The genetics behind AM symbiosis: the case of Tomato Wild-Relatives

Matteo Chialva[1], Stefania Stelluti[1], Mara Novero[1], Paola Bonfante[1], Luisa Lanfranco[1]

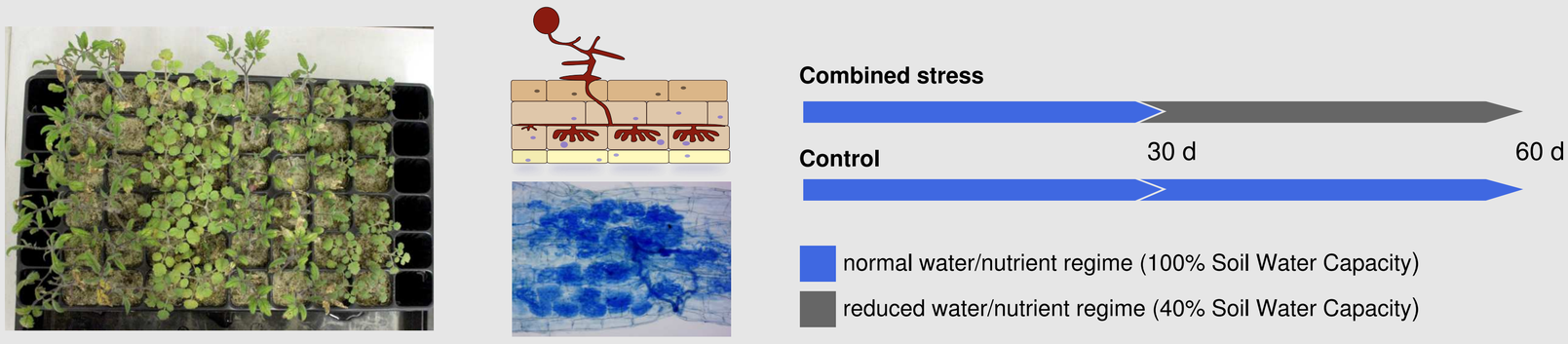
[1]Department of Life Sciences and Systems Biology, University of Torino, Viale P.A. Mattioli 25, I-10125, Torino, Italy

Background and aim

Arbuscular mycorrhiza (AM) is the most widespread mutualistic symbiosis established between land plants and Glomeromycotina, a group of soil fungi [1]. Among mycorrhizal crops, tomato has been extensively investigated for AM interactions being a valuable plant model. Genetics behind AM symbiosis responsiveness in tomato has been mostly faced using functional genomics approaches (RNAseq and microarray). However, precious genetic resources are available to afford this topic, such as wild relatives, introgression lines and mutants. In particular, tomato wild relatives offer an effective genetic reservoir for cultivated tomato [2,3], but have rarely been investigated for their susceptibility and responsiveness to the AM symbiosis at root and systemic level.

In this work, we compared the responses of two wild-relative species, *Solanum pennellii* and *Solanum neorickii* with the cultivated tomato (*Solanum lycopersicum*) cultivar 'M82' to the colonization by the AM fungus *Funneliformis mosseae*. AM-responsiveness was also tested under the growth condition of a combined stress, obtained by reducing nutrients and irrigation water to 40% Soil Water Capacity for one month.

Experiment set-up and timeline

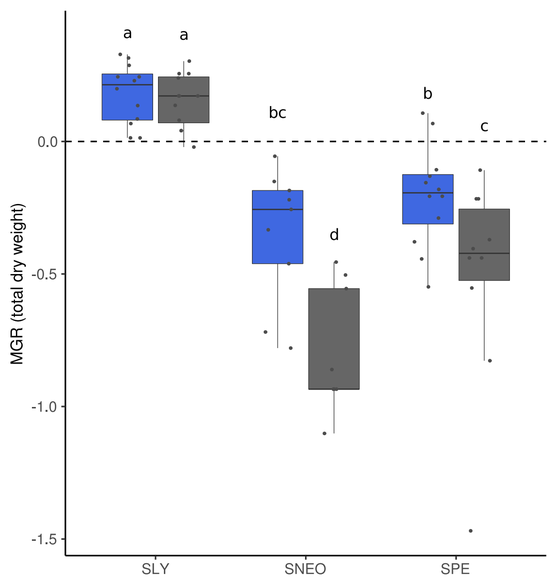


Alveolar trays (10x6 wells)

Funneliformis mosseae, BEG12

Results

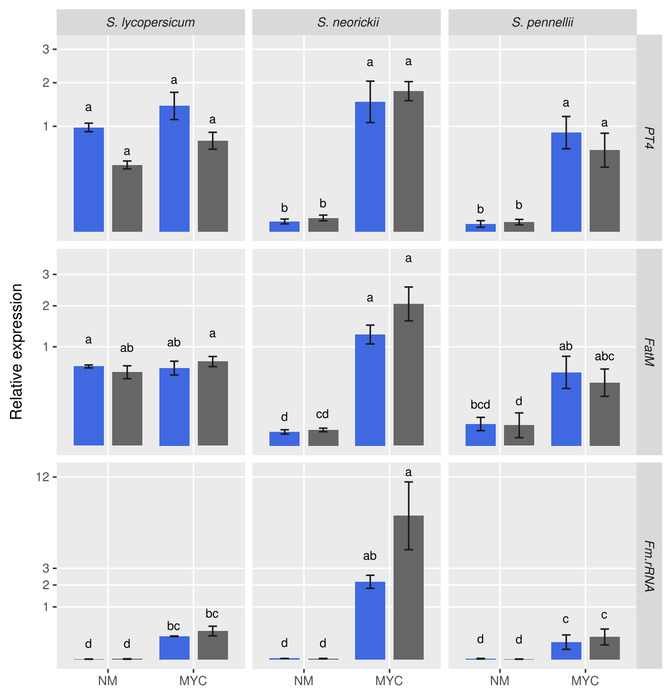
Growth response



Letters indicate statistically supported differences across treatments and species according to pairwise Kruskal-Wallis test (p<0.05). SLY= S. lycopersicum cv 'M82'; SNEO= S. neorickii; SPE= S. pennellii. WW= control conditions; SS= combined stress.

We calculated Mycorrhizal Growth Response (MGR) on total dry weight in *Solanum lycopersicum* (SLY), *Solanum neorickii* (SNEO) and *Solanum pennellii* (SPE) under control (WW) and combined stress (SS) conditions. Results showed that in tomato MGR was slightly >0, indicating that *F. mosseae* had a positive (even if weak) impact on plant biomass. By contrast, both wild-relatives displayed a MGR value always <0 indicating a lack of mycorrhiza responsiveness at systemic level. Under stress condition only wild species showed significantly lower values compared to plants grown at normal condition.

Marker genes expression

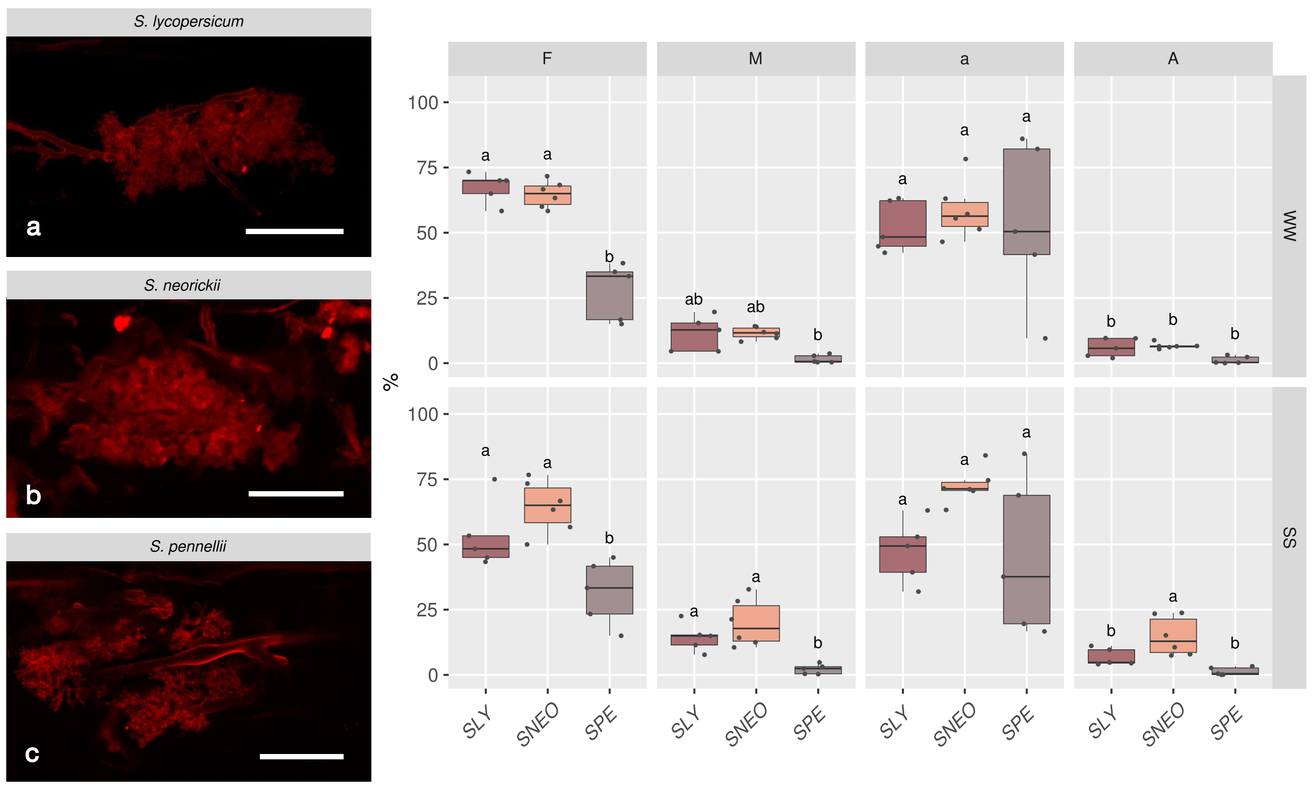


Expression values relative to the ubiquitin-3 housekeeping gene of AM marker genes in Solanum lycopersicum, Solanum neorickii and Solanum pennellii. Differences are indicated across species with different letters according to Tukey's HSD posthoc test after ANOVA (p<0.05). SS= combined stress, WW= control conditions. Genes for AM symbiosis functioning: PT4, FatM; and a F. mosseae reference gene (rRNA) were tested. Y-axis is square root-scaled .

The expression of two AM plant markers and one fungal reference gene was measured using RT-qPCR. In both wild relatives PT4 and FatM genes were highly up-regulated under AM colonization indicating that symbiosis functioning is also maintained at low colonization levels as in the case of *S. pennellii*. No difference was observed between control (WW) and combined stress (WW) conditions.

Interestingly, the up-regulation of PT4 and FatM upon AM colonization was evident only in the two wild relatives but not in the cultivated tomato.

AM colonization



AM colonization features in S. neorickii and S. pennelli wild-relatives compared to tomato 'M82'. (a, b, c) WGA-FITC staining of arbuscules in the three species grown under normal conditions (WW). Scale bar = 30 µm Right panel, quantitative evaluation of mycorrhizal colonization after 'cotton blue' staining. F= frequency of mycorrhization, M=intensity of mycorrhization, a=arbuscules abundance in mycorrhized fragments, A=arbuscules abundance in whole root apparatus. Letters indicate statistically supported differences across species and treatments according to Tukey's HSD posthoc test after ANOVA (p<0.05). SLY= S. lycopersicum cv 'M82', SNEO= S. neorickii, SPE= S. pennellii; WW= control conditions; SS= combined stress.

Results indicate that, although in all species arbuscule morphology is maintained, in *S. pennellii* mycorrhization level was reduced while in *S. neorickii* was similar to tomato 'M82'. Under combined stress mycorrhizal colonization displayed similar values even if *S. neorickii* showed a higher 'a' value indicating that more arbuscules were produced.

Conclusions

- *S. neorickii* and *S. pennellii* wild-relatives showed a negative mycorrhiza growth response compared to the cultivated tomato 'M82', especially under combined stress conditions.
- Since arbuscule morphology is maintained genotype variations probably do not involve the AM signalling pathway (common symbiotic pathway).
- Expression of PT4 and FatM AM markers revealed that symbiosis functioning is maintained across all the three species.
- The high level expression of both PT4 and FatM in tomato 'M82' under non mycorrhizal conditions (already documented in another tomato genotype by Volpe et al. (2018) [4]) can be probably related to higher sensitivity to nutrient starvation in cultivated tomato.
- Taken in the whole, wild relatives result to be less responsive to AM symbiosis, suggesting that - at least for tomato - human breeding has promoted these genetic traits.

Acknowledgments

Research was supported by TOMRES from the European Union's Horizon 2020 research and innovation programme under grant agreement no. 727929.

References

[1] P. Bonfante, A. Genre 2010 Nature Communications, 1:48441.
[2] A. Bolger et al. 2014 Nature Genetics, 46, 1034-1038
[3] N.P. Castañeda-Álvarez et al. 2016 Nature Plants, 2:6022
[4] V. Volpe et al. 2018 Frontiers in Plant Sciences, 9:1482