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Arbuscular mycorrhiza reduces the negative effects of *M. phaseolina* on soybean plants in arsenic-contaminated soils

Federico Spagnoletti[a,c,*], Marcelo Carmona[b], Natalia E.Tobar Gómez[b,c], Viviana Chiocchio[a,c], Raúl S. Lavado[c]

[a] Cátedra de Microbiología Agrícola, Buenos Aires, Argentina
[b] Cátedra de Fitopatología, Facultad de Agronomía, Universidad de Buenos Aires. Av. San Martín 4453, C1417DSE Buenos Aires, Argentina
[c] Instituto de Investigaciones en Biociencias Agrícolas y Ambientales – INBA (CONICET-UBA), Buenos Aires, Argentina

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ABSTRACT

All crops are negatively affected by several abiotic and biotic stresses, alone or jointly; however, some microorganisms, such as arbuscular mycorrhizal fungi (AMF), are able to alleviate them. Here, we investigated the effects of the AMF *Rhizophagus intraradices* (N.C. Schenck & G.S. Smith) C. Walker & A. Schüßler on soybean plants (*Glycine max* L.) grown in arsenic-contaminated soils and infected by the fungus *Macrophomina phaseolina* (Tassi) Goid., (charcoal rot). Two pots experiments were carried out in a glasshouse, and three levels of As (0, 25, and 50 mg As kg^{-1}) were evaluated. Plant and mycorrhizal parameters, disease severity, glomalin content, and arsenic content in roots and leaves were analyzed. Both arsenic and the pathogen negatively affected soybean biomass and morphological parameters. Moreover, both stresses adversely affected mycorrhizal symbiosis. Low levels of AMF colonization and vitality were observed in high As concentration and in pathogen presence; however AMF inoculation not only reduced the disease but also lowered arsenic accumulation rate in soybean biomass. On the other hands, disease severity was reduced by arsenic. Total glomalin content, produced by the AMF was increased in arsenic-enriched substrates, but was not modified in the presence of the pathogen. Increases in glomalin production could be one of the reasons by which soybean plants accumulate low arsenic amounts while the competition between AMF and the pathogen plays an important role in reducing the disease severity.

1. Introduction

Soybean (*Glycine max* L.) is the fourth most important grain produced in the world, with the USA, Brazil, China and Argentina as the main producers (Masuda and Goldsmith, 2009). As that observed in all other crops, the normal growth and development of soybean are affected by several abiotic and biotic stresses. Because of that, the effects of abiotic stresses like water, salinity, temperature, and toxic elements and compounds on plants have been extensively studied. Abiotic stresses also include the high concentration of Arsenic (As) found in some soils and waters. Arsenic is widely distributed in all types of rocks of the earth crust and it is a significant constituent of groundwater in many countries (Smedley and Kinniburgh, 2002). As a consequence of rock weathering and anthropogenic inputs, As can also be found in soils (Dahal et al., 2008). In Argentina, as well as in other countries, due to the expansion of soybean to marginal areas, the crop is sometimes irrigated with As-contaminated groundwater (Bundschuh et al., 2012; Bustingorri et al., 2015). Arsenic at high concentrations inhibits germination and decreases the chlorophyll content and photosynthesis rate in plants, which in turn lead to decreases in root and aerial biomass growth, including reduced height, tillering or ramifications. Arsenic can also reduce yields and, in extreme cases, plants may die affecting negatively crop production and, sometimes, food safety (food chain contamination) (Rahman et al., 2007; Pigna et al., 2008).

Biotic stressors (weeds, insects, pathogens, and viruses) can also reduce crop yield significantly. Plant pathogens, especially phytopathogenic fungi, are a primary cause of soybean losses worldwide, which have been estimated at an average of 11% per year (Hartman et al., 1999; Oerke 2006). *Macrophomina phaseolina* (Tassi) Goid, is the most common and extensively spread root pathogen and is part of a group of fungi that cause major losses in the global soybean production (Khan, 2007).

In Argentina, at least 40 infectious diseases have been reported to cause severe damage to soybean (Ploper, 2004; Wrather et al., 2010).

* Corresponding author at: Cátedra de Microbiología Agrícola, Buenos Aires, Argentina.
E-mail address: spagnole@agro.uba.ar (F. Spagnoletti).

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Currently, the most prevalent and severe pathologies in soybean are late-season diseases and the root rot caused by *Macrophomina phaseolina* (Carmona et al., 2015; Simonetti et al., 2015). This fungus attacks not only soybean but also more than 500 crops (Gupta et al., 2012; Chamorro et al., 2015). The disease is more damaging during the growing season when the weather is warm and dryer (Mengistu et al., 2011). The most common and frequent signs caused by this disease are the brown discoloration of the pith, stem and taproot and the presence of numerous dark microsclerotia.

Abiotic and biotic stresses usually interact in natural conditions. Llugany et al. (2007) stated that both the pathogen virulence and the plant susceptibility could be affected positively, neutrally or negatively by the simultaneous presence of toxic elements. However, in contrast to pathogenic fungi, other groups of fungi such as arbuscular mycorrhizal fungi (AMF) have shown beneficial effects to crops that growing in biotic and abiotic stressed environments (Smith and Read, 2008).

The arbuscular mycorrhizal symbiosis plays an outstanding positive role both in ecosystems and in agrosystems. AMF can improve plant mineral nutrition and plant-water relations and alleviate contamination effects (Smith and Read, 2008). Sometimes, AMF immobilize toxic elements at the root level, reducing their translocation to the aerial biomass (del Val et al., 1999; Pawlowska et al., 2000; Spagnoletti and Lavado 2015). Also, AMF can sequester those elements by biosorption, a process carried out by glomalin-related soil protein (GRSP), a glycoprotein produced by these fungi, together with extraradical mycelia. Glomalin is able to bind pollutants present in the soil, like Cu, Cd, Pb and Zn, or may immobilize them by passive adsorption to the hyphal cell walls (Vodnik et al., 2008; González-Chávez et al., 2009).

The ability of AMF to sequester and accumulate toxic elements in a non-toxic form may help to increase plant fitness in polluted areas. The metal accumulation and translocation within the plant may vary depending on the toxic element considered and its availability, the occurrence of AMF, the host plant and its root density, and the soil characteristics (Plouznikoff et al., 2016).

On the other hand, AMF have shown interesting results in the biocontrol of several plant pathogens (Saldajeno and Hyakumachi 2011; Elsharkawy et al., 2012; Cruz et al., 2014). In previous research we found that *R. intraradices* is most likely to get involved in the defense response against *M. phaseolina*, but also in the reduction of arsenate to arsenite as a possible detoxification mechanism in AMF associations in soybean (Spagnoletti et al., 2016). The effect of toxic elements like As on mycorrhiza and pathogenic fungi is already known, as well as the relationship between mycorrhiza and pathogen (Hanson et al., 2003; Spagnoletti et al., 2015). However, to our knowledge, little is known about the *crop-pathogen-toxic element-AMF* relationships system and in particular the glomalin role in this interaction. Accordingly we hypothesize that AMF inoculation could partially reverse the toxic effect of As and pathogen disease on soybean plants. The aim of the present research was to investigate the effect of AMF (*Rhizophagus intraradices*) (N.C. Schenck & G.S. Smith) C. Walker & A. Schüßler on soybean plants subjected to As (abiotic stress) and *M. phaseolina* (Tassi) Goid, (biotic stress).

2. Materials and methods

2.1. Characteristics of the substrate and As concentrations

The substrate used in the present study was a mix of sterilized soil: sand: perlite (7:3:2). The soil used to prepare the substrate was a loamy A horizon of a Typic Argiudoll (US Soil Taxonomy) from Solís, Buenos Aires province, Argentina (34°18′ S, 59°20′ W). Before the experiment, the soil was passed through a 2-mm sieve. The physiochemical properties of the substrate were: organic carbon 10.4 g kg^{-1}, available phosphorous 25.3 mg kg^{-1} (Kurtz and Bray No 1 method), pH 6.9, electrical conductivity 0.43 dSm^{-1}, clay 20%, silt 10% and sand 70%. Sodium arsenate was incorporated to the substrate after its tyndallization (three times for 1 h at a temperature of 100 °C) in the concentrations of 0 mg As kg^{-1} (without As), 25 mg As kg^{-1} and 50 mg As kg^{-1}. The concentrations of As were in the range of those causing significant effects on soybean plants, according to Bustingorri et al. (2015). To resemble actual contaminated soils, the interaction between the soluble As applied and the substrate matrix was forced by wetting/drying weekly cycles carried out for two months, as previously described by Bustingorri et al. (2015).

2.2. Pathogenic fungal isolate and culture conditions

A pure culture of *M. phaseolina* was provided by the Plant Pathology Department of the School of Agriculture, University of Buenos Aires (UBA), Buenos Aires, Argentina, from their Fungi Bank. The fungus was grown in Petri dishes with potato dextrose agar (PDA, Difco, Scientific Laboratory Supplies) and incubated in darkness at 28 °C for six days. The fungal mass was multiplied by placing sterilized rice (*Oryza sativa*) seeds in conical flasks and inoculating them with 5 mm mycelial discs with microsclerotia taken from the active periphery of a 7-day-old pure culture of the pathogen. Then, conical flasks were incubated for 3 weeks at 28 °C ± 1 °C.

2.3. Arbuscular mycorrhizal fungi

The AMF (*R. intraradices*) was obtained from a non-As-contaminated area at the Campus of the School of Agriculture, UBA. The strain (VCh 0011) belonged to the culture collection of the Microbiology Department of the School of Agriculture, UBA. The fungus was propagated in pot culture with *Trifolium repens* and *Sorghum bicolor* plants grown in a sterile sandy loam soil for four months in 250 cm^3 pots. These mass-multiplied AMF inocula (colonized root fragments, spores and rhizospheric soil − general inoculum) were prepared to inoculate soybean seeds.

2.4. Greenhouse experiment

Two pots experiments were carried out in a glasshouse at the School of Agriculture, UBA (34°36′S, 58°29′W). Each experiment was considered a block and within them the experimental design was factorial (3 × 2 × 2) at completely randomized design with ten plants (replicates) for each treatment. Three levels of As in soils (0, 25 and 50 mg As kg^{-1}), two levels of inoculation with the mycorrhizal fungus *R. intraradices* (inoculated and non-inoculated) and two levels of infection with the pathogenic fungus *M. phaseolina* (infected and non-infected) were applied to soybean plants.

Soybean (cultivar NIDERA 4990, rhizobial free) was seeded in 1000 cm^3 pots containing 1 kg of the above-described substrate. The substrate was maintained between 70%–80% of field capacity using distilled water. The soybean seeds were superficially sterilized using ethanol 70% and sodium hypochlorite 3% for 3 min each, rinsed several times with sterilized distilled water, and placed in each pot. Before sowing the soybean seeds in pots, 10 g of AMF general inoculum (containing colonized root fragments, rhizospheric soil and approximately 100 spores g^{-1} dry soil) was added to the corresponding treatment at a soil depth of 3–4 cm. The control non-inoculated plants received 10 g of heat-sterilized inoculum (the inoculum was autoclaved twice at 121 °C for 25 min, 24 h apart) plus 10 ml of microbial filtrate (0.45 µm pore size). Soybean plants were partially removed and the pathogen inoculum was applied below each soybean plant at a rate of 8 g after 15 days of growth. Propagule concentration was determined by plating the inoculum on PDA medium. The population of *M. phaseolina* was 1×10^4 colony-forming units (CFU) g^{-1}. The control non-infected plants received 8 g of heat-sterilized *M. phaseolina* inoculum (the inoculum was autoclaved twice at 121 °C for 25 min, 24 h apart). Soybean plants were harvested 70 days after seeded, when they reached the R4 phenological stage (plants with pods completely formed). The

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