IDENTIFICATION OF ARBUSCULAR MYCORRHIZAL FUNGI SPECIES PREFERENTIALLY ASSOCIATED WITH GRAPEVINE ROOTS INOCULATED WITH COMMERCIAL BIOINOCULANTS

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Abstract:

Context and purpose of the study – Arbuscular mycorrhizal fungi (AMF) form symbiotic associations with plant roots and can help plants acquire nutrients from the soil in exchange for photosynthetic carbon. Commercial bioinoculants containing AMF are widely available and represent a potential opportunity to reduce the dependence of grapevines on agrochemicals. However, which commercially available AMF species colonize vine roots and affect vine growth remains unknown. The aim of this study was to identify the AMF species from commercial bioinoculants that colonize grapevine roots using high-throughput sequencing, and to evaluate the performance of five commercial bioinoculants and their effects on own-rooted Cabernet sauvignon.

Material and methods – Two-year-old own-rooted Cabernet sauvignon vines were potted into a non-sterile orchard-collected soil and placed in a greenhouse. The silt loam soil was low in available nitrogen, phosphorus, potassium and organic matter and had a neutral pH. Pots were inoculated with one of five commercial bioinoculants. Root length colonized by AMF, petiole nitrogen concentration, plant biomass and root morphology were evaluated. The AMF community present in the grapevine roots growing in non-inoculated (control) and inoculated soil were profiled using high-throughput sequencing of 18S and ITS2 rRNA gene regions.

Results – The proportion of roots colonized with AMF fungal structures significantly increased for plants in inoculated soil, but the degree of colonization differed among commercial bioinoculants. Petiole nitrogen concentration increased and carbon to nitrogen ratio decreased for plants in inoculated soil. Shoot and root dry weight were increased for plants in inoculated soil when compared with plants in non-inoculated soil. Root diameter decreased and root length density and specific root length increased with greater AMF root colonization for plants in inoculated soil. The most predominant genus of AMF species from commercial bioinoculants colonizing roots in the inoculated soil was *Rhizophagus, Glomus* and *Funneliformis*, while *Diversispora, Paraglomus*, and *Mortierella* showed a low relative abundance respectively. A high relative abundance of the genus *Glomus* and *Rhizophagus* were found in roots of plants growing in the non-inoculated soil controls while *Funneliformis*, *Paraglomus* and *Mortierella* were less predominant. Interestingly the endophytic fungus *Mortierella* from the order *Mortierellales* is considered as a beneficial root colonizing fungus. These results suggest that the native AMF community of non-inoculated soil controls had limited effect on grapevine adaptative traits while inoculated soil with commercial bioinoculants containing specific species from the genus *Glomus* and *Rhizophagus* can modify root traits under soil nutrient deficiency and may be considered an alternative to replace chemical fertilizers .

Keywords: Grapevine, bioinoculants, nitrogen, biomass, root traits, meta-barcoding.

1. Introduction

Plant-soil microbial community interactions have the potential to improve grapevine responses to climate change and diseases (Torres et al., 2018) representing a sustainable solution to reduce the dependency of the grape and wine industry on agrochemicals. Arbuscular mycorrhizal fungi (AMF) are among these beneficial microorganisms that are attracting worldwide interest as bioinoculants. AMF are plant symbionts that play various roles in plant and soil health by improving plant nutrient acquisition, increasing plant tolerance to biotic and abiotic stress and promoting soil aggregation (Rillig et al., 2015; Torres et al., 2018). However, the adoption of bioinoculants on specialty crops such as grapevines have been slow because of their inappropriate formulation, poor viability and packaging techniques (Berrutti et al., 2016) along with the inconsistent results improving plant fitness and health (Holland et al. 2018; Rosa et al. 2020) Furthermore, there is no knowledge if commercial available bioinoculants with diverse AMF taxa will maximize the benefits that AMF can provide (Berrutti et al., 2016; Berdeja et al., 2023) as well as which specific AMF species from these bioinoculants are able to colonizes grapevine roots and influence above and below-ground traits. The present study aimed to identify the specific AMF species from commercial bioinoculants colonizing cv. Cabernet sauvignon roots and evaluate their effects on grapevine growth and physiology.

2. Material and methods

Plant material and growing conditions

Plant material and soil - Two-year-old potted cv. Cabernet sauvignon own rooted was grown in Cornell Orchard soil in a completely randomized block design under greenhouse conditions in 2018. The silt loam soil had low organic matter, low mineral nutrient content and adequate pH. Plants were inoculated with five commercial bioinoculants (Table 1) with a non-inoculated orchard soil as a control. The bioinoculants were designated as product 1 (four AMF species), product 2 (nine AMF species), product 3 (nine AM species), product 4, (nine AMF species) and product five (four AMF species). Plants were watered twice a week and supplied with a low-P fertilizer solution to ensure adequate growth. Twenty weeks after inoculation plants were destructively harvested for further analysis.

Plant measurements – Fine root diameter and total root length was measured with image analyzer system (Winrhizo; Regent Instruments Inc., Québec City, QC, Canada). Fine roots were stained according to Koske and Gemma (1989) and root length colonization (RLC) by AMF was quantified according to McGonigle et al (1990). Leaf carbon (C) and nitrogen (N) concentration was determined via combustion analysis (Primacs; Skalar Inc., Buford GA). Root, and shoot biomass were also calculated.

Molecular Analysis – DNA extraction of fine roots samples followed the protocol for the Dneasy Plant mini kit (Qiagen; Science Inc; Germantown, MD). Each DNA sample was prepared for Illumina sequencing of PCR amplicons generated to target fungi with 18S primer WANDA/AML2 (Lee et al., 2008; Dumbrell et al., 2011) and ITS2 primers fITS7:fITS7o/ITS4 (Ihrmark et al., 2012; Taylor et al., 2016). Samples were indexed, multiplexed and sent to the Cornell Institute of Biotechnology (Ithaca, NY) to be sequenced using Illumina MiSeq 300 bp.

Bioinformatic and statistical analysis – The 18S and ITS2 rRNA amplicon sequencing datasets were processed and aligned with the AMPtk PIPELINE V 1.4.1 (Palmer et al., 2018). Operational taxonomic units (OTUs) were determined at the ≥ 97% identity level using the UNOISE3 (Edgar et al., 2010) algorithm. Taxonomic identification of OTUs was performed using the Silva and the built-in UNITE ITS database in AMPtk. All downstream data analysis was conducted in R version 4.0.3 (R Core Team, 2020) with packages Vegan and Phyloseq. Nonmetric multidimensional scaling (NMDS) ordinations were performed with the metaMDS function in vegan and plotted using GGPLOT2. To compare the similarity or differences in fine root AMF composition across all the treatments a PERMANOVA was performed using the adonis2 function in the vegan package. Taxonomy bar plots and alpha diversity metrics for all fungi were conducted in Phyloseq. AMF colonization, leaf nutrient concentration, plant biomass and root morphology parameters were checked for normality and homogeneity of variance

and analyzed using a linear mixed-model analysis of variance and Tukey's honest significant differences, using LME4 and LMERTEST packages in R.

3. Results and discussion

3.1. *Performance of bioinoculants on grapevine roots and plant growth*

The effectiveness of AMF inoculants depend on many factors such as the fungal colonization ability, inoculum with one or more AMF taxa, host plant, soil characteristics and compatibility with native AMF community in the soil (Holland et al., 2018; Rosa et al., 2020; Berdeja et al., 2023). Our results showed that all grapevine roots were effectively colonized. Plants treated with product 5, 4 and 3 resulted in the highest percentage of RLC when compared to control plants with 88%, 84% and 81% respectively (data not shown). Plants inoculated with product 5, 4 and 3 have the greater increase respectively for leaf N concentration and root and shoot biomass (data not shown). These results support the view that AMF benefit plants, either via physiological changes of roots or by a greater translocation of nutrients and water from the fungus to the plant via the extraradical mycelium (ERM) (Torres et al., 2018; Berdeja et al., 2023). The greatest decrease in root diameter and higher root length (data not shown) observed in plants inoculated with products 5, 4 and 2 respectively suggest that AMF symbiosis and significant root morphological plasticity are strategies that grapevines can use to acquire nutrients and water (McCormack and Iversen, 2019; Berdeja et al., 2023) under low soil nutrient conditions.

3.2. *Identification of arbuscular mycorrhizal fungi species from bioinoculants colonizing grapevine roots*

The alpha diversity indices (Chao1, Simpson and Shannon) for the 18S were not significant and the overall fungal richness was similar across all treatments (data not shown). Similarly, the NMDS analysis for the ITS and 18S region showed that there are no differences in the fungal communities between treatments (data not shown), indicating that grapevine roots harbored similar AMF communities. Our results agree with previous studies revealing that inoculation with bioinoculants can instead increase or not impact soil and rhizosphere alpha diversity (Strauss et al., 2015; Jaiswal et al., 2017), one explanation for these contradictory results may be the individual genetic and phenotypic characteristics of the AMF species present in the bioinoculant and the abiotic and edaphic conditions of the environment being inoculated (Garbeva et al., 2004). Regardless of the treatment the AMF community colonizing grapevine roots was represented for a total of five families, including *Glomeraceae*, *Claroideoglomeraceae*, *Diversisporaceae, Paraglomeraceae* and Mortierellaceae. Similar compositional patterns, denoting a clear dominance of Glomeraceae in the soil and root of vines, have been reported (Massa et al., 2020, Carbone et al., 2021). *Rhizophagus intraradices*, *Glomus sp*. and *Funneliformis mosseae* respectively were the most abundant AMF specie colonizing roots of plants growing in the inoculated soil whereas *Paraglomus brasilianum, Claroideoglomus etunicatum* and *Mortierella sp.* were less abundant (Fig 1). A high abundant of *Glomus* sp. and *Rhizophagus intraradices respectively* were found in roots of non-inoculated plants while *Funneliformis mosseae*, Paraglomus laccatum, and *Mortierella sp*. were less predominant. The bioinoculants used in this study contained *Rhizophagus intraradices* and *Funneliformis mosseae and they* are considered generalist species (Berrutti et al., 2016) that are fast, early-stage colonizers and adapted to disturbed systems like agricultural fields and therefore it is not surprising they represented the majority of the AMF community of inoculated and non-inoculated grapevine roots in this study. Interestingly, recent findings suggest that the genus Mortierella plays an important role in soil and plant health, including higher resistance at soil-borne pathogens and improving plant growth (Ozimek and Hanaka 2021).

4. Conclusions

This study showed that not all AMF species from commercial bioinoculants are equal in their ability to increase colonization, nutrient uptake, and biomass accumulation on grapevines. Our findings also revealed that commercial AMF species such as *Rhizophagus intraradices* and *Funneliformis mosseae* from inoculated soil had a stronger effect on grapevine root traits than native AMF community from non-inoculated soil controls under low soil nutrient conditions. As AMF functional traits vary between soil types and environmental conditions, extensive and rigorous field evaluation is needed to validate bioinoculants as an alternative to enhance viticulture sustainability under a changing climatic scenario.

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Table 1: Characteristics of commercial bioinoculants applied to roots of Cabernet sauvignon grown under greenhouse conditions in 2018.

Product no	Arbuscular mycorrhizal fungi species	Other components
1	*Funneliformis mosseae, Rhizophagus aggregatum, Rhizophagus intraradices, Claroideoglomus etunicatum*	N-P-K, humic acids, softwood biochar and worm castings
2	*F. mosseae, Funneliformis monosporum, C. etunicatum, R. aggregatum, R. intraradices, Rhizophagus clarus, Septoglomus deserticola, Gigaspora margarita, Paraglomus brasilianum*	Clay
3	*F. mosseae, F. Monosporum, C. etunicatum, R. aggregatum, R. intraradices, R. clarus, S. deserticola, G. margarita, P. brasilianum*	Ectomycorrhizae (7 species), bacteria (6 species), kelp, humic acids
4	*F. mosseae, F. monosporum, C. etunicatum, R. aggregatum, R. intraradices, R, clarus, S. deserticola, G. margarita, P. brasilianum*	Ectomycorrhizae (7 species), bacteria (4 species), Clay, N-P-K
5	*F. mosseae, C. etunicatum, R. aggregatum, R. intraradices*	Clay

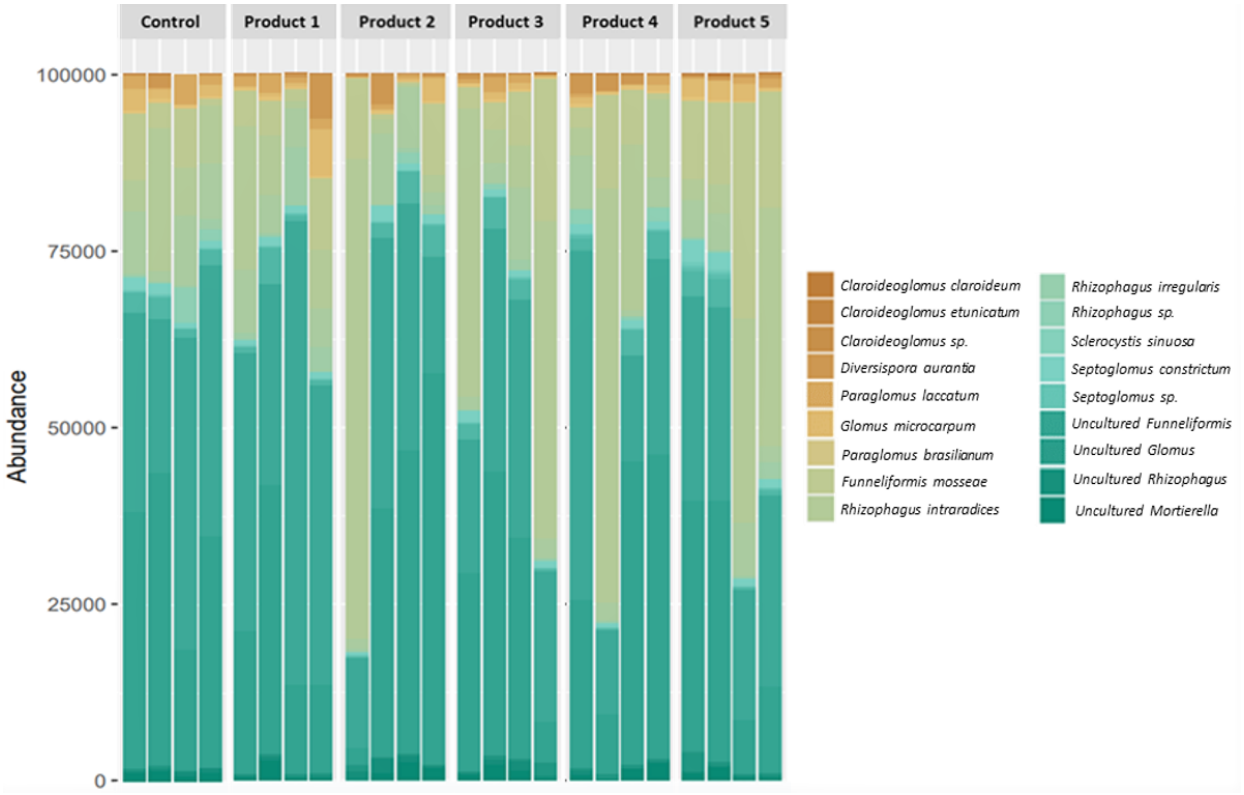

Figure 1: Relative abundance (>1%) of major arbuscular mycorrhizal species colonizing the roots of Cabernet sauvignon inoculated or not with five bioinoculants under greenhouse conditions (n=4) in 2018.