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The role of inoculum identity in drought stress mitigation by arbuscular mycorrhizal fungi in soybean
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The role of inoculum identity in drought stress mitigation by arbuscular mycorrhizal fungi in soybean

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Abstract

It is well known that arbuscular mycorrhizal fungi (AMF) effects on plant growth largely depend on fungus identity. The objective of this study was to test whether three individual AMF isolates and their mixture, mitigate drought stress (DS) differentially in soybean (*Glycine max*) genotype, predicting that under DS, the mixture of the AMF isolates would provide greater benefits to soybean plants than individual ones. In a greenhouse experiment, a drought-susceptible soybean genotype was inoculated with *Septoglomus constrictum*, *Glomus* sp. *Glomus aggregatum*, known to be among the most abundant in agricultural and natural soils from central Argentina, and their mixture (*Mx*). Whereas under well watered (WW) conditions individual isolates and *Mx* treatment were similarly infective, under DS conditions, the *Mx* treatment showed lower rates of root colonization. Between WW and DS conditions, biomass was decreased in all treatments, although this effect was more marked in non-AM plants. Moreover, AMF strains improved water content and P and N concentration. Under DS, the *Mx* treatment was unable to exceed the highest contents that were recorded by AMF isolates. However, under WW conditions the *Mx* treatment showed a higher N content than individual isolates. Under

both watering conditions, AM-plants reduced oxidative damage evaluated as malondiadehyde and chlorophyll content and keep constant osmotic metabolites such as soluble sugars and proline content, without significant differences between AMF isolates and the *Mx* treatment. These results show that AMF play an important role in mitigating drought impacts on soybean, but that mixtures of AMF isolates did not perform as well as the best single strain inoculum, excluding complementarity effects and suggesting selection effect of AMF on DS alleviation in soybean.

Keywords: Drought tolerance . Oxidative stress . *Glycine max* . Glomeromycota mixed inocula

Introduction

It is widely recognized that arbuscular mycorrhizal fungi (AMF) community composition can influence plant performance (van der Heijden et al. 2003; Jansa et al. 2008). However, some previous studies indicated that maximun benefits to plants might be achieved with a single, most efficient AMF species (Edathil et al. 1996). More recently, it has been suggested that the benefits of AMF species mixtures on plants may be greater than those of individual isolates (Hoeksema et al. 2010; Verbruggen and Kiers 2010). These benefits can be the result of niche differentiation and facilitation between fungal species (complementary effect) (e.g. Maherali and Klironomos 2007) or the dominant effect of a particular productive single species (selection effect) (e.g. Vogelsang et al. 2006).

Drought is one of the major abiotic stress factors negatively affecting the productivity of soybean (*Glycine max* (L.) around the world. Particularly, Argentina, one of the world leading soybean production countries, has extended its production to less fertile and arid areas than traditionally used, which exposes soybean to disease and drought stress (DS) (Pérez Brandán et al. 2012). The possibility of enhancing drought resistance in plants via inoculation with AMF has been largely investigated (Augé 2001; Rapparini and Peñuelas 2014; Saia et al. 2014). It has been recently shown that AMF inoculations of important agroforestry plants such as *Phaseolus mungo*, *Triticum aestivum*, *Eucalytus tereticornis* and *Albizia procera*, could be more important than soil moisture in improving plant growth (Shukla et al. 2013). Particularly, effects of the inoculation of single AMF isolates on soybean performance exposed to DS have been shown in Porcel and Ruiz-Lozano (2004). However, as far as we known, no study compared the effects of individual AMF isolates and their mixture under well watered (WW) and DS conditions in soybean genotype.

In the present study, we selected isolates of three AMF species, *Septoglomus constrictum* (*Sc*), *Glomus* sp. (*Gs*p.), and *Glomus aggregatum* (*Ga*), known to be among the most abundant in agricultural and natural soils from central Argentina (Urcelay et al. 2009; Grilli et al. 2012; Longo et al. 2014). We compared individual isolates and their mixture effects, through mechanisms of drought avoidance, related to plant growth, mineral nutrition and water absorption (Rapparini and Peñuelas 2014), or drought tolerance related to improved osmotic adjustment by proline (Pro) or soluble sugars (SS) and oxidative damage mitigation (Rapparini and Peñuelas 2014). We have previously shown that AM soybean plants suffer less of oxidative stress due to application of paraquat (PQ) (Bressano et al. 2010). In addition, we showed that the concentration of a product of lipid peroxidation, malondialdehyde (MDA), was decreased in AM soybean plants after PQ treatment.

In this study, we have evaluated under DS whether a mixture of AMF isolates would provide more benefits to soybean plants than individual one. The benefits of AMF make utilization of the symbiosis attractive to sustainable agricultural systems, designed to minimize synthetic inputs (Douds et al. 2010). Since, conventional agroecosystems are enriched with high amount of pesticides and inorganic fertilizers, the AM fungus activity is lower in relation with conservation management systems. Given that agricultural practices affect AMF composition in soils, it is highly relevant to disentangle the effects of fungal identity and composition on DS mitigation and tolerance in cultivated plants (Verbruggen et al. 2013).

Materials and methods

Isolation of AMF and inocula preparation

The selected AMF isolates were *Septoglomus constrictum* (*Sc*) (Trappe) Sieverd, G. A. Silva & Oehl, *Glomus* sp. (*G*sp), and *Glomus aggregatum* (*Ga*) N.C. Schenck & G.S. Sm. All isolates were obtained from the same ecosystem: the experimental field station in Córdoba, Central Argentina (see Urcelay et al. 2009 for details). The spores were extracted from 50 g of each soil sample, according to Daniels and Skipper (1982) and identified under a light microscope, using the morpho-taxonomic criteria of the International Collection of Vesicular and Arbuscular Mycorrhizal Fungi (http://invam.caf.wvu.edu/). To initiate the monosporic cultures, the spores were individually put in pots containing a sterile sand: soil mix (2:1 v/v), employing *Sorghum allepense* as host plant. The pots were put under controlled conditions in the greenhouse at 20-25ºC, and were watered daily with distilled water. After 6 months the newly formed spore populations were assessed to confirm the presence of the

targeted isolate. Each inoculum was then propagated in pots containing soybean plants. Roots and soil from these pots were the source of inoculum for the present experiment.

Growth conditions of an AMF-inoculated, drought -susceptible soybean genotype under different water regimes

The AMF inoculation experiment was setup in a completely randomized 5 x 2 factorial design, with five inoculation treatments and two water regimes: WW and DS conditions. The drought-susceptible soybean genotype 48 belonging to the group IV, was chosen. The five inoculation treatments were: a mock-inoculation control (C = non-AM), single strain inoculation treatments with a single strain of *Sc*, *G*sp and *Ga* and the mixture (*Mx)* of the three AMF species. Each treatment was replicated five times in five liter pots with two soybean plants. The mycorrhizal inoculum consisted of 20 g of soybean root fragments colonized to about 60% of the root length. It was added to the centre of the pot, near the soybean root, at transplantation; non-AM plants treatments received the same amount of autoclaved inoculum. Before autoclaving, the inoculum was filtered with deionized water through a 37-µm sieve (Schleicher & Schuell, Germany). The filtrate was added to the non-AM planting pots to provide them with the microbial populations accompanying the AMF. Plants were grown in a greenhouse at 20-25ºC and watered with distilled water twice a week, to maintaining soil water content close to field capacity during the first 45 days of plant growth. Then half of the pots were normally watered to maintain the substrate water content close to field capacity throughout the entire experiment. The other half of the pots were allowed to dry until 7 % of volumetric soil moisture over 7 days as previously determined in the genotype selection trial. Soil moisture was maintained by weighting the pots and re-supplying any lost water every day. Arbuscular mycorrhizal plants were harvested 60 days after planting.

Biomass, mineral nutrient concentrations, water status and AMF root colonization

Plant biomass was measured as shoot dry mass (SDM), root dry mass (RDM) and total dry mass (TDM) (after drying in a 70ºC oven to constant weight). Shoot diameter (SD) was determined with calipers above the node of the second leaves. Leaf area (LA) was estimated from the fourth trifoliate leaves, the leaflet outlines were traced on a paper, cut out and weighed; those weights were compared with the weight of a known area of paper (1 cm^2). Mineral nutrients were total nitrogen (N) determined following the Kjeldahl method and phosphorous (P) determined by UV-Vis molecular absorption spectrometry as described by Murphy and Riley (1962). Shoot

water content (SWC) was calculated as differences between shoot fresh mass (SFM) and SDM: (SFM-SDM) / SDM x 100. Relative water content (RWC) was calculated as differences between SFM, SDM and shoot turgid mass (STM): RWC = (SFM – SDM)/ (STM – SDM) x 100 according to Barrs and Weatherley (1962). The AMF structures in the roots were stained and colonization measured according to Phillips and Hayman, (1970) and McGonigle et al. (1990), respectively.

Oxidative damage and osmotic metabolites

About 100 mg of the fifth soybean leaves were processed. Oxidative damage was evaluated as lipid peroxidation, and it was estimated as the content of 2-thiobarbituric acid-reactive substances and expressed as equivalents of malondialdehyde (MDA), according to Hodges et al. (1999). Total chlorophyll (TCh) was estimated by extracting the leaf material in 80% ethanol after incubation for 15 min at 80 ^{o}C. Absorbance was recorded at 665, 645 and 470 nm and TCh was calculated according to Arnon (1949). Soluble sugar (SS) content was determined by the anthrone sulfuric acid method described by Mokrasch (1954), by mixing the leaf extract with the anthrone reagent. Absorbance at 620 nm was mesured after boiling the mixture for 5 minutes. Proline concentration was determined by the modified method of Bates et al. (1973) and glutathione concentration was measured as described by Lascano et al. (2001).

Statistical analysis

Data were statistically analyzed by analyses of variance (ANOVA). Differences among means were compared by Fisher least significant difference tests at the significance level of $p \leq 0.05$. All statistical analyses were performed by the InfoStat Professional version 2011. A principal component analysis (PCA) was performed to detect differences among soybean genotypes under three water regimes (3% and 15 of soil moisture, and drought recovery), separating experimental units into subgroups with the aim of identifying the soybean genotype most sensitive to drought

Results

Physiological and biochemical characterization of drought-susceptible soybean genotype

To select a drought-susceptible soybean genotype, an exploratory analysis was performed with the three soybean (*Glycine max* L. Merrill) genotypes 0, 9, and 48 (maturity group III, V and IV, respectively) (see Appendix 1 in supplementary material). As genotype 48 was the most influenced by DS treatment, as shown by its projection on PC 1 and oxidative damage evaluated as MDA content (for details see Appendix 1), it was selected as the most sensitive to DS.

Mycorrhizal colonization and drought avoidance mechanisms in isolates vs mixture AM-drought susceptible soybean genotype

Under both watering conditions inocula of individual isolates and *Mx* strains were infective, with *Ga* strain showing lower root colonization by hyphae and vesicles than *Sc* and *Gsp*. Under WW conditions, the *Mx* treatment was similarly infective to *Sc* and *G*sp treatments (Table 1). When DS developed, the level of colonization did not change among individual isolates as compared to WW condition. However, there was a significant decrease in root colonization by vesicles and arbuscules of the *Mx* strain. Moreover, root colonization by arbuscules in *Mx* strain was lower than *G*sp strain and this showed the greatest ability to increase root colonization by arbuscules under DS. Non-AMF plants showed no root colonization by AMF (Table 1).

Biomass evaluated as root and shoot dry mass was significantly decreased by watering conditions in both non-AM and AM plants (Table 2 and 3). However, non-AM plants were more severely influenced by DS in shoot dry mass, because it was 42 % decreased, while in all AM-plants, it diminished from 22 to 30% (Table 3). By contrast DS affected root dry mass in AM plants , thus while root dry mass decreased about 30 % in all AM plants, in non-AM plants the decrease was 18%. Under both watering conditions shoot and root dry mass in the *Mx* treatment were similar to other isolates, particularly the treatment with *G*sp strain that showed a higher root dry mass than the other isolates. The R/S ratio was significantly increased by DS in non-AM plants, while AM plants showed similar values to those under moister conditions (Table 2 and 3). Moreover, under DS, R/S ratio was significantly lower in AM plants than non-AM plants. No differences in the R/S ratio were evident between individual isolates and the *Mx* treatment, under both watering conditions (Table 3). Arbuscular mycorrhizal fungi identity affected shoot water content under DS but not under WW conditions (Fig. 2). Under DS, *Sc* and *Gsp* treatments increased shoot water content as compared to non-AM plants, but not *Ga* and *Mx* treatments (Fig. 2a). Relative water content was only negatively affected by water regime (Table 2, Fig. 2b).

Regarding nutrients, AM-plants increased mineral nutrients concentration under both WW and DS conditions, as compared to non-AM plants (Table 2). Under WW condition *Ga* and *G*sp treatments significantly increased N concentration, whereas the *Sc* treatment was similar to control. Under DS condition only *G*sp plants exhibited a significant increase in N concentration. Interestingly, plants inoculated with *Mx* under WW condition showed a significantly higher N content than plants inoculated with individual isolates. Under DS, the *Mx* treatment was able to keep high N concentration level, but without to exceed the range of N concentration of plants inoculated with the respective individual isolates (Table 3). Phosphorus concentration was increased under WW condition in all AM-treatments and this level was maintained under DS condition. In both watering conditions the *Mx t*reatments, showed a similar P concentrations to plants inoculated with AMF (Table 3).

Drought tolerance mechanisms in isolates vs mixture AM- drought susceptible soybean genotype

According to ANOVA, oxidative damage evaluated as MDA and TCh content was significantly affected by AMF treatments but not by watering conditions (Table 2). In non-AM plants, the MDA level increased significantly under DS (Fig. 3a), while it remained constant in AM-plants, at both watering conditions, with no differences between isolates and the *Mx* treatment (Fig. 3a). A similar level of TChl content was observed in plants inoculated with AMF isolates and *Mx* strains under WW and DS conditions (Fig. 3b). In turn, osmotic metabolites were significantly affected by watering conditions but not by AMF (Table 2). In AM-plants under DS, whereas no differences in SS content were evident between isolates and Non-AM plants (Fig. 3c), the Pro content was higher in *G*sp and *Ga* inoculated plants (Fig. 3d). As with oxidative damage evaluation, osmotic metabolites in plants inoculated with *Mx* strains, never exceeded the level of SS and Pro content of plants inoculated with individual isolates (Fig. 3c,d).

Discussion

The influence of fungal identity on AM-plants symbiosis is extensily studied (Jansa et al. 2008; Hoeksema et al. 2010; Wagg et al. 2011) and the relationship between AMF richness and plant communities under abiotic stress could be crucial, since AMF may act as insurance under altered environmental conditions (Shukla et al. 2013; Wagg et al. 2011). In this study, we examined the role of three AMF isolates and their mixture on a soybean genotype previously characterized to be susceptible to DS. The three AMF species were selected because they

are abundant and widely distributed in native and agricultural soils in the studied region (Urcelay et al. 2009; Grilli et al. 2012; Longo et al. 2014). To our best knowledge, this is the first report on the role of AMF isolates vs mixture in mitigating DS in a drought- susceptible soybean genotype. Contrarily to our expectations, under DS no complementary effects among isolates were observed on several variables related to growth, tissue nutrient concentration, water balance and alleviation of oxidative stress in the studied soybean genotype.

The percentage of mycorrhizal colonization by hyphae and vesicles was differentially affected by AMF identity (Table 1). In turn, mixed isolates did not show higher root colonization than those observed for individual isolates. These results support previous findings showing that the percentage of AMF colonization by mixtures was not the result of the additive colonization by individual isolates (Jansa et al. 2008). There is some evidence showing that mycorrhizal colonization could be reduced by low soil moisture level (Shukla et al. 2013)). Nevertheless, in our study, all isolates maintained AMF colonization levels under DS stress, albeit in *Mx* plants a decreased in colonization by vesicles and arbuscules was observed (Table 1), showing that DS was able to affect mycorrhizal colonization as observed by Shukla et al. (2013) and suggesting a lower efficiency of root colonization by the *Mx* treatment.

The plant resistance to drought mediated by AMF colonization has often been associated with the AMF promotion of plant growth observed mostly in pot-experiments (Shukla et al. 2013; Rapparini and Peñuelas 2014) but also in field-experiments (Saia et al. 2014). In our study, DS decreased SDM, both in AM and Non-AM treatments, albeit this effect was higher in the latter (Table 2 and 3). In turn, under both watering conditions, RDM diminished in all isolates and the mixture (Table 3). It has been reported that AMF colonization under WW and DS can change specific root length, root architecture and R/S ratio (Auge 2001). Moreover, it is known that, a relatively larger, more finely divided or more efficient root system improves access to soil water and enhances leaf hydration, but this is not needed under stress conditions, because the presence of AMF enhances root functions, as suggested by Cruz et al. (2004). In our study, a reduced RDM in AM plants, was correlated with changes in R/S ratio by comparing Non-AM and AM plants under DS (Table 2 and 3). This is in agreement with findings by Varesoglou et al. (2012), who found that the R/S ratio tends to be lower in AM than in non-AM plants. When DS developed on soybean plants inoculated with the *Mx* strain, biomass evaluated as RDM and SDM and the R/S ratio, were comparable to those inoculated with the single strain inocula (Table 3), suggesting no complementary effect. A similar result, but under WW condition, was observed by Jansa et al. (2008) where the effects of AMF mixtures on plant growth were mostly within the range of the effects exerted by the respective single AMF species. Also, van der Heijden et al. (2003) reported that increased mycorrhizal diversity

did not result in a greater biomass of two naturally coexisting plant grass species and Vogelsang et al. (2006) showed that increasing AMF richness promoted plant diversity, but that this effect was small relative to the effects of individual AMF species.

The role of AM fungal hyphae in water uptake when water is limiting is still a matter of debate (Augé 2001; Smith et al. 2010). In a recent study on barley plants inoculated with *Glomus intraradices*, Ruth et al. (2011) estimated the hyphal water flow at approximately 20 % of the total water uptake of the plant. Here we observed that, in comparison to WW conditions, not surprisingly, DS negatively affected water content in soybean (Table 2; Fig. 2). However, while the effects of AMF inocula did not differ under WW, some AMF strains tended to mitigate the negative effects of DS on water content. But, contrary to our expectations, *Mx* did not perform better than any individual inocula (Fig. 2).

Arbuscular mycorrhizal fungi effects on host growth under DS are often related to improved P plant uptake, as the availability of P in soils is reduced by soil drying (e.g. Auge 2001). In our study, AMF improved both P and N accumulation in plants, as compared to Non-AM treatment, under WW and DS conditions (Table 3). This probably due to the greater surface area for absorption provided by fungal hyphae (Smith and Read 2008; Abbaspour et al. 2012). However, and contrary to our expectations, *Mx* did not perfom better in increasing P level under DS, than any individual inocula (Table 3). Accordingly, van der Heijden et al. (2003) and Vogelsang et al. (2006) found that mixed inoculation with AMF did not improve plant P uptake. In contrast, Jansa et al. (2008) provided direct evidence of functional complementarity among species within the AMF community colonizing a single root system. On the other hand, we did observe a synergistic effect of mixture treatment, under WW condition; for example, the N concentration was greater in the *Mx* treatment than individual isolates, which confirms the complementary effect of mycorrhizal species on plant N uptake (Koide 2000). Interestingly, high N concentration in the *Mx* treatment was maintained under DS. A recent review (Smith and Smith 2011) suggests that mycorrhizal plants can improve N uptake and N transfer from mycorrhizae to the roots when exposed to water-limited conditions. Moreover, in a field experiment, Saia et al. (2014) found that AMF symbiosis increased N content and N fixation of berseem clover subjected to DS.

It is known that DS induces the production of oxidative stress characterized by oxidative damage with an increase in lipid peroxidation and protein oxidation (Cruz de Carvalho 2008). Malondialdehyde is often regarded as the product and an indicator of the degree of membrane lipid peroxidation. Own results showed that mycorrhizal soybean plants were able to mitigate MDA increase after an oxidative stress treatment by Paraquat (Bressano et al. 2010). Chlorophyll concentrations have often been higher in leaves of watered AM-plants than

non AM plants, and also after DS chlorophyll concentrations were usually higher in AM-plants than non-AM plants (Auge 2001). In this study, both AMF isolates and the *Mx* treatment decreased MDA level and increased TCh content under DS (Table 2, Fig. 3a,b), indicating a general mycorrhizal capacity to mitigate oxidative damage under DS conditions. This is consistent with Wu et al. (2006) who reported that DS increased MDA in leaves and roots of *Poncirus trifoliate* in non-AM plants, and that the inoculation with AMF reduced MDA content as well as H_2O_2 and O_2^- in roots and leaves. Similar results about the oxidative damage under DS evaluated as MDA were reported in AM soybean (Porcel and Ruiz Lozano 2004), *Zea mays* (Zhu et al. 2011), and *Casuarina equisetifolia* (Zhang et al. 2010). Our results showed higher TCh content in *Sc* plants under DS than in plants treated with the other isolates (Fig. 3b). This is in agreement with Gong et al. (2013), who found that *Sc* was more efficient at improving ΦPSII of *Sophoradavidii* seedlings than *G. mosseae* under both WW and DS conditions. Regarding the *Mx* treatment, the level of TCh content never exceeded the level of plants inoculated with *Sc* strains (Fig. 3b) and MDA content was similar to plants inoculated with the other individual isolates, suggesting no complementary effects in oxidative stress regulation under DS.

It has been observed that AM-plants accumulate a high concentration of low molecular mass organic solutes, such as SS, Pro or other amino acids to tolerate DS and to regulate the osmotic potential of cells, and this can improve water uptake under DS (Wu et al. 2006). Our data indicated that the concentrations of SS and Pro in leaves increased during DS in both AM and non-AM plants (Table 2).These results are in agreement with previous report (Porcel and Ruiz-Lozano 2004; Abbaspour et al. 2012), whereas no differences in SS concentration between plants inoculated with different AMF isolates were observed (Fig. 3c), Pro concentration was affected by AMF identity under DS, with plants inoculated with *Gsp* and *Ga* showing the higher concentration as compared to non-AM plants (Fig. 3d). The enhanced accumulation of Pro has been linked to AM-induced drought tolerance with Pro acting as osmoprotectant or an effective scavenger of reactive oxygen species (Porcel and Ruiz Lozano 2004; Rapparini and Peñuelas 2014). Conversely, plants treated with *Mx* showed similar SS and Pro content as non-AM plants; in addition they were unable to exceed the highest contents recorded by plants inoculated with AMF isolates, suggesting no complementary effect, although a selection effect cannot be discharged. A lower accumulation of Pro has been observed in AM-plants relative to non-AM plants counterparts suggesting greater drought tolerance or less injury in AM soybean plants (Abbaspour et al. 2012).

It has been shown in a recent meta-analysis that benefits of mixed inocula are in general higher than those of individual isolate inocula (Hoeksema et al. 2010). Such an effect was even observed when only isolates

of the same phylogenetic clade were studied (Gustafson and Casper 2006; Wagg et al. 2011). In this study no complementary effects were observed on variables related to growth, tissue nutrient content, water balance and alleviation of oxidative stress. Then, these results are in line with other recent studies in which the richest assemblage of AMF isolates did not promote either higher plant growth response (Doubková et al. 2013) nor greater tolerance against pathogen stress (Lewandowski et al. 2013) than individual isolates. Despite that several lines of evidence suggest that the existence of functional complementarities among AMF does not exclusively depend on phylogenetic dispersion among isolates (Jansa et al. 2008; Doubková et al. 2013; Lewandowski et al. 2013), we cannot rule out the possibility that the lack of complementarity observed in our study, could be related to the phylogenetic affiliation of isolates (Maherali and Klironomos 2007). On the other hand, the possibility exists that in mixed inocula not all isolates colonized the roots (Wagg et al. 2011) or that only some of them formed arbuscules (Berruti et al. 2013). Whatever the mechanism, our results suggest a selection effects (Wagg et al. 2011).

Conclusion

Despite some differences between the AMF treatments, there were not consistent differences between plants treated with individual and mixed isolates. In contrast to our starting hypotheses, no complementary effect among isolates was observed. Our results suggest that the choice of a particular inoculum provide equal or greater benefits than increasing AMF richness on DS alleviation in soybean, supporting the selection effect of AMF. Finally, despite the fact that conclusions could not be extended to field conditions, the approach adopted here is the best way to answer the questions we asked. In fact, most of the evidences obtained on the effects of AMF on water stress or stress tolerance, including those cited here, were obtained from pot experiments. Our results serve as a first step in our understanding of the role of AMF on stress tolerance.

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Figures

Fig. 1 Physiological and biochemical characterization of the 0, 9, and 48 soybean genotypes subjected to drought (7% soil moisture). **a** Biplot of principal component scores showing the correlative relationships among water content (WC), relative water content (RWC), ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) (GSH/GSSG), shoot diameter (SD), shoot dry weight (SDW), leaf area (LA), shoot length (SL) and malondialdehyde (MDA); **b** percentage of change of malondialdehyde (MDA) and glutathione (GSH/GSSG) concentrations due to the drought stress treatment. Bars with the same colour and the same letter are not significantly different, according to least significant difference tests at $p < 0.05$.

Fig. 2 Effects of inoculating arbuscular mycorrhizal fungal isolates and their mixture on the water content in the shoots of soybean plants, under well-watered and drought (7% soil moisture) conditions: **a** water content; **b** relative water content. *Septoglomus constrictum* (*Sc*), *Glomus* sp. (*G*sp.), *Glomus aggregatum* (*Ga*) and their mixture (*Mx*). Treatments labeled with different letters are significantly different, according to least significant difference tests at $p < 0.05$.

Fig. 3 Effects of inoculating arbuscular mycorrhizal fungal isolates and their mixture on parameters of oxidative damage and osmoregulation in soybean plants, under well-watered and drought (7 % soil moisture) conditions. **a** malondialdehyde concentration; **b** total chlorophyll concentration; **c** soluble sugar concentration; **d** proline concentration. *Septoglomus constrictum* (*Sc*), *Glomus* sp. (*G*sp), *Glomus aggregatum* (*Ga*) and their mixture (*Mx*). Treatments labeled with different letters are significantly different, according to least significant difference tests at $p < 0.05$.

Fig. 4 Mycorrhizal colonization by isolates and their mixture of soybean plants after drought stress treatment. Arbuscular mycorrhizal plants were evaluated 60 days after planting. Letters in the figure are: a and b: *Septoglomus constrictum* (*Sc*), c and d: *Glomus* sp. (*G*sp), e: *Glomus aggregatum* (*Ga*) and f: their mixture (*Mx*).