SUSTAINABLE AND INTEGRAL EXPLOITATION OF AGAVE

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MYCORRHIZATION OF *Agave cupreata* FOR PREVENTION OF WILT CAUSED BY *Fusarium oxysporum*

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) promotes plant growth on most cases through the transport of nutrients from the soil to the roots. In recent decades, they have also been used as biological control agents against various plant pathogens and the results show decreased incidence and / or severity of the disease due to the mycorrhizal symbiosis. Therefore, the aim of this study was to evaluate the effect of mycorrhizal plants *Agave cupreata* in bioprotection against *Fusarium oxysporum*. *A. cupreata* plants seven month old, mycorrhizal and non-mycorrhizal under greenhouse conditions were used to evaluate the effect of bioprotection against *F. oxysporum*. The severity of the disease (SE) based on the observed damage in the leaves (chlorosis and wilting) of plants *A. cupreata* was evaluated by an ordinal scale, at 20 and 90 days after inoculation of the plant pathogen. The results showed colonization of fungi (AMF) in agave plants, regarding bioprotection, plants of *A. cupreata* presented initial wilt symptoms and severity of disease averaged 1.3 to 1.82 (first yellow leaf low, plant damage between 8-21%) with no significant differences (Kruskal-Wallis, $p \leq 0.05$) between plants with and without AMF at 90 d after inoculation with *F. oxysporum* strain FPC. This result was probably due to the physiology of the plant, so more time action pathosystem (mycorrhized agave-*F. oxysporum*) is required to assess the bioprotection of AMF against *F. oxysporum*.

Keywords: *Mycorrhiza, bio-protection, wilt agave.*

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INTRODUCTION

Agave cupreata is a species of economic importance to Mexico, because from the fermentation of stems ("pineapples") of adult plants produced mezcal (Martínez-Palacios y col. 2011). During 2013, mezcal region from Michoacán entered the Denomination of Origin of Mezcal (DOM); which will bring benefits to the mezcal industry. Also, an increase in the cultivated area *A. cupreata* expected, so better agronomic and phytosanitary management will be important to maintain the sustainability of this crop (Martínez-Palacios y col. 2011). However, the increase in crop area could increase the incidence of disease, as has happened in plantations of A. tequilana (Vega-Ramos y col. 2013). One of the phytosanitary problems that significantly affect productivity agave is wilt, caused by the plant pathogenic fungus *Fusarium oxysporum*. The infection process of *F. oxysporum* starts at the roots, then a curl of leaf, death of leaf tips is presented and finally a wilting occurs throughout the plant (Qui-Zapata y col. 2011; Vega-Ramos y col. 2013). This problematic occurs in plant producing regions such as mezcal A. cupreata. Conventional control of F. oxysporum based fungicides has not been effective, also generate environmental problems, damage the soil and increase production costs (Bernal-Alcocer y col. 2005). Therefore, alternatives are required to control power *F. oxysporum* and thus decrease loss of agave. The use of microorganisms has been an alternative for biological control of various plant pathogens, as in the case of arbuscular mycorrhizal fungi (AMF), which are obligate symbionts that need roots plant to complete its life cycle. From this bidirectional symbiosis, the mycorrhizal fungi promotes plant growth by absorbing nutrients (P and N) of the soil to the roots of the plant and the plant turn provides the AMF from a source carbon (Smith and Read, 2008). Furthermore AMF are effective as bioprotectors against various phytopathogens. The results of several studies suggest that in most cases there is a reduction in the incidence and / or severity of disease due to the mycorrhizal symbiosis. For example, Steinkellner y col. (2012) found a bioprotector effect *Funneliformis mosseae* in tomato varieties (*Solanum lycopersicum* L.) against *F. oxysporum* f. sp. *lycopersici* (Fol); Hage-Ahmed y col. (2013) also showed that commercial AMF (Symbivit®) reduced the severity of disease caused by Fol. Hu y col. (2010) by inoculating cucumber (*Cucumis sativus* L.) and a native *F. caledonium* AMF consortium found decreased incidence of F. oxysporum inoculation with native consortium. Jaiti y col. (2007) showed that inoculation with native AMF consortium date palm (*Phoenix dactylifera* L.) decreased plant mortality effect of *F. oxysporum* f. sp. *albedinis*. In Maradol papaya (*Carica papaya* L.), Hernandez-Montiel y col. (2013) found significant difference in reducing the severity of disease caused by *F. oxysporum* when papaya plants were inoculated with a consortium of AMF. These works show that AMF may have bioprotector effect against *Fusarium*. Wherefore, the aim of this study was to evaluate the effect of mycorrhization of *Agave cupreata* by four consortia AMF native and a commercial inoculum (mycorrhizal INIFAP®, MI) in bioprotection against *Fusarium oxysporum*.

METHODOLOGY

A. cupreata seeds were placed *in vitro* conditions for the production of agave seedlings. After ten days, agave plants were placed in trays (38 wells) for growth on a substrate mixture of sand-perlite (4: 1, v: v) sterilized (121°C for 6 h), then was inoculated with 100 spores of AMF under study and placed in the greenhouse until 7 months. After three months after planting, seedlings were fertilized with low in phosphorus nutrient solution (Jarstfer and Sylvia, 1992) biweekly. From the sixth month after planting was stopped applying nutrient solution and watered with distilled water until the end

of the experiment. At the seventh month agave plants were inoculated with *F. oxysporum* at a final concentration of $1x10^4$ cfu g^{-1} (250 mL container with a mixture of sand-sphagnum peat-perlite substrate 4: 1: 1, v: v: v) and remained confined under conditions of growth chamber (8 h photoperiod at 30°C). The evaluation of the experiment was performed at 20 and 90 days after inoculation with the pathogen.

AMF and *Fusarium oxysporum* inoculum

Four native consortia from rhizosphere of *A. cupreata* Michoacán were used: El Huizachal (EH, N 19° 25' 31.4", O 101° 12' 51.4"), Cerro del Metate (CM, N 19° 34' 22.3", O 100° 56' 27.5"), Paso Ancho (PA, N 19° 30' 1.7", O 100° 54' 51.1") y Agua Dulce (AD, N 19° 32' 4.4", O 101° 04' 39.4") and commercial inoculum "Micorriza Inifap" (MI), based de *Glomus intraradices*. F. oxysporum strain FPC at CIATEJ collection was used, which causes wilt in A. tequilana (Qui-Zapata y col. 2011) and previously evaluated as phytopathogenic in A. cupreata (Trinidad-Cruz y col. 2013).

Experimental design and statistical analysis

Experimental design was completely randomized with 12 treatments: 6 levels of AMF: CM, PA, EH, AD, MI without AMF; 2 levels of pathogen: with and without *F. oxysporum*. Each *A. cupreata* plant was a experimental unit and nine replicates of each treatment were evaluated. The degree of severity (SE) of the disease was evaluated following the ordinal scale proposed by De Cal y col. (2000), briefly: 1 = healthy plant; 1.1 to 1.9 = one yellow leaf; 2 = 2.9 more a yellow leaf and a dead leaf, 3 to 3.9 = dead lower leaves and upper yellow leaves; 4 to 4.9 = lower leaves dead and upper leaves wilted and 5 = plant dead. Data from this scale were analyzed using the Kruskal-Wallis test ($p \leq 0.05$) and confidence intervals for the median ($p \leq 0.05$) were calculated using the statistical package StatGraphics Centurion XV.

RESULTS AND DISCUSSION

At 20 days after inoculation (DDI) with *F. oxysporum* significant differences (Kruskal-Wallis, $p \leq 0.05$) between the control treatment with *F. oxysporum* and *F. oxysporum* treatments without (Figure 1 **i**) were found. Subsequently 90 DDI, significant differences between CM, EH and MI treatments more *F. oxysporum* with respect to treatments without plant pathogen were found (Figure 1 **ii**). Was observed that the severity of disease (SE) on the foliage of plants of *A. cupreata* advanced slowly over time. Due to this, the damage at the root caused by *F. oxysporum* (FPC) in agave was visually analyzed in plants with and without AMF (Figure 1 **iii**). Smaller root damage due to FPC was observed in plants with AMF (CM + FPC) compared to non mycorrhizal seedlings (C + FPC) (Figure 1 **iii**). In this regard, Gardezi y col. (2001) evaluated the effect of a native consortium (*Glomus* sp. Zac-19) and *G. aggregatum* in plant bioprotection gladiola (*Gladiolus grandiflorus*) against *Fusarium* sp and found that root rot in plants AMF significantly decreased with respect to plants without AMF.

Montoya-Martínez (2014) *A. tequilana* plants inoculated with native consortium (the rhizosphere of *A. cupreata*) and a commercial for evaluating the effect of AMF bioprotector against *F. oxysporum* strain FPC, found that in plants with and without AMF (100 days after inoculation) showed initial symptoms of disease as curl and necrotic at the tips of the lower leaves and indicated that *A. tequilana* a longer-term development is needed in order to have greater accuracy in results. This result is reinforced in a study of Avila-Miranda y col. (2010) evaluating the virulence of strains of *F. oxysporum* in *A. tequilana* plants and observed initial symptoms (chlorotic leaf curl and wilt) from 200 days after inoculation. So

it physiology agave plants, can delay the symptoms of wilt and therefore the period of observation of these experiments should be extended to longer.

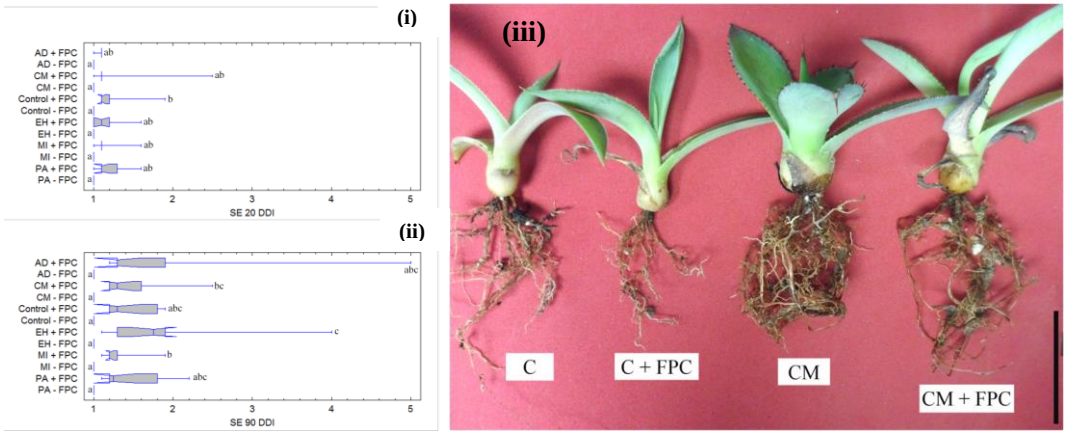

Figure 1. Effect of mycorrhization on mezcal agave plant wilt three months after inoculation with *F. oxyporum* (FPC). **(i)**, **(ii)** Box and whisker graphs of the distribution of the severity scale wilt (SE) of *A. cupreata*, at 20 and 90 days after infection (DDI). Control = without AMF; + FPC, -FPC: with and without *F. oxysporum*; AD: Agua Dulce; CM: Cerro del Metate; EH: El Huizachal; PA: Paso Ancho; MI: Micorriza commercial. **(iii)** Symptoms of wilt in plant *A cupreata* roots inoculated with F. oxysporum at 90 DDI. C: without AMF; C + FPC: without AMF + *F. oxysporum*; CM: Cerro del Metate; CM + FPC: Cerro del Metate + *F. oxysporum*; Bar = 5 cm.

CONCLUSION

Mezcal agave plant (*Agave cupreata*) with and without AMF had similar foliar wilt symptoms to 90 days after inoculation with *Fusarium oxysporum*. A root level damage agave mycorrhizal plant was lower compared to without mycorrhiza plants.

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