candidate *VviLOXs* by means of Stable Genetic Transformation

Overexpression of candidate resistance factors *VviLOX2*, *VviLOX7*



- Coding sequences were amplified from 'NY95_39' and cloned into the binary expression vector pK7WG2 under the 35S promoter



Agrobacterium mediated transformation of somatic embryos of the highly transformable cv. 'Sugraone' and plant regeneration

CRISPR/Cas9 knock-out of *VviLOX2*, *VviLOX7*, *VviLOX12* and *VviLOX13*



- Specific gRNA were designed on 'Sugraone' sequenced targets sites
- gRNA were cloned into a binary vector with Flp/FRT recombination sites, for cassette removal



Agrobacterium mediated transformation of nodal explants from (R) is under development



Conclusions

- The phylogenetic analysis of the *VviLOX* family on the 12X.v2 genome assembly and v3.Vcost annotation allowed to define a correct nomenclature for the isoforms, besides confirming the two groups formed by 13S- and 9S-LOXs.
- The *in silico* transcriptomic data analysis based on VESPUCCI showed distinguished modulation patterns of the *VviLOX*s in different plant-pathosystems, while the gene network analysis based on OneGenE identified genes involved in plant defense response as correlated to some *VviLOX* isoforms, supporting further investigations.
- Finally, a differential isoforms modulation has been experimentally observed in a resistant and a susceptible grapevine varieties inoculated with *E. necator*, leading to the identification of interesting candidates to be functionally characterized.
- Ongoing work concerns the generation of grapevine mutant lines to better characterize these isoforms at the functional level and evaluate their role in plant defence.