Arbuscular mycorrhizal fungi alter oxidative stress response in virus infected grapevine

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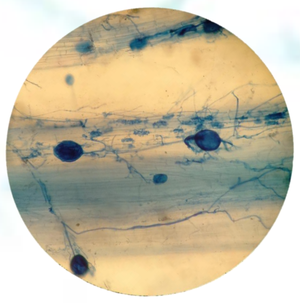

Why this particular research?

- grapevine virus infections are worldwide spread and have potential severe consequences
- arbuscular mycorrhizal fungi (AMF) may have beneficial impact on plants under various biotic stresses and easily colonize grapevine roots
- insights into bioprotection efficiency of AMF against plant viruses are so far inconsistent and vastly underexplored in perennial fruit crops
- antioxidative defence mechanisms are first line of defence in grapevine coping with virus infection

Hypothesis

AMF have potential to alleviate virus-induced oxidative stress in grapevine.

Experimental set up

Merlot cv. (*Vitis vinifera* L.) scions grafting on Kober 5BB rootstock (*Vitis Berlandieri* Planch. × *Vitis riparia* Michx.)

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plants establishment in 6 L pots in sterilized substrate

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determination of virus status and selection of virus-free plants and with GRSPaV only

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infection with GLRaV-3 and GPGV by „chip budding" transmission

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AMF inoculation

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collecting of five samples of each treatment five months after AMF inoculation

Final 15 treatments of the experiment

Treatment	Virus status	AMF status
No	No virus	No AMF
		R. irregularis
		AMF Mix*
R	grapevine rupestris stem-pitting associated virus (GRSPaV)	No AMF
		R. irregularis
		AMF Mix*
RL	grapevine rupestris stem-pitting associated virus (GRSPaV)	No AMF
	grapevine leafroll-associated virus 3 (GLRaV-3)	*R. irregularis*
		AMF Mix*
RP	grapevine rupestris stem-pitting associated virus (GRSPaV)	No AMF
	grapevine pinot gris virus (GPGV)	*R. irregularis*
		AMF Mix*
RLP	grapevine rupestris stem-pitting associated virus (GRSPaV)	No AMF
	grapevine leafroll-associated virus 3 (GLRaV-3)	*R. irregularis*
	grapevine pinot gris virus (GPGV)	AMF Mix*

*AMF Mix – inoculum is consisted of *Rhizophagus irregularis, Funeliformis mosseae* and *F. caledonium*

Virus and AMF detection: For the initial virus status, presence of ten most common grapevine viruses was determined in wood scrapings applying CTAB based RNA extraction (Gambino 2015), reverse transcription to cDNA and quantitative PCR detection. After the „chip budding" infection, virus transmission was checked for GLRaV-3 and GPGV (Gaši et al. 2023). AMF olonization was assessed according to Brundrett et al. 1996.

Oxidative stress: Lipid peroxidation – ***LPO***, ***proline***, ascorbate - ***AsA***, superoxide dismutase - ***SOD***, ascorbate- ***APX*** and guaiacol peroxidases - ***GPOD***, polyphenol oxidase – ***PPO*** and glutathione reductase – ***GR*** were measured spectrophotometrically (Stolfa et al. 2017).

Results

Fig. 1. *Heatmap and cluster analysis summarizing the response of oxidative markers to mycorrhizal status of experimental plants. Non mycorrhizal grapevines (No) and vines inoculated with Rhizophagus irregularis (Rhiz) and R. irregularis, Funneliformis mosseae and F. caledonium (Mix) are displayed with eight oxidative and antioxidative markers. Color scale indicates range between maximum (red, 1) and minimum values (blue, -1). Distance was determined by Euclidean method and clustering was performed using Ward method.*

- AMF inoculated treatments clustered separately in heatmap from the AMF-free controls, indicating a relevant relation to the AMF status
- AsA, SOD and proline responded the most significantly to AMF colonization
- reduced SOD activity in AMF colonized plants suggests lower degradation of superoxides into H_2O_2, and consequently lower accumulation of H_2O_2; this was accompanied with lower accessibility of AsA as APX substrate, and inconsistent APX reduction of H_2O_2 and GR activity → reduced activation of ascorbate-glutathion cycle
- other observed antioxidant parameters were not significantly influenced by AMF or performed inconsistent pattern
- proline concentrations depended strongly on type of AMF inoculum

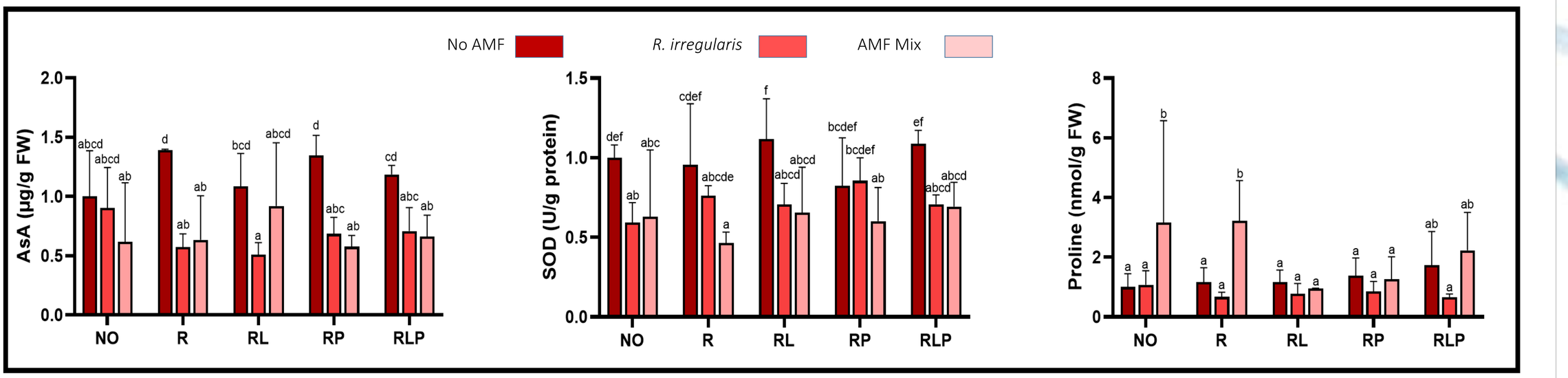

Fig. 2. *Antioxidative parameters that were significantly changed due to AMF inoculation: AsA, SOD and proline. Data were normalized to control treatment that contained no virus and no AMF. Statistical analysis was carried out using two-way ANOVA, with virus and AMF status as independent variables. Different letters indicate a significant difference between treatments (p<0.05, Duncan post-hoc test).*

Conclusions

- AMF is integral part of reactive oxygen species homeostasis in virus-infected grapevine
- The reduced need for the activation of the ascorbate-glutathione cycle, coupled with an enhanced growth of AMF-inoculated grapevines, suggests a lower level of oxidative stress.

References:

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