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ORIGINAL ARTICLE

# Effect of inoculation of symbiotic fungi on the growth and antioxidant enzymes' activities in the presence of *Fusarium subglutinans* f. sp. *ananas* in pineapple plantlets

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**Abstract** The inoculation with symbiotic fungi, Arbuscular mycorrhizal fungi (AMF) and/or *Piriformospora indica* on the growth, nutrient absorption, and induction of antioxidant enzyme activities in plantlets of pineapple 'Imperial' (fusariosis-resistant) and 'Pérola' (fusariosis-susceptible) in the presence of *Fusarium subglutinans* f. sp. *ananas* was investigated. The experiment was comprised by two cultivars, with or without fungal inoculation (*Claroideoglomus etunicatum*, *Rhizophagus clarus*, and *P. indica*, a mixture of all the fungi, and the control—absence of fungal inoculation), with or without applying *Fusarium* conidia, and with four replicates. In both cultivars, nutrient absorption was higher in the AMF plantlets compared to those inoculated with *P. indica* or the control ones, although it was more efficient in 'Imperial' than in 'Pérola'. Inoculation with AMF and/or *P. indica* as well as the pathogen influenced differently the activities of superoxide dismutase, catalase, glutathione reductase, peroxidase, and polyphenol oxidase, in the shoots or roots of pineapple plantlets in both cultivars. Inoculated plantlets with mixture of all the fungi also exhibited a better growth and nutrient absorption, and generally, the 'Imperial' responded better than 'Pérola'. In addition, these plantlets developed better than the control even in the presence of pathogen, indicating that inoculation with AMF and/or *P. indica* may contribute to the production of more resistant propagative material. Increased antioxidant enzyme activity is a potential strategy for managing this plant for explore biological control as an alternative to reduce environmental and health impacts by reducing the use of fungicides.

**Keywords** Arbuscular mycorrhizal fungi · Biological control · *Claroideoglomus etunicatum* · AMF · *Piriformospora indica* · *Rhizophagus clarus*

## Introduction

Biotic and abiotic stresses caused by unfavorable weather conditions and attack by pests and diseases are among the main causes of reduced agricultural crop yield (Waller et al. 2005). Plants are often challenged by a wide variety of pathogens, such as fungi, bacteria, viruses, and nematodes, and the main control strategies include the development of resistant cultivars, crop rotation, application of chemical pesticides and biological controls (Thakur and Sohal 2013), and the production of healthy and high-quality propagation material.

One of the main diseases of the pineapple crop (*Ananas comosus* (L.) Merril) is caused by *Fusarium subglutinans* f. sp. *ananas* (Jacobs et al. 2010). It is estimated to cause 30–40 % fruit production losses and approximately 20 % loss of propagative material (Ventura et al. 2009).

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Vegetative propagation using different propagules of the plant may spread the disease to new areas, causing even higher losses (Be and Debergh 2006; Souza et al. 2013). Thus, using techniques that aid in producing high phytosanitary quality planting material or that make plants more tolerant or even resistant to pathogens in the field is of great economic and environmental importance for the pineapple crop.

The use of microorganisms as biological control agents has been extensively tested to confer resistance to a range of plant pathogens while simultaneously reducing the risk of environmental contamination with chemical pesticides (Pereira et al. 2011; Vanitha and Umesha 2011; Hernández-Montiel et al. 2013; Widmer 2014). Among these microorganisms, arbuscular mycorrhizal fungi (AMF) and *Piriformospora indica* have been identified as alternatives for increasing resistance to pathogens that act as plant growth-promoting agents (Pozo et al. 2002; Waller et al. 2005; Kumar et al. 2009; Hernández-Montiel et al. 2013; Wang et al. 2015) in addition to reducing the stress that micropropagated plants undergo during the acclimatization period (Sahay and Varma 1999; Rai 2001; Kapoor et al. 2008; Yadav et al. 2013).

Among AMF, *Claroideoglomus etunicatum* (W.N. Becker and Gerd.) C. Walker and A. Schüssler and *Rhizophagus clarus* (Nicolson and Schenck) C. Walker and A. Schüssler have been used with success, both for biomass production and nutrients absorption to host plant and to increase plant resistance against biotic and abiotic stresses (Lambais et al. 2003; Santos et al. 2011; Hernández-Montiel et al. 2013; Sennoi et al. 2013). These characteristics were also found in plants colonized by *P. indica* (Waller et al. 2005; Varma et al. 2012; Wang et al. 2015).

Several mechanisms are involved in plant defense stimulated by fungi, among them: increasing in nutrient absorption by plants and compensation for pathogen-caused damages; interactions with soil microbial populations; competition for colonization and infection sites; competition for host photo-assimilates; morpho-anatomical alterations in the root; and activation of plant defense responses (Azcón-Aguilar and Barea 1996; Harrier and Watson 2004; Pozo and Azcón-Aguilar 2007; Wehner et al. 2010; Tahat et al. 2010); and also increasing antioxidant enzyme activity (Pozo et al. 2002; Kumar et al. 2009).

Reactive oxygen species (ROS), such as superoxide anion ($O_2 \cdot^-$) and hydrogen peroxide ($H_2O_2$), are natural byproducts of plant metabolic processes, such as photosynthesis and glycolysis (Nanda et al. 2010; Lushchak 2011), or they can be generated by apoplastic peroxidases and NADPH hydrogenases located in the plasma membrane (Apel and Hirt 2004). These enzymes play an important role in the production of these radicals during the oxidative burst to defend cells from pathogen invasion (Nanda et al. 2010). Their production is usually induced under stress conditions (Thakur and Sohal 2013), and increased levels of ROS may activate various signaling cascades that induce the transcription of defense proteins, have direct toxic effects on the pathogenic agents, and induce the cell death response, which triggers the hypersensitivity process (De Gara et al. 2003; Apel and Hirt 2004; Barna et al. 2012).

Excess ROS is harmful to several plant molecules, such as lipids, proteins, and nucleic acids. Therefore, plants increase antioxidant activity via enzymatic and non-enzymatic pathways to avoid oxidative destruction of plant cell structures (Apel and Hirt 2004; Barna et al. 2012). Increased activity of superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), glutathione reductase (GR, EC 1.8.1.7), peroxidase (POX, EC 1.11.1.7), and polyphenol oxidase (PPO, EC 1.14.18.1) is crucial to reaching an equilibrium stage with the ROS and preventing oxidative damage to cell structures (Kumar et al. 2009; Hernández et al. 2010; Vanitha and Umesha 2011).

AMF and *P. indica* increase the activity of these enzymes in plants (Kumar et al. 2009; Huang et al. 2010; Li et al. 2012; Rozpadek et al. 2014), and plants previously colonized are predisposed to respond more quickly to a possible pathogen attack (Azcón-Aguilar and Barea 1996; Harrier and Watson 2004; Pozo and Azcón-Aguilar 2007; Wehner et al. 2010). Thus, the study aimed to evaluate the effects of inoculation with AMF and *P. indica* on growth, nutrient absorption, and induced antioxidant enzyme activity in the micropropagated 'Imperial' and 'Pérola' pineapple cultivars (fusariosis-resistant and fusariosis-susceptible, respectively), in the presence of *Fusarium subglutinans* f. sp. *ananas*.

## Materials and methods

### In vitro culture

'Imperial' and 'Pérola' pineapple plantlets were subcultivated on Murashige and Skoog (1962) basal medium (MS) for multiplication. The medium was supplemented with 30 g $L^{-1}$ sucrose, 1.8 mg $L^{-1}$ α-naphthaleneacetic acid (NAA), 2 mg $L^{-1}$ indole-3-butyric acid (IBA), and 2.1 mg $L^{-1}$ kinetin (KIN) at pH 5.5. The cultures were grown in 250-mL glass jars containing 15 mL of culture medium and sealed with rigid polypropylene covers. Cultures were kept in a growth room at 26 ± 2 °C under a photoperiod of 16 h light/8 h dark and under an irradiance of 36 µmol $m^{-2}$ $s^{-1}$, provided by white fluorescent lamps. Subcultures were performed every 40 days.

### Fungal inoculants

Isolates of the AMF *Claroideoglomus etunicatum* RJN101A (=*Glomus etunicatum*) and *Rhizophagus clarus* RJN102A (=*Glomus clarum*) were obtained from the International Culture Collection of Glomeromycota (ICCG, http://www.furb.br/cicg) of the Fundação Universidade Regional de Blumenau (FURB), Santa Catarina state, Brazil. Single cultures were established following the procedures adopted at the CICG. Briefly, spores were extracted from trap cultures, separated by morphotypes, and inoculated on the roots of 15-day-old *Sorghum bicolor* seedlings that had been grown on sterilized substrate. Sorghum seedlings were then transplanted to cones (270 $cm^3$) in a sterilized sand:expanded clay:soil (2:2:1 v:v:v) mix and grown for 4 months under greenhouse conditions. After that period, cones were checked for sporulation. Plants were allowed to dry in situ, and the contents of cones were stored in zip lock plastic bags at 4 °C for 6 months. The in vitro *P. indica* culture was obtained from the microbial collection of the Laboratório de Associações Micorrízicas of the Universidade Federal de Viçosa (UFV), Minas Gerais state, Brazil, and was maintained and multiplied in Kaefer medium (KM) (Hill and Kaefer 2001) and stored in the dark at 30 °C (Kumar et al. 2011) for 30 days. The *Fusarium subglutinans* f. sp. *ananas* inoculum was obtained from the Laboratório de Fitopatologia da Central de Laboratórios da Agropecuária, of the Empresa Baiana de Desenvolvimento Agrícola (EBDA), Salvador, Bahia state, and multiplied in potato dextrose agar (PDA) medium and maintained in the dark at 30 °C for 30 days. These spores' suspension was obtained by adding distilled water on the cultures growing in *Petri* dish, transferred to an Erlenmeyer flask. Aliquots from this solution were used for counting spores in Neubauer camera. The conidia concentration was $7.2 \times 10^5$ spores $mL^{-1}$.

### Experimental design and inoculation

The experiment was conducted in a greenhouse, beginning in January (summer time, with average maximum temperature of 41 °C, minimum temperature of 19.7 °C, and humidity 72.4 %) to July (winter time, with maximum temperature of 33.4 °C, minimum temperature of 17 °C, and humidity of 74 %) under a completely randomized design in factorial arrangement ($2 \times 5 \times 2$) containing two pineapple cultivars ('Imperial' and 'Pérola'), five fungal inoculation treatments (*C. etunicatum*, *R. clarus*, and *P. indica*, a mixture of all the fungi (Mix) and an uninoculated control), with or without applying *F. subglutinans* f. sp. *ananas* conidia, and with four replicates.

The substrate was sterilized in autoclave for 1 h at 121 °C, consisted of a soil:sand mixture (1:1 v:v). After nitro-perchloric digestion, Melich 1 extractor was used to analyses *P* and *K*; the extractor KCl 1 mol $L^{-1}$ for Ca, Mg, and Al; Organic Matter (OM) was organic carbon × 1.724—Walkley–Black, obtaining the following characteristics: $pH_{(water)} = 4.9$; $P = 1.9$ mg $dm^{-3}$ (Mehlich 1); $K = 125$ mg $dm^{-3}$; Ca = 0.3 and Mg = 0.1 $cmol_c$ $dm^{-3}$, and Al = 0; sum of bases (SB) = 0.72 $cmol_c$ $dm^{-3}$, organic matter (OM) = 1.2 dag $kg^{-1}$, and $P_{remaining} = 24.7$ mg $L^{-1}$.

Pineapple plantlets (average height of 4.4 cm) with 12–15 leaves were transplanted into 1 kg plastic pots. One plant per pot and four pots per treatment were used. At the moment, the plantlets were transferred into the substrate, and the root was inoculated with a volume of soil containing approximately 200 spores of each AMF, whereas inoculation with *P. indica* corresponded to four 1 cm diameter disks of KM medium containing fungal structures (hyphae and chlamydospores). For the mixed inoculum, the volume of soil used containing approximately 100 spores of each AMF and two 1 cm diameter disks of KM medium containing *P. indica* were used. The control plants did not receive any fungal inoculum. Plants that were not inoculated with *P. indica* received four 1 cm diameter disks containing only KM culture medium.

The moisture content of the substrate in the pots was periodically corrected with distilled water, and 50 mL of Clark's nutrient solution was applied every 20 days (Clark 1975).

After 180 days of cultivation, half of the seedlings in each treatment (cultivar × mycorrhizal fungi) were sprayed with a suspension of *F. subglutinans* f. sp. *ananas* spores containing $7.2 \times 10^5$ spores $mL^{-1}$, whereas the other half was sprayed with distilled water. After applying the spores, the seedlings were covered with transparent polyethylene bags, forming a humid chamber, with minimal wetting time for infection of 96 h, to promote pathogen spore germination. At the end of this period, the seedlings were kept in a greenhouse for 45 more days, after which the evaluations were performed.

### Plant growth and nutritional content evaluations

The following parameters were determined: mean plant height (H), leaf number (LN), shoot fresh, and dry matter (SFM and SDM, respectively). SDM was determined after drying at 70 °C in a forced-air oven until reaching constant weight. Next, SDM was ground in a Willey mill with 0.420 mm sieve and subjected to nitric-perchloric acid digestion (Johnson and Ulrich 1959) to determine nutrient content. P content was determined by the vitamin C method as modified by Braga and Defelipo (1974). For the analysis of N, the mineralization was performed dry way by direct incineration and the sample in muffles and its content was determined by the Kjeldahl method (Embrapa 1999). Ca, Mg, Zn, Fe, and Mn levels were determined by

atomic absorption spectrophotometry. K content was determined by flame photometry, and S content was determined colorimetrically at 420 nm. A portion of the root (500 mg) was used to quantify mycorrhizal colonization, whereas the remaining materials, together with fragments of middle leaves of the seedlings, were used to quantify antioxidant enzyme activity.

### Fungal colonization

Approximately 500 mg of root, cut into 1–2 cm pieces, was treated with 10 % KOH (w:v) for 12 h. Thereafter, the root pieces were washed several times with sterilized distilled water and acidified with 2 % HCl (w:v) for 5 min. Finally, the root pieces were stained with 0.05 % trypan blue in lactoglycerol (w:v) at 70 °C for 15–20 min and storage in lactoglycerol (Phillips and Hayman 1970; Brundrett et al. 1996). Fungal colonization was estimated by the gridline intersect method (Giovannetti and Mosse 1980) under a stereoscopic microscope.

### Extraction and enzymatic activity analyses

For extraction and enzymatic analyses, 500 mg of leaves and root were frozen in liquid nitrogen and triturated in a porcelain mortar with 5 mL of potassium phosphate buffer (50 mM, pH 6.0) containing 1 % of polyvinylpyrrolidone (w:v) at 4 °C. The homogenate was centrifuged at 16,000 *g* for 20 min at 4 °C (NT 805-Microcentrifuge, Nova Técnica, Piracicaba-SP, Brazil). The supernatant was used to measure the enzymatic activities of superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), peroxidase (POX), and polyphenol oxidase (PPO). Protein content was determined by the Bradford method using bovine serum albumin (BSA) as standard (Bradford 1976). The activity of all the enzymes was measured in triplicate at 25 °C using a Multiskan GO Microplate Spectrophotometer (Thermo Scientific), and the results were expressed as U $mg^{-1}$ protein.

To evaluate SOD activity, 980 µL of reaction mixture consisting of sodium phosphate buffer at 50 mM (pH 7.8), 100 mM EDTA, and 10 mM pyrogallol was supplemented with 20 µL of enzymatic extract, and the absorbance was read at 420 nm for 120 s at 60 s intervals (Roth and Gilbert 1984).

The decrease in $H_2O_2$ concentration due to CAT activity was measured according to Beers and Sizer (1952). One 20 µL aliquot of enzymatic extract was added to 980 µL of reaction mixture containing sodium phosphate buffer at 0.05 mM (pH 7.0) and $H_2O_2$ at 1 mM. The decrease in $H_2O_2$ concentration was observed by absorbance decrease at 240 nm with readings every 10 s for a 90 s period.

GR activity was determined, as described by Nordhoff et al. (1993). The reaction mixture contained potassium phosphate buffer at 100 mM (pH 7.8), 2 mM EDTA, 0.2 mM NADPH, and 0.5 mM oxidized glutathione (GSSG). For every 990 µL of the reaction mixture, 10 µL of enzymatic extract was added. NADPH oxidation was observed at 340 nm for 120 s at 60 s intervals.

POX activity was evaluated according to Cakmak and Marschner (1992) with modifications. The reaction solution consisted of 100 µL of enzymatic extract, 125 µL of deionized water, 250 µL of 30 mM $H_2O_2$, 250 µL of 30 mM guaiacol, and 750 µL of 50 mM potassium phosphate buffer (pH 6.0). Activity was determined by the increase in absorbance at 470 nm for 180 s due to the oxidation of the guaiacol.

The reaction mixture for determining PPO activity contained 1.5 mL of sodium phosphate buffer at 100 mM (pH 6.5) and 200 µL of enzymatic extract in addition to 200 µL of catechol at 10 mM to start the reaction. The increase in absorbance was determined at 420 nm for 60 s (Mayer et al. 1966).

### Statistical analyses

This experiment was performed after previews assays to assure the reproducibility of our data. The data were subjected to analyses of variance (ANOVA) at a significance level ($\alpha$) of 5 %, and the means were compared by Student–Newman–Keuls test ($p \leq 0.05$). All results were included factorial analyses with two or three factors ($p \leq 0.05$). The data on mycorrhizal colonization were previously normalized via arcsine$\sqrt{(x/100)}$ transformation prior to ANOVA.

## Results

### Vegetative growth

'Imperial' pineapple plantlets responded positively to AMF inoculation, favoring almost all the parameters evaluated (Table 1). AMF-inoculated 'Imperial' plantlets exhibited better results regarding H, LN, SFM, and SDM compared to 'Pérola' plantlets ($p \leq 0.0245$; $p \leq 0.0044$; $p \leq 0.0402$ and $p \leq 0.044$, respectively) (Table 1), except for H when "Pérola" was inoculated with *R. clarus* or *P. indica*, in the absence of the pathogen, and *R. clarus* or Mix in the presence of *F. subglutinans*, as well as for SFM and SDM when inoculated with Mix. Conversely, 'Pérola' plantlets inoculated with Mix exhibited higher H, LN, SFM, and SDM values in the presence of the pathogen when compared with the treatments of the same cultivar without fungal inoculation (Table 1).

**Table 1** Effect *Claroideoglomus etunicatum, Rhizopagus clarus* and *Piroformospora indica* on plant height (H), leaf number (LN), shoot fresh matter (SFM), and shoot dry matter (SDM) of two pineapple cultivars, 'Imperial' and 'Pérola' grown for 230 days, and inoculated (+) and non-inoculated (−) with *Fusarium subglutinans* f. sp.*ananas* conidia, under greenhouse conditions

| Cultivar | Fungi | *F. subglutinans* | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Plant height (cm) | | Leaf number | | Shoot fresh matter (g) | | Shoot dry matter (g) | |
| | | − | + | − | + | − | + | − | + |
| Imperial | No | 7.28 dA | 6.60 eA | 11.50 bA | 12.50 eA | 2.501 eA | 1.872 cA | 0.449 eA | 0.302 cA |
| | *R. clarus* | 12.67 abA | 13.30 abA | 20.65 aA | 22.00 aA | 19.747 bA | 20.665 aA | 2.520 bA | 2.741 aA |
| | *C. etunicatum* | 14.30 aA | 12.28 abA | 22.50 aA | 18.50 bcB | 26.492 aA | 17.789 aB | 3.886 aA | 2.393 abB |
| | *P. indica* | 7.95 dA | 6.90 eA | 15.75 bA | 13.00 eB | 5.277 deA | 3.060 cA | 0.852 deA | 0.526 cA |
| | Mix | 12.33 abA | 12.78 abA | 20.00 aA | 21.00 abA | 15.040 cA | 19.014 aA | 1.937 bcA | 2.532 abA |
| Pérola | No | 10.33 bcA | 9.18 deA | 15.25 bA | 12.25 eB | 4.532 deA | 4.434 cA | 0.921 deA | 0.823 cA |
| | *R. clarus* | 11.63 abA | 11.93 abA | 15.25 bA | 17.00 cdA | 9.317 dA | 11.592 bA | 1.450 cdA | 1.886 bA |
| | *C. etunicatum* | 8.30 dB | 10.50 bcA | 12.50 bA | 14.75 deA | 4.854 deA | 7.370 bcA | 1.101 deA | 1.120 cA |
| | *P. indica* | 11.85 abA | 9.88 cdA | 12.50 bA | 13.25 eA | 7.248 deA | 4.957 cA | 1.410 cdA | 1.023 cA |
| | Mix | 9.35 cdB | 14.20 aA | 15.50 bB | 18.75 bcA | 6.238 deB | 18.632 aA | 1.020 deB | 2.631 abA |

Means followed by the same lowercase letters, within a column, and the same uppercase letters in each row, within each factor, are not significantly ($p < 0.05$) different by Student–Newman–Keuls tests

The interaction between the cultivars and the AMF showed that the fungi contribute significantly ($p \leq 0.044$) to higher growth of the 'Imperial' plantlets as compared to the 'Pérola' cultivar, exhibiting a 252 % increase ($p \leq 0.044$) in SDM when inoculated with *C. etunicatum* and a 73.80 and 89.9 % increase ($p \leq 0.044$) in SDM when inoculated with *R. clarus* or Mix, respectively, when comparing both cultivars colonized by the same fungus under treatments uninoculated with *F. subglutinans* (Table 1).

Inoculation with *F. subglutinans* negatively affected the LN, SFM, and SDM values ($p \leq 0.0044$; $p \leq 0.0402$ and $p \leq 0.044$, respectively) in plantlets inoculated with *C. etunicatum* in 'Imperial' cultivar, but only LN was negatively affected ($p \leq 0.0044$) for the same cultivar when they were inoculated with *P. indica*. In contrast, for 'Pérola' cultivar inoculated with this pathogen, there was increased ($p \leq 0.00245$) H in plantlets inoculated with *C. etunicatum* and increased H, LN, SFM, and SDM ($p \leq 0.0245$; $p \leq 0.0044$; $p \leq 0.0402$ and $p \leq 0.044$, respectively) for the Mix treatment, but reduced ($p \leq 0.0044$) LN in the plants without any fungal inoculation (Table 1).

## Plant nutritional content evaluation

The evaluation of the nutrient content absorbed by plantlets revealed that there was no interaction ($p > 0.05$) among the three factors (cultivar × fungal inoculation × application of the pathogen), or was there an effect of inoculation with *F. subglutinans* when analyzed separately. However, the interaction cultivar × fungal inoculation was significant ($p \leq 0.01$), showing that inoculation with these fungi exerted different effects on nutrient absorption between the cultivars studied.

There was no difference ($p \leq 0.01$) in nutrient content between the cultivars in the plantlets uninoculated with fungal inoculation except for Mn, whose content was higher ($p \leq 0.001$) in 'Pérola' (Table 2). Nutrient absorption was unaffected by inoculation with *P. indica* in both cultivars (Table 2).

In general, the nutrient content was higher ($p \leq 0.001$) in the plantlets inoculated with *R. clarus* followed by *C. etunicatum*, or Mix in both cultivars, being higher ($p \leq 0.001$) than the plantlets noinoculated in almost all evaluated nutrients (Table 2). The effect of the absorption of nutrients in plants inoculated with AMF was larger ($p \leq 0.001$) in the 'Imperial' cultivar than in 'Pérola'. The N content of the 'Imperial' cultivar increased ($p \leq 0.001$) by 392, 436, and 299 % when inoculated, respectively, with *R. clarus*, *C. etunicatum*, or Mix, whereas it increased ($p \leq 0.001$) by 79, 15, and 120 % for 'Pérola' when compared to their respective controls within each cultivar. The P content in 'Imperial' increased ($p \leq 0.001$) by 1348, 1265, and 1098 %, and the K content increased ($p \leq 0.001$) by 766, 761, and 594 % for *R. clarus*, *C. etunicatum*, and Mix, respectively. For 'Pérola', the P content increased ($p \leq 0.001$) by 230, 103, and 298 %, and the K content increased ($p \leq 0.001$) by 86, 12, and 114 %, respectively (Table 2).

For Ca, Mg, S, and Mn, the *R. clarus*, *C. etunicatum*, and Mix treatments continued to be more efficient

**Table 2** Effect of *Claroideoglomus etunicatum*, *Rhizopagus clarus* and *Piroformospora indica* on nutrient content of two pineapple cultivars, 'Imperial' and 'Pérola', grown for 230 days under greenhouse conditions

| Fungi | Imperial N (mg plant$^{-1}$) | Pérola N (mg plant$^{-1}$) | Imperial P (mg plant$^{-1}$) | Pérola P (mg plant$^{-1}$) | Imperial K (mg plant$^{-1}$) | Pérola K (mg plant$^{-1}$) | Imperial Ca (mg plant$^{-1}$) | Pérola Ca (mg plant$^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| No | 5.80 cA | 9.51 bA | 0.10 bA | 0.26 bA | 10.93 cA | 24.01 bA | 1.65 bA | 3.91 bA |
| *R. clarus* | 28.56 aA | 17.10 aB | 1.41 aA | 0.87 aB | 94.71 aA | 44.84 aB | 14.14 aA | 7.44 abB |
| *C. etunicatum* | 31.14 aA | 10.95 bB | 1.33 aA | 0.54 bB | 94.19 aA | 27.07 bB | 14.77 aA | 4.76 bB |
| *P. indica* | 8.71 cA | 11.59 bA | 0.23 bA | 0.39 bA | 24.28 cA | 30.59 bA | 2.85 bA | 4.29 bA |
| Mix | 23.17 bA | 20.99 aA | 1.16 aA | 1.05 aA | 75.95 bA | 51.49 aB | 11.89 aA | 8.54 aB |

| Fungi | Imperial Mg (mg plant$^{-1}$) | Pérola Mg (mg plant$^{-1}$) | Imperial S (mg plant$^{-1}$) | Pérola S (mg plant$^{-1}$) | Imperial Fe (μg plant$^{-1}$) | Pérola Fe (μg plant$^{-1}$) | Imperial Mn (μg plant$^{-1}$) | Pérola Mn (μg plant$^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| No | 0.65 cA | 1.52 bA | 1.40 cA | 4.83 aA | 1.21 bA | 1.64 aA | 148.71 cB | 369.72 aA |
| *R. clarus* | 8.31 aA | 3.03 abB | 16.72 aA | 10.63 aB | 2.30 aA | 1.73 aA | 758.10 bA | 371.77 aB |
| *C. etunicatum* | 8.82 aA | 1.69 bB | 9.64 bA | 5.25 aA | 1.22 bA | 1.18 aA | 1009.18 aA | 205.62 aB |
| *P. indica* | 1.40 cA | 1.81 bA | 2.12 cA | 4.85 aA | 1.34 bA | 1.02 aA | 269.59 cA | 269.10 aA |
| Mix | 5.76 bA | 3.47 aB | 15.77 aA | 7.49 aB | 2.39 aA | 1.13 aB | 591.15 bA | 265.11 aB |

Means followed by the same lowercase letters, within a column, and the same uppercase letters in each row, within each factor, are not significantly ($p < 0.05$) different by Student–Newman–Keuls tests

($p \leq 0.001$; $p \leq 0.001$; $p \leq 0.003$; $p \leq 0.001$, respectively) at absorbing nutrients and accumulating them in the 'Imperial' plantlets. However, for 'Pérola', only those inoculated with Mix exhibited higher Ca and Mg absorption, whereas the content of the remaining nutrients did not differ from the controls (Table 2).

Plantlets inoculated with *R. clarus* were overall more efficient ($p \leq 0.001$) at absorbing nutrients in both cultivars (Table 2). The plantlets inoculated with *C. etunicatum* were as efficient ($p \leq 0.001$) at absorbing N, P, K, Ca, Mg, and Mn as those inoculated with *R. clarus* in 'Imperial', and they were as efficient at absorbing Ca, Mg, S, Fe, and Mn as those inoculated with *R. clarus* in 'Pérola'. In turn, the plantlets inoculated with Mix exhibited increased efficiency for P, Ca, S, and Fe ($p \leq 0.001$; $p \leq 0.001$; $p \leq 0.003$; $p \leq 0.0052$, respectively) absorption in 'Imperial' and for absorption of all the macronutrients, except for S, in 'Pérola' as well as in plantlets inoculated with *R. clarus* (Table 2).

## Enzymatic activity

Inoculation of both cultivars with these fungi, with or without applying *F. subglutinans*, affected antioxidant enzyme activity in the root and in the shoots differently, wherein the activity resulting from the treatments applied was specific for each enzyme (Fig. 1).

There was an interaction between the factors cultivar, fungal inoculation, and application of *F. subglutinans* for SOD enzymatic activity in both the root ($p \leq 0.002$) and the shoots ($p \leq 0.006$). Regarding SOD activity in the root, 'Imperial' and 'Pérola' cultivar plantlets that were uninoculated did not differ from each other regardless of pathogen inoculation status; only the 'Pérola' control exhibited higher ($p \leq 0.002$) SOD activity when inoculated with *F. subglutinans* spores compared to the uninoculated 'Pérola' control (Fig. 1a). Without *F. subglutinans* application, 'Pérola' inoculated with *P. indica* provided the highest ($p \leq 0.002$) SOD activity, whereas there was no difference ($p < 0.05$) among the other treatments (Fig. 1a).

'Imperial' plantlets inoculated with *R. clarus* and 'Pérola' plantlets inoculated with *C. etunicatum* or Mix, which were also inoculated with the pathogen, had the most increased SOD activity in the root. In comparing the same treatment with the only difference being the application or not of the pathogen, the presence of *F. subglutinans* increased ($p \leq 0.002$) SOD activity in 'Imperial' inoculated with *R. clarus* and in 'Pérola' that were uninoculated with these fungi or inoculated with *C. etunicatum* or Mix. In turn, in 'Pérola' inoculated with *P. indica*, SOD activity was higher ($p \leq 0.002$) without *F. subglutinans* than in the presence of the pathogen (Fig. 1a).

In 'Pérola', SOD activity in the shoots when inoculated with any fungus was higher than 'Pérola' control and all the 'Imperial' treatments in the absence of the pathogen ($p \leq 0.002$) (Fig. 1b). In the presence of *F. subglutinans*, only 'Pérola' inoculated with *P. indica* exhibited higher ($p \leq 0.006$) SOD activity compared to the other treatments (Fig. 1b).

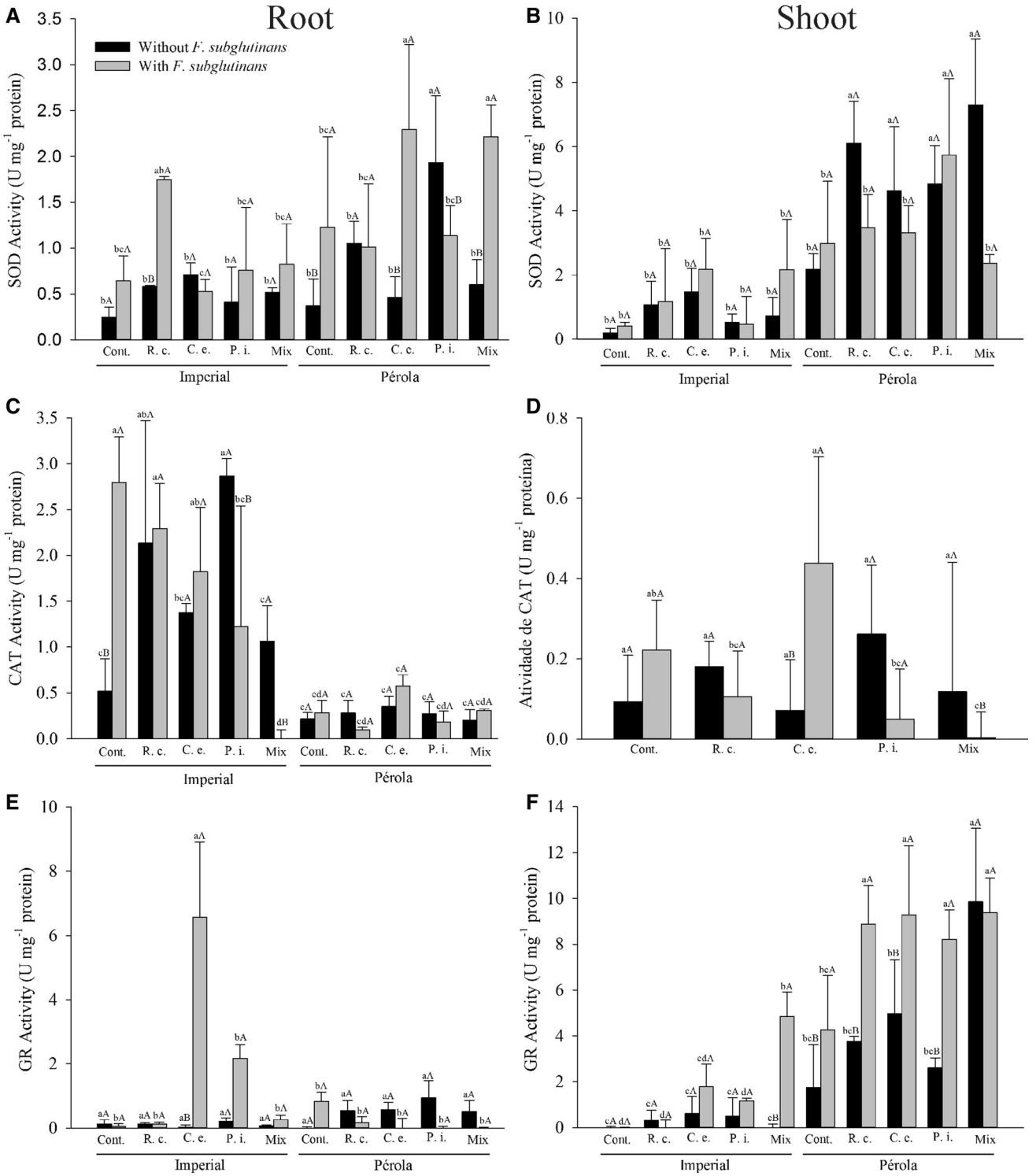


**Fig. 1** Enzymatic activity of superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR) in the root and shoots of pineapple plantlets ('Imperial' and 'Pérola') at 230 days after fungal inoculation. Cont. (control), R.c. (*Rhizophagus clarus*), C.e. (*Claroideoglomus etunicatum*), P.i. (*Piriformospora indica*), and Mix. Means followed by the *same lowercase letter* are not significantly ($p < 0.05$) different by Student–Newman–Keuls test and compare all of the treatments of both cultivars with or without inoculation with the pathogen separately. Means followed by the *same uppercase letter* are not significantly ($p < 0.05$) different by Student–Newman–Keuls test and compare a given treatment in 'Imperial' with the same treatment in 'Pérola' regarding the effect of inoculation with the pathogen

CAT activity in the root was higher ($p \leq 0.001$) in 'Imperial' plantlets inoculated with *P. indica* or *R. clarus* compared to the uninoculated 'Imperial' or inoculated with *C. etunicatum* or Mix and compared to all the 'Pérola' cultivar treatments in the absence of the pathogen (Fig. 1c). There were no differences ($p < 0.05$) in CAT activity in the root among the 'Pérola' treatments, even when applying *F. subglutinans* conidia (Fig. 1c). In contrast, applying the pathogen increased CAT activity in 'Imperial' plantlets uninoculated and decreased CAT activity when they were inoculated with *P. indica* or Mix. There were no differences ($p < 0.05$) among the other treatments in either cultivar (Fig. 1c).

Regarding CAT activity in shoots, there were no differences ($p < 0.05$) among the cultivars occurring only interaction between the plantlets inoculated these fungi and the application or not with *F. subglutinans* (Fig. 1d). There was no difference ($p < 0.05$) in the treatments without the application of the pathogen's conidia. In contrast, when the plantlets were inoculated with the pathogen, there was increased ($p \leq 0.0022$) CAT activity in those inoculated with *C. etunicatum* and in the controls compared to the other treatments, but only the plantlets inoculated with *C. etunicatum* exhibited higher ($p \leq 0.0022$) activity compared to the same treatment without the pathogen (Fig. 1d).

GR enzyme activity in the root of the plantlets remained unaffected by fungal inoculation in both cultivars in the absence of the pathogen. However, after applying *F. subglutinans* conidia, the 'Imperial' plantlets with *C. etunicatum* exhibited increased enzyme activity that was higher ($p \leq 0.0005$) than all the remaining treatments (Fig. 1e). GR activity in shoots was higher ($p \leq 0.0005$) for 'Pérola' with or without the application of the pathogen. In the presence of *F. subglutinans*, only the 'Imperial' cultivar treatment inoculated with Mix exhibited increased ($p \leq 0.0005$) GR activity compared to the other 'Imperial' treatments, matching the 'Pérola' control (Fig. 1f). After applying the pathogen, the 'Pérola' plantlets inoculated with *R. clarus, C. etunicatum*, and *P. indica* showed increases of 2.36, 1.86, and 3.66 times compared to the respective treatments without *F. subglutinans*, which were, together with Mix, significantly ($p \leq 0.0005$) higher than the 'Imperial' cultivar plantlets (Fig. 1f).

There was no effect of the cultivars on POX activity in the root, although there was an interaction between fungal inoculation and presence of the pathogen. There was no difference ($p \leq 0.05$) among the treatments that received the fungal inoculation and the control in the absence of *F. subglutinans*. However, the plants inoculated with *C. etunicatum* or the Mix, in the presence of the pathogen, exhibited higher ($p \leq 0.0389$) POX activity compared to the other treatments and also compared to inoculation with the same fungus, but in the absence of *F. subglutinans* (Fig. 2a). POX activity in the shoots remained unaffected by fungal inoculation even in the presence of the pathogen (Fig. 2b). The only difference observed for POX activity in the shoots was the higher activity ($p \leq 0.0001$) found in 'Imperial' (87 %) compared to 'Pérola' regardless of fungal inoculation or the presence of *F. subglutinans*.

Inoculation with *F. subglutinans* did not affect PPO activity in the root, in which only the interaction between cultivars and fungal inoculation was affected. The 'Imperial' treatment inoculated with Mix exhibited lower ($p \leq 0.0051$) PPO activity compared to the seedlings inoculated with *C. etunicatum*. Whereas for 'Pérola', the plantlets inoculated with *R. clarus* and *P. indica* had the lowest ($p \leq 0.0051$) PPO activity (Fig. 2c). When comparing seedlings of different cultivars inoculated with the same fungus, 'Imperial' inoculated with *R. clarus* or *C. etunicatum* exhibited significantly ($p \leq 0.0051$) higher PPO activity than 'Pérola' under the same conditions, whereas there was no difference among the other treatments (Fig. 2c). In contrast, there was no effect on the PPO activity in shoots of seedlings regardless of any treatment (cultivar, fungal inoculation, or inoculation with *F. subglutinans*) (Fig. 2d).

## Fungal colonization

Fungal colonization was obtained for plants inoculated with AMF and with *P. indica* in both cultivars. The controls did not exhibit structures characteristic of fungal colonization.

Inoculation of the plantlets with *F. subglutinans* did not affect fungal colonization in either cultivar, but the interaction between cultivar and these fungi inoculated was observed.

Colonization with *R. clarus* or Mix was higher ($p \leq 0.0018$) in the 'Imperial' cultivar plantlets, whereas the highest percentages of mycorrhizal colonization in 'Pérola' were obtained in all plantlets inoculated with all AMF (Fig. 3). In comparing the cultivars when colonized by the same fungus, *R. clarus* and Mix resulted in significantly higher ($p \leq 0.0018$) colonization percentage for 'Imperial' than for the 'Pérola' cultivar. In contrast, 'Pérola' plantlets exhibited higher ($p \leq 0.0018$) colonization percentage than 'Imperial' plantlets when colonized by *C. etunicatum* under the same conditions. Regarding colonization with *P. indica*, there was no difference between the cultivars. The colonization percentage of *P. indica* was always lower ($p \leq 0.0018$) compared to the seedlings inoculated with AMF (Fig. 3).

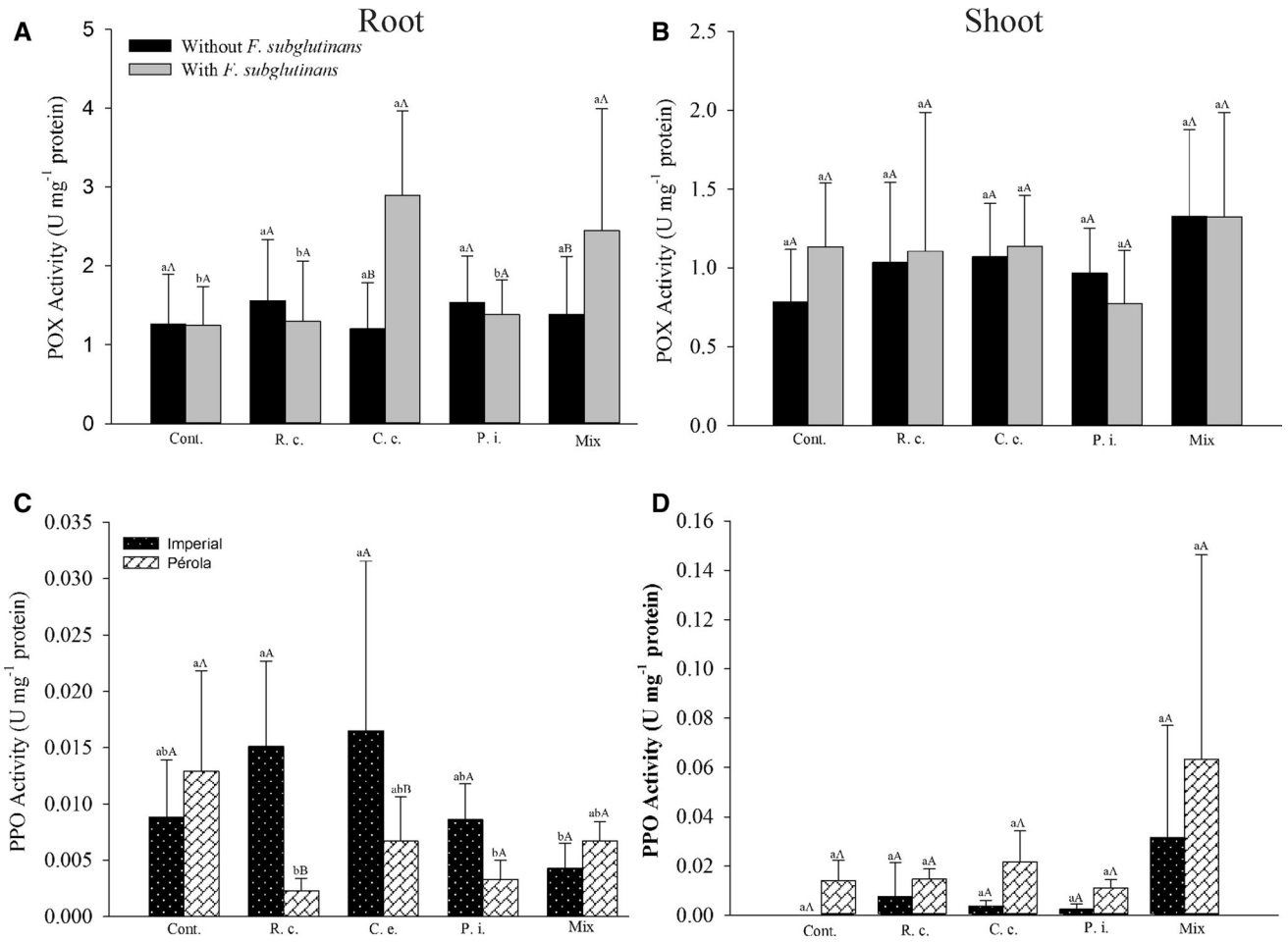


**Fig. 2** Enzymatic activity of peroxidase (POX) and polyphenol oxidase (PPO) in the root (**a**, **c**) and shoots (**b**, **d**) of pineapple plantlets ('Imperial' and 'Pérola') at 230 days after fungal inoculation. Cont. (control), R.c. (*Rhizophagus clarus*), C.e. (*Claroideoglomus etunicatum*), P.i. (*Piriformospora indica*), and Mix. In **a** and **b**, means followed by the *same lowercase letter* are not significantly ($p < 0.05$) different by Student–Newman–Keuls test and compare the effect of inoculation or not with *F. subglutinans* on the treatments with AMF or *P. indica* regardless of the cultivar, and means followed by the *same uppercase letter* are not significantly ($p < 0.05$) different by Student–Newman–Keuls test and compare the effect of inoculation with *F. subglutinans* f. sp. *ananas* within the same treatment with AMF or *P. indica*. In C and D, means followed by the *same lowercase letter* are not significantly ($p < 0.05$) different by Student–Newman–Keuls test and compare the effect of inoculation with AMF or *P. indica* within each cultivar separately, and means followed by the *same uppercase letter* are not significantly ($p < 0.05$) different by Student–Newman–Keuls test and compare the effect of each treatment between the pineapple cultivars

## Discussion

This study is the first to compare the effects of AMF and *P. indica* on the growth, nutrient absorption, and production of enzymes related to the increased resistance to *F. subglutinans* f. sp. *ananas* of both pineapple cultivars, one resistant to the pathogen and one susceptible. Other studies in the literature have demonstrated beneficial effects of AMF on the growth and nutrition of pineapple plantlets (Gutiérrez-Oliva et al. 2009; Rodríguez-Romero et al. 2011; Santos et al. 2011; Kunze et al. 2014) and only Moreira et al. (2015) considering association with AMF and *P. indica*.

The highest vegetative growth in colonized by fungi pineapple plantlets, in the present study, directly reflects the benefits of the fungal association. These fungi have shown numerous benefits in various studies in the literature as plant growth-promoting agents (Azcón-Aguilar et al. 1997; Kumar et al. 2011), being able to exploit higher soil volume (Smith and Read 1997) and improve the water relationships of plants (Augé 2001; Varma et al. 2012) and their nutrient uptake (Smith et al. 2010; Varma et al. 2012), especially that of phosphorus (P) (Waller et al. 2005; Smith et al. 2010; Santos et al. 2011).

The presence of the pathogen affected the relationship between the cultivars and the studied fungi. For 'Pérola'

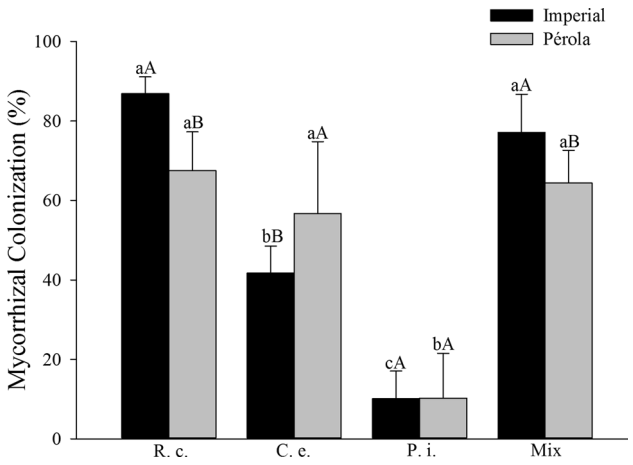


**Fig. 3** Fungal colonization rate (%) of pineapple plantlets at 230 days growing in a greenhouse. Cont. (control), R.c. (*Rhizophagus clarus*), C.e. (*Claroideoglomus etunicatum*), P.i. (*Piriformospora indica*), and Mix. Means followed by the same lowercase letter are not significantly ($p < 0.05$) different by Student–Newman–Keuls test and compare the effect of inoculation with AMF or *P. indica* within each cultivar separately. Means followed by the *same uppercase letter* are not significantly ($p < 0.05$) different by Student–Newman–Keuls test and compare the effect of each treatment between the pineapple cultivars

plantlets, in the absence of the pathogen, the treatments did not differ from the control for many of the parameters evaluated. However, when the pathogen was presented, the pineapples plantlets inoculated with Mix showed a better development compared to controls, reducing the harmful effect of the pathogen on susceptible cultivar. This indicates that the fungal colonization minimized the decrease in growth caused by inoculation with *F. subglutinans*. Different responses to fungal colonization may be obtained depending on plant variety (Gupta et al. 2002) and are conditioned by symbiotic fungus-plant compatibility (Pouyu-Rojas et al. 2006).

Nutrient absorption was considerably affected by association with AMF in both the 'Imperial' and 'Pérola' cultivars. It has already been reported that association with *Funneliformis mosseae* (=*Glomus mosseae*) (Nicolson and Gerdemann) C. Walker and A. Schüessler in 'Smooth Cayenne' pineapple plantlets increases the absorption of N, P, and K (Rodríguez-Romero et al. 2011) as well as the association with mixed inoculum *R. clarus* and *Gigaspora margarita* Becker and Hall also increased the content of these nutrients in relation to non-inoculated plants in experiments with cultivars 'Smooth Cayenne', 'Pérola', and 'Jupi' (Santos et al. 2011). This shows that mycorrhization is important for this crop's nutritional status. Several nutrients are absorbed better in mycorrhized plants and have been addressed in several studies, such as P (Rodríguez-Romero et al. 2011; Tong et al. 2013; Xie et al. 2014), N (Oelmüller et al. 2009; Rodríguez-Romero et al. 2011; Tong et al. 2013), K (Rodríguez-Romero et al. 2011), S (Oelmüller et al. 2009), Cu and Zn (Karagiannidis et al. 2011; Tong et al. 2013), Ca, Fe, and Mn (Karagiannidis et al. 2011), although results vary depending on the inoculated fungus and plant variety (Karagiannidis et al. 2011).

Increased nutritional status, root growth, and improved water and nutrient absorption in the root system of plants associated with mycorrhizal fungi, as noted for pineapple plantlets of both cultivars, can be considered as a process to compensate for pathogen-caused damage (Harrier and Watson 2004). Adequate nutrition provided by the mycorrhizal colonization results in healthier plants, which are able to more effectively tolerate pathogens (Xavier and Boyetchko 2002), as shown in tomato and eggplant (Karagiannidis et al. 2002). Moreover, a higher richness of AMF colonizing the same plant can improve the host's nutritional supply due to complementarity among different AMF species, thus favoring responses to possible pathogen attacks (Wehner et al. 2010). This behavior may explain the reason why the Mix treatment was efficient at absorbing many of the nutrients evaluated in both cultivars.

Increased antioxidant enzyme activity in pineapple plantlets, in both the root and the shoots, is an important response to fungal colonization for producing plantlets that are more resistant to stress conditions. Colonization by AMF and *P. indica* has already been shown to improve, via this mechanism, plant resistance to water or salt stress conditions (Waller et al. 2005; Wu et al. 2006; Latef and Chaoxing 2011; Li et al. 2012) and to high heavy metal levels (Shahabivand et al. 2012; Rozpadek et al. 2014; Garg and Chandel 2015) by alleviating oxidative stress.

Higher GR activity in barley plants colonized by *P. indica* confers local resistance against the pathogen *Fusarium culmorum*, which causes root rot, and systemic resistance against *Blumeria graminis* (barley powdery mildew) on leaves of the plant, without affecting grain production (Waller et al. 2005). *P. indica* was also responsible for increasing the activities of CAT, SOD, GR, and glutathione S-transferase in the shoots of corn plants, conferring systemic resistance against *Fusarium verticillioides*, and also leading to increased plant growth (Kumar et al. 2009). According to these authors, the presence of *P. indica* reduces the pathogen colonization. Moreover, when the plants are colonized by both fungi, there is an increase in biomass production and alterations in root and shoot morphology compared to plants only colonized by the pathogen, thus suggesting that *P. indica* increases the plant resistance to the pathogen. For pineapple plantlets, the inoculation with *P. indica* alone proved to be effective to increase the activity of SOD, CAT, and GR, but was less efficient when compared with the AMF in several other parameters evaluated (Figs. 1, 2).

It is well documented that plants colonized by AMF are less susceptible to stress conditions due to increased activity of enzymes that eliminate ROS such as SOD, CAT (Huang et al. 2010; Li et al. 2012; Rozpadek et al. 2014; Garg and Chandel 2015), and POX (Rozpadek et al. 2014). However, inoculation with AMF does not always result in similar responses, which can vary if different AMF species are individually colonizing the same plant species (Lambais et al. 2003). Therefore, it is natural to find differences between the enzymatic activities between 'Imperial' and 'Pérola' inoculated with these fungi. The bioprotective effect of AMF against pathogens is also related to induce local and/or systemic resistance (Cordier et al. 1998; Pozo et al. 2002; Elsen et al. 2008). Moreover, these defense mechanisms may be effective against multiple pathogens, because when activated, they can protect the plant for prolonged periods (Pozo et al. 2002).

Inoculation with *Rhizophagus intraradices* (Schenck and Smith) C. Walker and A. Schüssler and *F. mosseae* in tomato seedlings reduces the progression of the disease caused by *Phytophthora parasitica*, increasing enzymatic activities of chitinase, chitosanase, β-1,3-glucanase, and SOD. In papaya, plants inoculated with an AMF complex (*R. intraradices*, *C. etunicatum*, *F. mosseae*, and *Gigaspora albida* Schenck & Smith), and *Pseudomonas* sp. strains simultaneously were used as biological control agents against *Fusarium oxysporum*. The results showed improved colonization for both (AMF complex and *Pseudomonas* sp. strains) when inoculated in combination as well as increased seedling biomass (Hernández-Montiel et al. 2013). However, the signaling pathways that confer plant resistance against pathogens are still unknown, both for *P. indica* and for AMF (Waller et al. 2005; Huang et al. 2010).

As inoculated by fungi pineapples plantlets exhibit higher antioxidant enzyme activity, they can withstand higher ROS concentration in their tissue without undergoing cell damage. This can facilitate chemical communication in the host, which can react faster to pathogen attack (Hamilton et al. 2012). Some of these molecules, such as $H_2O_2$, readily diffuse through the cell membranes and function as inter- and intracellular messengers for activating plant defense systems (Barna et al. 2012). Once these signaling cascades are activated, it results in direct effects on pathogens and alterations in cell structures, such as callose deposition, senescence, and programmed cell death, in the case of the hypersensitive response (Cordier et al. 1998; Apel and Hirt 2004).

It has been proven that colonization by AMF and *P. indica* can activate plant defense systems, sending signals to the host that activates genes related to defense protein synthesis. Thus, plants previously colonized by these microorganisms are predisposed to a faster defense response to possible pathogen attack, and this defense system is used for controlling diverse pathogen species covering diverse groups of fungi, oomycetes, bacteria, and nematodes (Azcón-Aguilar and Barea 1996; Harrier and Watson 2004; Wehner et al. 2010; Tahat et al. 2010).

Antioxidant enzyme activity is usually higher when both the pathogen and microorganism used for inducing resistance are present (Kumar et al. 2009; Pereira et al. 2011; Vanitha and Umesha 2011). Our results corroborate these authors when SOD activity in 'Imperial' plantlets colonized by *R. clarus* and in 'Pérola' plantlets colonized by *C. etunicatum* or Mix is analyzed in the root. Moreover, the CAT activity in shoots when plantlets were inoculated with *C. etunicatum*, regardless of the cultivar, also corroborates the above-mentioned studies by the authors. Similarly, GR activity in shoots of 'Imperial' plantlets inoculated with Mix and for 'Pérola' plantlets in all treatments with fungal inoculation was higher when the fungi and pathogen were present (Fig. 1). This behavior may be one of the factors that accelerate plant defense response against phytopathogens.

When comparing antioxidant enzyme activity between cultivars resistant and susceptible to certain stress conditions (biotic or abiotic), some authors have found that the activity of some enzymes is higher in the resistant cultivar (Vanitha and Umesha 2011; Madadkhaha et al. 2012). When two melon cultivars with different resistance responses to *Fusarium oxysporum* f. sp. *melonis* (race 1) were tested, the POX and PPO enzyme activity were higher in the resistant cultivar compared to the susceptible cultivar (Madadkhaha et al. 2012). Similarly, the activities of phenylalanine ammonia lyase, POX, and PPO were found to be higher in tomato cultivars resistant to *Ralstonia solanacearum* compared to the susceptible cultivar (Vanitha and Umesha 2011).

The better results for CAT and PPO in some of the treatments in the root systems of 'Imperial' cultivar plants regarding 'Pérola' (Figs. 1, 2) behaved differently from higher activities of SOD and GR in the shoots of 'Pérola' cultivar plantlets. This may be because the activities of these enzymes are related to the fungal-plant interaction, as different enzyme activity responses can be observed depending on the fungal species inoculated (Lambais et al. 2003). This interaction can also be found regarding mycorrhizal colonization, in which colonization with *R. clarus* or Mix was higher for 'Imperial', whereas the higher mycorrhizal colonization for 'Pérola' was obtained by inoculation with *C. etunicatum*.

## Conclusions

Pineapple plantlets of both cultivars ('Imperial' and 'Pérola') inoculated with AMF and/or *P. indica* exhibited higher growth, nutrient absorption, and antioxidant

enzymes activities compared to no inoculated plantlets. Overall, the 'Imperial' cultivar exhibit higher growth and development when inoculated compared to the 'Pérola' cultivar.

The pineapple plantlets inoculated with AMF and/or *P. indica* develop better than no inoculated ones in the presence of *F. subglutinans* f. sp. *ananas*, indicating that the colonization contributes to produce more resistant propagation material that could aid in lower losses in the field. The higher antioxidant enzyme activities are related to plant defense responses and they increase when plants are in fungal association, becoming a potential tool in pineapple crop management, aiming to explore biological control as an alternative for reducing environmental and health impacts using less fungicide.

**Author contribution statement** Dr. Bruno Coutinho Moreira: responsible for the experimental design; assembly and experiment analysis in all steps, writing, and organization article. Ms. Paulo Prates Junior: obtaining and maintaining fungi, aid in the conduct and analysis of the experiment, aid in enzymatic analysis. Thuany Cerqueira Jordão: assistance in conducting the experiment and aid in enzymatic analysis. Dr. Marliane de Cássia Soares da Silva: assembly and analysis of experiment, writing, and organization article. Dr. Sidney Luiz Stürmer: co-advisor, assistance in experimental design, assistance in article writing, and obtaining inoculum of AMF. Dr. Luiz Carlos Chamhum Salomão: co-orientador, auxilio no desenho experimental, auxílio na escrita do artigo. Dr. Wagner Campos Otoni: aid in writing and organization of the article. Dr. Maria Catarina Megumi Kasuya: advisor, responsible for the experimental design; assembly and experiment analysis in all steps, writing, and organization article.

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