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Identification of arbuscular mycorrhizal fungi in agricultural soil irrigated with tequila vinasses

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1. Introduction. During the production of tequila is generated a waste call vinasse and is estimated that in 2013 were generated 1647 million liters of tequila vinasses. Because of the high concentration of organic matter, it is considered that the application of vinasses can be beneficial to soil, which is a common practice in many tequila factories. However, there is not information about the effects on populations of beneficial organisms as arbuscular mycorrhizal fungi (AMF). Actually, there is no registers that indicate if there are AMF in soils irrigated with tequila vinasses. This fungi (AMF) are symbiotic organisms which establish mutual symbiosis with most higher plants, providing a link between the soil and plant roots (1). The objetives of this work were determine presence of AMF populations and their identifications; and determine abundance and richness of the species of AMF in an agricultural soil irrigated with tequila vinasses frequently.

2. Methods. The study site was located in the "El Arenal", Jalisco (20° 46' 7"N y 103° 41' 18" O), which has been irrigated with tequila vinasses frequently since 5 years. Two samples of soil were taken: with the addition of tequila vinasses (VS) and without addition of vinasses (CS). The soil was classified as Phaeozem, with predominant vegetation of sugarcane (*Saccharum officinarum*) (2), the soils had sandy loam textural classification, with pH 6.5 and 7, organic matter 93,712 and 74,437 mg kg^{-1}, total nitrogen 971 and 1259 mg kg^{-1}, phosphates 0.099 and 0.126 mg kg^{-1} for CS and VS respectively. The tequila vinasses had a pH 3.5, BOD 25,367 mg L^{-1}, COD 57,762.5 mg L^{-1}, settleable solids 120 ml L^{-1}, total volatile solids 37.3 mg L^{-}, TOC 16,800 mg L^{-} and total nitrogen 4,165 mg L^{-}. The AMF spores were isolated from CS and VS using wet sieving method, decanting and centrifugation in water and 50% saccharose (w/v), according to Brundrett et al. (3). The spores were analyzed under a stereomicroscope and mounted in preparations of PVLG and PVLG+Melzer (1:1 v/v) for their identification in an optical microscope followed the descriptions of the International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi I(NVAM) (4).

3. Results. Twenty-one species were identified from CS and VS. The most abundant species were *Acaulospora mellea, A. scrobiculata, Funneliformis geosporum, F. mosseae and Paraglomus occultum* (Figure 1 y 2).

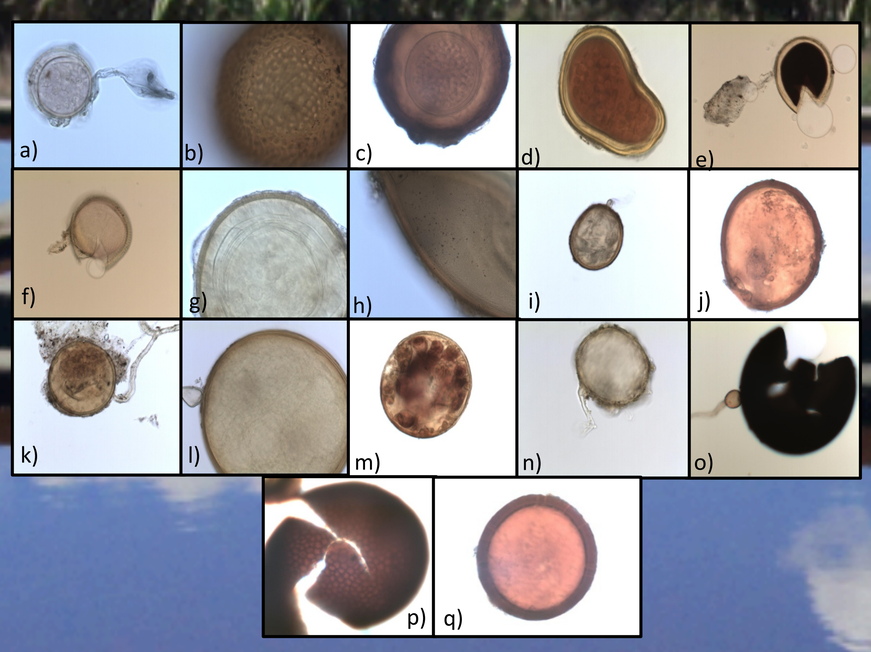

Fig 2. AMF species identified in CS and VS. a) *Acaulospora delicata,* b) *A. foveata,* c) *A.* aff. *laevis*, d) *A. mellea,* e) *A. morrowiae,* f) *A. scrobiculata,* g) *A.* sp., h) *A. spinosa,* i) *Claroideoglomus etunicatum,* j) *Funneliformis geosporum,* k) *F. mosseae,* l) *G. margarita,* m) *Glomus microagregatum,* n) *Paraglomus occultum,* o) *Racocetra gregaria,* p) *Scuttelospora reticulata,* q) *Septoglomus constrictum.*

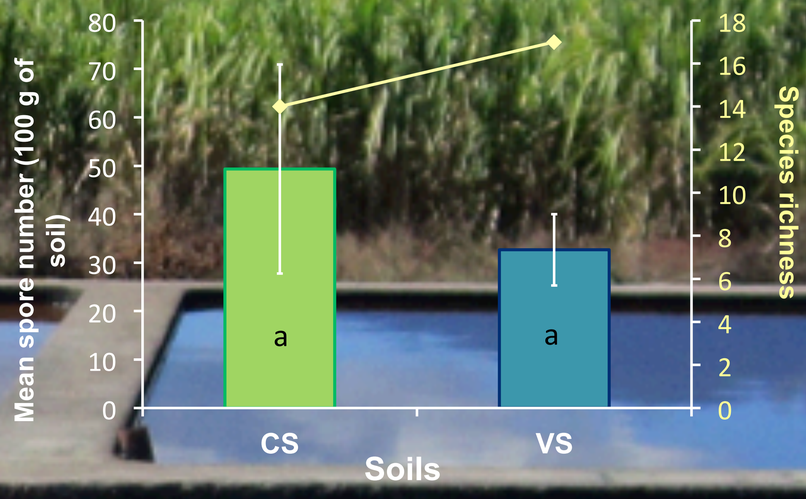

Fig 3. Mean spore number (bars) and species richness (line) of AMF found in vinasse and control soil. Bars with the same letter are not significant different ($P \leq 0.05$).

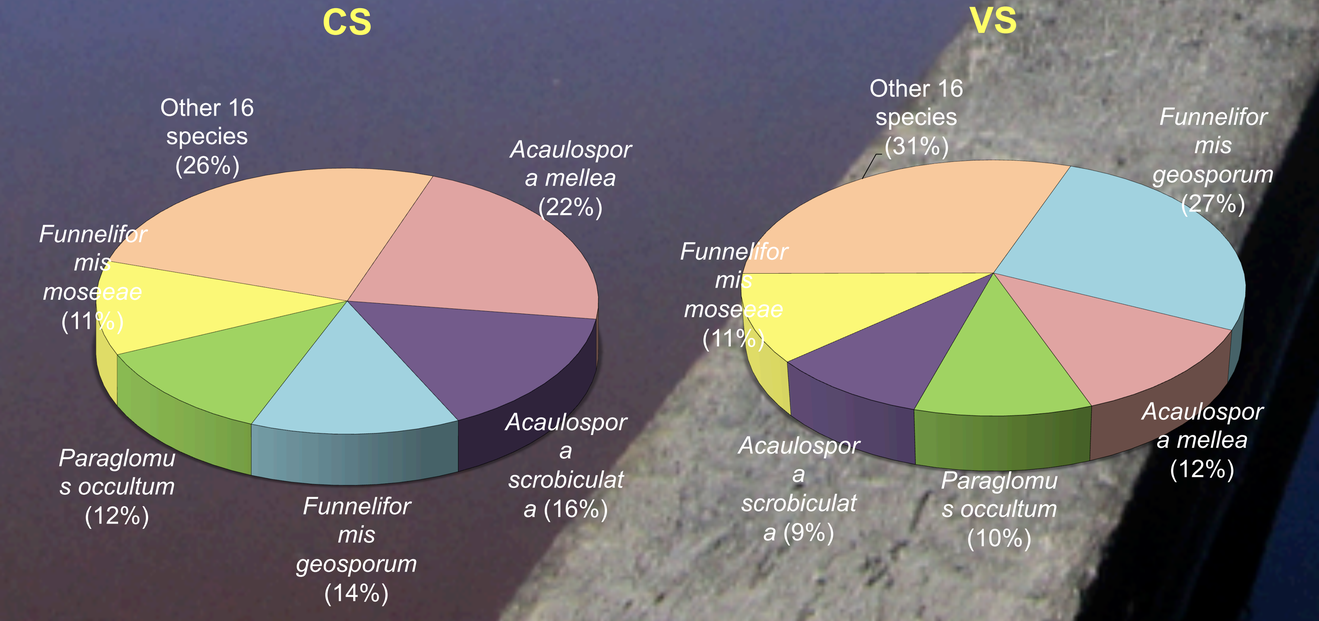

Fig. 1. Relative abundance (% of total spores) of AMF species present in CS and VS.

Species diversity of HMA expressed with the index of Shannon-Wiener (H ') found in SC and SV was not significantly different. However calculating the Shannon-Wiener weighted index (Hp) found that the diversity of the SV was significantly higher than the SC ($P \leq 0.05$) (Table 1).

The control soil had more spores than the vinasse soil, however was not significant different (P ≤ 0.05) (Figure 3 and Table 1). The SV had higher species richness than the SC (Figure 3), where species such as *Clareidoglomus etunicatum*, *Glomus microagregatum* and *Septoglomus constrictum* who were not found in SC.

Table 1. Total number of spores (100 g of soil) and diversity index of AMF in CS and VS.

	CS	VS
Total number of spores (100 g of soil)	1.677 ± 21.57^{A}	1.520 ± 7.37^{A}
Species number (S)	14	17
Shannon-Wiener index (*H'*)	2.24 ± 1.03^{A}	2.33 ± 0.83^{A}
Shannon-Wiener weighted (*Hp*)	0.975^{A}	1.014^{B}

Different letters between columns indicate significant differences ($P \leq 0.05$).

4. Conclusions. Twenty-one species of AMF were identified, where the predominant species were *Acaulospora mellea*, followed by *Acaulospora scrobiculata*, *Funneliformis geosporum*, *Funneliformis mosseaea and Paraglomus occultum.* Therefore, AMF are present in soil which is irrigated with tequila vinasses frequently.

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6. Reference.

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