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CROP PRODUCTION

Growth and physiological attributes of blueberry seedlings inoculated with arbuscular mycorrhizal fungi

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ABSTRACT: High-quality seedlings are one of the key factors in achieving high yield and precocity of blueberries. The inoculation of arbuscular mycorrhizal fungi (AMF) can enhance the development of seedlings in the nursery, ensuring more vigorous seedlings in a shorter time. This study evaluated the effect of inoculation of arbuscular mycorrhizal fungi on the development of 'PowderBlue' blueberryseedlings. The treatments were arranged in a 4 x 2 factorial scheme, in which the first factor was the arbuscular mycorrhizal fungi *Gigaspora rosea*, *Glomus clarum*, *G. rosea* + *G. clarum*, and a control level without mycorrhizae, while the second factor consisted of usingindole-3-butyric acid(IBA) and a control level without IBA. Semi-hardwood cuttings were planted in pots containing sterilized soil and kept in a greenhouse for 660 days. The percentage of rooted cuttings, plant height, root system length, shoot dry mass, root dry mass, total dry mass, peroxidase (POD) and superoxide dismutase (SOD) enzyme activities, SPAD index, mycorrhizal efficiency and dependence, number of spores, and soil basal respiration were evaluated. Plants inoculated with *G. clarum* without IBA and inoculated with *G. rosea* with IBA showed higher dry matter and SOD and POD enzyme activities, but the use of IBA had a negative effect on the fungus. The inoculation of blueberry cuttings with *G. clarum*may help seedlingdevelopment, thus improving biometric and biochemical parameters. Furthermore, the plant regulator IBAwas essential in establishing the symbiosis between blueberry and the AMF *G. rosea*.
Key words: IBA, *Gigaspora rosea*, *Glomus clarum*, SPAD index, peroxidase, superoxidedismutase.

Crescimento e atributos fisiológicos de mudas de mirtileiro inoculadas com fungos micorrízicosarbusculares

RESUMO: Um dos fatores primordiais para alcançar alta produtividade e precocidade de mirtileiros é o emprego de mudas de alta qualidade. A inoculação de fungos micorrízicos arbusculares (FMA) pode potencializar o desenvolvimento de plântulas no viveiro, garantindo mudas mais vigorosas e em menor espaço de tempo. O estudo objetivou avaliar o efeito da inoculação de fungos micorrízicosarbusculares no desenvolvimento de mudas de mirtileiro cv. Powderblue. Os tratamentos utilizados foram arranjados em um esquema fatorial 4 x 2, sendo o primeiro fator os diferentes fungos micorrízicos arbusculares *G. rosea*, *G. clarum*, *G. rosea* + *G. clarum* e um nível controle sem micorrizas, o segundo fator foi com uso de AIB e um nível controle sem AIB. Estacas semi-lenhosas foram plantadas em vasos contendo solo esterilizado, mantidos em casa de vegetação durante 660 dias. A porcentagem de estacas enraizadas, altura de plantas, comprimento de sistema radicular, e massa seca de parte aérea, sistema radicular e planta toda, atividade das enzimas peroxidase e superóxido dismutase, índice SPAD, eficiência e dependência micorrízica, número de esporos e respiração basal do solo foram avaliadas. Plantas inoculadas com *G. clarum* sem AIB e inoculadas com *G. rosea* com AIB apresentaram maior matéria seca e atividade das enzimas SOD e POD, em contrapartida, o uso de AIB teve efeito negativo sobre esse fungo. A inoculação de estacas de mirtilo com *G. clarum* pode auxiliar no desenvolvimento das mudas, melhorando assim, parâmetros biométricos e bioquímicos, além disso, o regulador vegetal AIB demonstra-se essencial no estabelecimento da simbiose entre o mirtileiro e o FMA *G. rosea*.
Palavras-chave: AIB, *Gigaspora rosea*, *Glomus clarum*, índice SPAD, peroxidase, superóxido dismutase.

INTRODUCTION

Blueberry belongs to the family Ericaceae and is a temperate fruit and an exotic species in Brazil. Its cultivation is concentrated in the states of the South region, in addition to São Paulo and Minas Gerais, regions that have edaphoclimatic conditions for producing it from December to February, making it possible to offer the fruit in the off-season in the United States and Europe (PERTUZATTI et al., 2021).

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Seedling supply is one of the most important factors that limit blueberry production in some areas due to the propagation difficulties of this species (COLOMBO et al., 2018). Propagation by seeds is rarely used commercially due to genetic variation (GOYALI et al., 2018). Propagation by cuttings is traditionally the most used among the available techniques (FAN et al., 2017). However, this method has some limitations, such as low production of branches to obtain cuttings and variation in results according to the cultivar, physicochemical characteristics of the substrate, and concentration of plant hormones (AN et al., 2018).

Auxins play an important role in root initiation of stem cuttings. This hormonal group stimulates the differentiation and division of pericycle cells. The dividing cells form the lateral root, which grows through the root cortex and epidermis (GILANI et al., 2019). Some cultivars have endogenous contents of auxins sufficient for differentiation and induction of cell division (FISCHER et al., 2008). However, some varieties require the application of synthetic analogs of this hormone for rooting to occur (MIHALJEVIĆ & SALOPEK-SONDI, 2012).

Another limiting factor for the crop is related to the morphology of its root system. The roots of this species are not deep and devoid of root hairs, meaning that the water absorption capacity is limited and management techniques are necessary to improve crop development (SPINARDI & AYUB, 2013).

Inoculation with arbuscular mycorrhizal fungi (AMF) can contribute to better plant nutrition and reduce development time, in addition to increasing resistance to stress during transplant acclimatization (MACHINESKI et al., 2018). These benefits are achieved because the presence of extramatricial hyphae increases the absorption of water and nutrients, especially those with slow diffusivity, such as phosphorus (P), zinc (Zn), and copper (Cu), made possible by the increase in the volume of explored soil (ANTONIOLLI & KAMINSKI, 1991).

Ericoid mycorrhizae are generally formed in plants of the family Ericaceae, but the presence of AMF has been recorded in the roots of this group of plants, which were illustrated for the first time in 1990 by KOSKE et al. (1990) in three species of the genus *Vaccinium* (Ericaceae). Since then, some authors have sought to present the effects of the AMF-blueberry interaction. FARIAS et al. (2014) evaluated seedling development and observed that micropropagated blueberry seedlings of the Woodard cultivar inoculated with *Gigaspora margarita* had higher root height and dry biomass. Treatments with *Scutellospora heterogama* and *G. margarita* showed the best results for root green biomass. Inoculation with *S. heterogama*, *Glomus etunicatum*, *Glomus clarum*, and *G. margarita* provided higher nitrogen (N) and P contents in the plant shoot.

Regarding biochemical characteristics, YANG et al. (2020) demonstrated that AMF inoculation of blueberries activated genes related to photosynthesis, hormonal metabolism, carbohydrate metabolism, amino acid metabolism, stress response, signal transduction, and antioxidation.

However, AMF inoculation efficiency is regulated by the balanced condition between fungi and plants. It can be influenced by the interaction between fungal species and the host genotype, which regulates the plant's ability to respond to symbiosis (SOARES et al., 2012; LANFRANCO et al., 2018). TU et al. (2019) indicated specific molecular changes in the symbiosis of blueberries inoculated with the mycorrhizal fungi species *Glomus mosseae*, *Glomus intraradices*, and *G. etunicatum*. The study evaluated the effect of inoculation of arbuscular mycorrhizal fungi of the species *Gigaspora rosea* and *G.clarum* on the rooting and development of 'Powder Blue' blueberry seedlings associated with indole-3-butyric acid (IBA) application.

MATERIALS AND METHODS

The substrate used for multiplication of the mycorrhizal inoculum was composed of a mixture of an Oxisol (Latossolo Bruno distrófico), sterilized in an autoclave three times at a temperature of 121 °C for one hour, and then placed in pots with a capacity of 3 dm^3. A 50 g sample of soil containing spores and roots colonized with AMF was added to each pot. Then, 20 seeds of the genus *Brachiaria* of different species were sown as hosts for AMF.

The number of viable spores was determined beforesetting up the experiment and after completing it, following the methodologies proposed by GERDEMANN & NICHOLSON (1963), using a channeled plate and stereoscopic magnifying glass (SZ51, Olympus, Japan).

Semi-hardwood cuttings from the Powder Blue cultivar with an approximate length of 20 cm and a mean diameter of 1 cm were used to form the seedlings. The cuttings were implanted in the Oxisol, which was chemically characterized after autoclaving. The soil had the following chemical characteristics: pH in H_2O = 5.26; P = 2.49 mg dm^{-3}; K = 0.19 $cmol_c$ dm^{-3}; Ca = 2.49 $cmol_c$ dm^{-3}; Mg = 0.89 $cmol_c$ dm^{-3};Al = 0.0 $cmol_c$ dm^{-3}; and CEC = 6.56

$cmol_c\ dm^{-3}$ (SILVA, 2009). The soil and washed sand were previously disinfected in an autoclave at 121 °C for one hour. This process was repeated three times.

The experimental design consisted of completely randomized blocks, with the sources of variation arranged in a 4 x 2 factorial scheme, in which the four levels of the first factor corresponded to the inoculation with the arbuscular mycorrhizal fungi *G. rosea*, *G.clarum*, *G. rosea* + *G.clarum*, and a control level without mycorrhizae. The two levels of the second factor correspond to the use of IBA and a control level without IBA. The treatments consisted of five replications and the experimental plot consisted of four pots, totaling 20 plants per treatment.

The experiment was conducted in a greenhouse for 660 days. Pots with a capacity of 3 dm^3 were previously sanitized with 1% sodium hypochlorite and filled with 3 kg of substrate composed of soil and washed sand (1:1 v/v). The treatments were applied via soil, in the planting furrow of the plant species with 27.17 g of soil per pot for the species *G. rosea* and 9.36 g of soil per pot for the species *G. clarum*, equivalent to 250 spores per pot for each AMF species. In treatments using IBA, the base of the cuttings was submerged in an alcoholic solution of the plant regulator at a concentration of 2000 mg L^{-1} for 10 seconds (FISCHER et al., 2014).

Physiological and biochemical characteristics were evaluated, initially determining the percentage of rooted cuttings, calculated by the total number of rooted cuttings divided by the total number of cuttings. Plant height was obtained as the distance from the collar to the apical bud. The root system length was determined by measuring the distance from the collar region to the root apex. The plants were divided into shoot and root systems and;subsequently, the plant materials were dried in a forced-air circulation oven at 65 °C for 72 hours. The shoot dry mass (SDM) and root dry mass (RDM) allowed the calculation of the total dry mass (TDM). The SPAD index was evaluated at 330 and 660 days after transplanting (DAT) using a SPAD-502 chlorophyll meter (Konica Minolta Holding Inc., Tokyo, Japan). The measurements were conducted on fully expanded leaves on all plants in the plot, three leaves per plant from 8:00 to 10:00 am.

The evaluation of root colonization was performed according to the technique described by PHILLIPS & HAYMAN (1970), modified by GIANINAZZI & GIANINAZZI-PEARSON (1992). Mycorrhizal efficiency (ME) and mycorrhizal dependence (MD) were calculated based on the shoot dry mass parameters using the equations proposed by PLENCHETTE et al. (1983).

Proteins contents and activity of the superoxide dismutase (SOD) (E.C.1.15.1.1) and peroxidase (POD) (EC.1.11.1.7) enzymes were determined. Protein content was determined using the methodology proposed by BRADFORD (1976). The SOD activity was determined using the methodology proposed by GIANNOPOLITIS & RIES (1977) and the POD activity was determined according to the conditions mentioned by TEISSEIRE & GUY (2000). Soil basal respiration (SBR) was determined using the methodology described by ALEF (1995).

The data were tested for normality by the Shapiro-Wilk test and subjected to analysis of variance. The means were compared by the Scott-Knott test at a 5% significance ($P \leq 0.05$) when the analysis of variance was significant using the statistical program SISVAR version 5.6 (FERREIRA, 2019).

RESULTS

There was an interaction between the factors and significant differences in cutting survival (Figure 1A), plant height (Figure 1B), and root system length (Figure 1C). Cuttings inoculated with *G. rosea* among treatments without the addition of IBA had a lower percentage of rooted cuttings (20%), plant height (4.95 cm), and root system length (16.38 cm).

Cuttings inoculated with *G. rosea* and *G. rosea* + *G. clarum* among treatments with IBA showed a higher percentage of rooting and higher plant height. IBA application reduced the survival, height, and length of roots of blueberry seedlings inoculated with *G. clarum* when compared to those with the same AMF and without IBA. Also, a reduction in rooting and height of seedlings treated with IBA and without inoculation was observed, when compared to the control without IBA.

Cuttings without IBA application showed higher SDM values in plants inoculated with *G. clarum* (Figure 2A). No difference was observed for SD Min seedlings with IBA treatment. However, seedlings inoculated with *G. rosea* and *G. rosea* + *G. clarum* treated with IBA had higher RDM than the other treatments (Figure 2B). The SPAD index at 330 DAT (Figure 3A) showed no difference between treatments. Inoculation with *G. clarum* and *G. clarum* + *G. rosea* provided an increase in the SPAD index at 660 DAT (Figure 3B) among the treatments without IBA. Plants treated with IBA and inoculated with *G. rosea* and *G. rosea* + *G. clarum* showed a higher SPAD index than the other treatments. Moreover, a reduction in SPAD values was observed between the first and second evaluations carried out in the

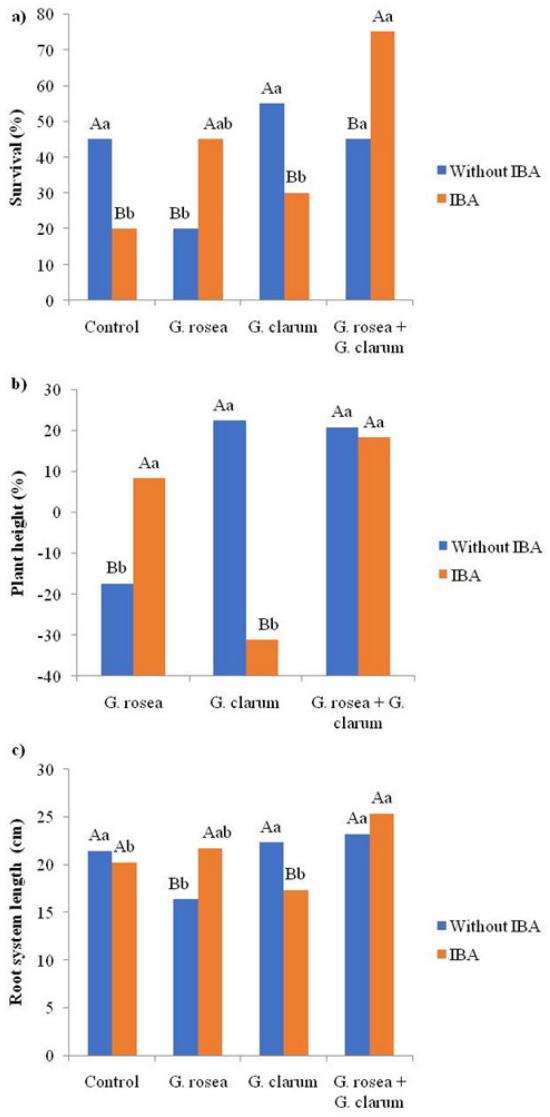

Figure 1 - Percentage of survival of cuttings (a), plant height (b), and root system length (c) of blueberry seedlings inoculated with different arbuscular mycorrhizal fungi and subjected to treatment with indole-3-butyric acid (IBA). *Means followed by the same letter do not differ from each other by Student's t-test for the factor indole-3-butyric acid (IBA) and Tukey's test for the factor mycorrhiza at a 5% probability level. Lowercase letters indicate differences between inoculation with different AMF species and uppercase letters between indole-3-butyric acid (IBA) treatments. Guarapuava, PR, 2021.

experiment, explained by the fact that the plants preceded the entry of dormancyat 660 DAT.

Root colonization in cuttings not treated with IBA was higher in seedlings inoculated with *G. clarum* (71.25%), with values of 26.25% and 31.25% higher than those observed for *G. rosea* and *G. rosea* + *G. clarum*. However, AMF inoculation associated with IBA promoted higher seedling colonization with *G. rosea* (66.25%) and *G. clarum* + *G. rosea* (65%) (Figure 4).

The activities of POD (Figure 5A) and SOD enzymes (Figure 5B) in cuttings not treated with IBA showed a significant increase in plants inoculated with *G. clarum* when compared to the other treatments. The fungus *G. rosea* provided an increase in POD and SOD activities in cuttings treated with IBA. The use of IBA also reduced the enzymatic activity of plants, except for those in association with *G. rosea*.

The number of spores (Figure 6A) in cuttings not treated with IBA was higher in treatments with *G. rosea* + *G. clarum*. The association of fungi showed a higher number of spores, which can be justified by the methodology employed, as 500 spores were placed per pot in the association, that is, 250 spores of *G. rosea* + 250 spores of *G. clarum*. The AMF *G. clarum* had the highest number of sporesfor treatments with IBA. The use of IBA increased the number of spores in pots inoculated with *G. clarum* and *G. rosea* + *G. clarum*, different from the control treatment.

DISCUSSION

Different AMF genotypes provided differences in survival, height, and root development for the plant species. Host plants directly influence AMF composition, as they regulate carbon allocation to roots and production of secondary metabolites and alter soil environmental conditions (EOM et al., 2000). The effectiveness of AMF species depends on the plant species and the occurrence of different ideal combinations of host plants, and AMF species are important to maintain the diversity of plant communities (KUMAR et al., 2017). Therefore, the differences observed between mycorrhizal species are related to the plant-AMF association. In this sense, the reduction in the percentage of survival, plant height, and root system length may be related to the incompatibility between the host species and the fungus *G. rosea*.

Phytohormones are central to the regulation of interactions and modulate the associations of plants and microorganisms, in addition to coordinating cellular and metabolic responses associated with the progression of microorganisms in different plant tissues (BOIVIN et al., 2016). Auxins are involved in the host-AMF interaction because of the pre-symbiotic exchange of signals, also contributing to

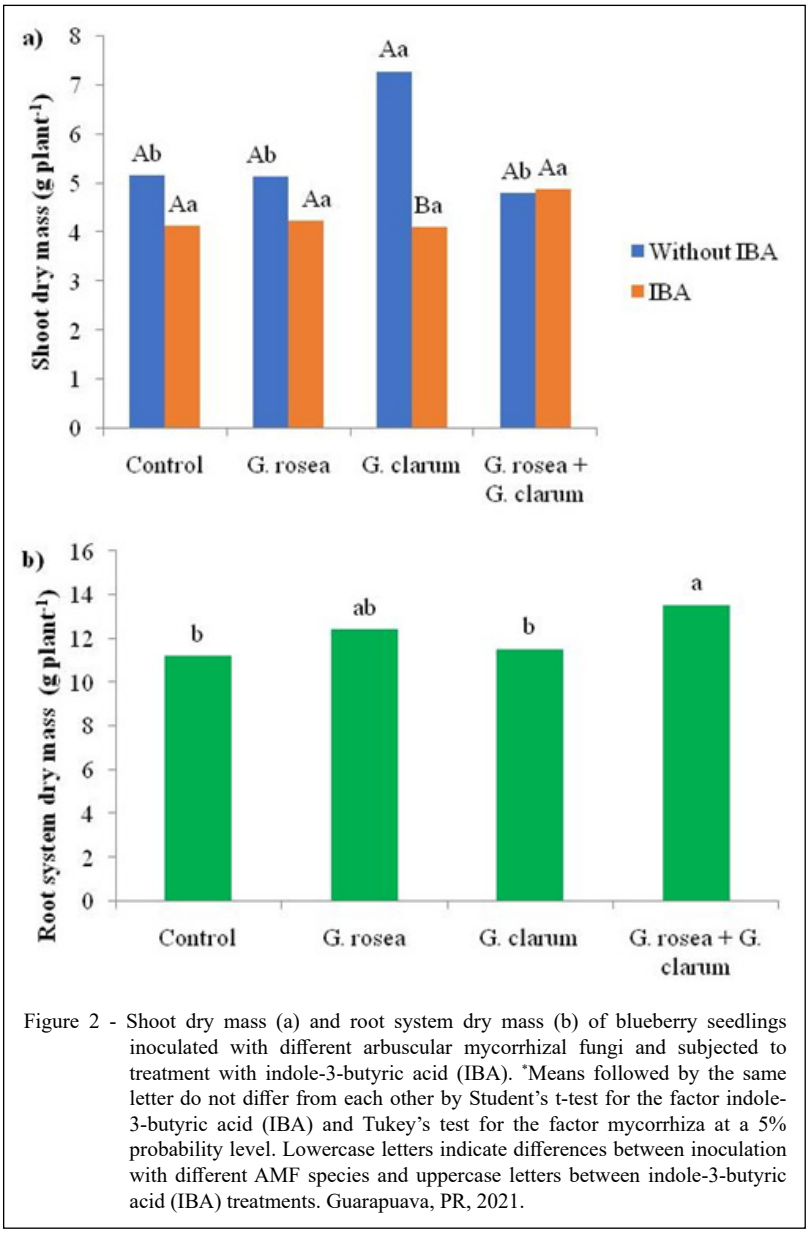

Figure 2 - Shoot dry mass (a) and root system dry mass (b) of blueberry seedlings inoculated with different arbuscular mycorrhizal fungi and subjected to treatment with indole-3-butyric acid (IBA). *Means followed by the same letter do not differ from each other by Student's t-test for the factor indole-3-butyric acid (IBA) and Tukey's test for the factor mycorrhiza at a 5% probability level. Lowercase letters indicate differences between inoculation with different AMF species and uppercase letters between indole-3-butyric acid (IBA) treatments. Guarapuava, PR, 2021.

the establishment of symbiosis, as they promote the development of lateral roots, which are the preferred sites of infection for fungi (LUDWIG-MÜLLER & GÜTTER, 2007). Thus, the exogenous application of IBA in plants colonized by mycorrhizae promotes an increase in the survival and development of seedlings, a result observed in the present study for the AMF species *G. rosea* and *G. rosea* + *G. clarum*. Thus, the use of the plant regulator had a beneficial effect on the association of blueberry with the AMF *G. rosea*, which may be related to the signals for the establishment of symbiosis and higher emission of lateral roots in the plant, thus promoting compatibility between the species and the symbiote.

However, different fungi have optimal levels of auxin for growth and establishment of the relationship with the plant, and the effects of exogenous application of auxin are dependent on the strain, with high phytohormone doses being harmful to the development of the microorganism and establishment of symbiosis (FU et al., 2015). This phenomenon may explain the negative effect of IBA on the fungus *G. clarum*, as the dose of 2000 mg L^{-1} was not ideal for the establishment of its symbiosis

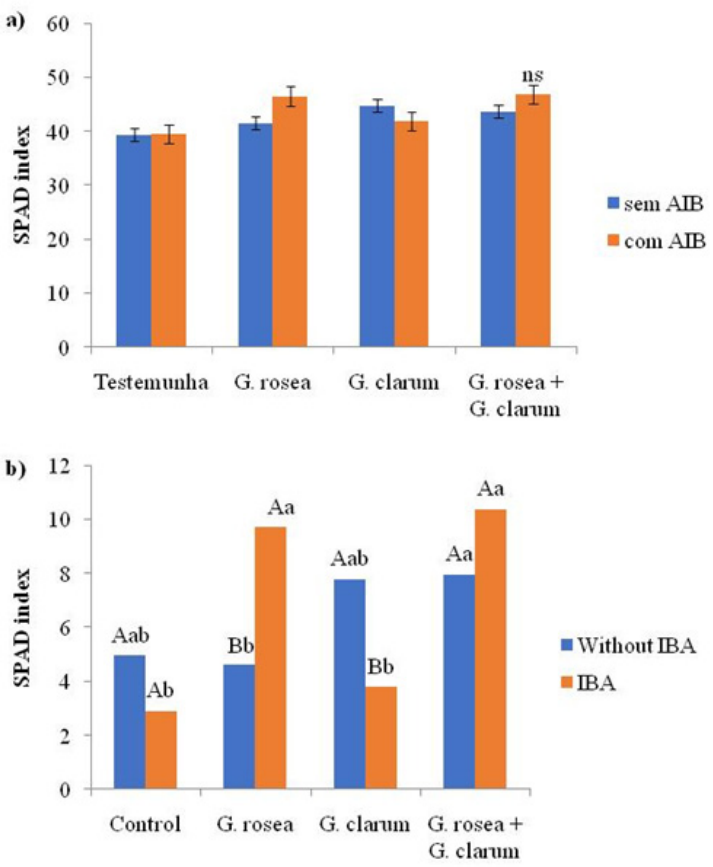

Figure 3 - SPAD index at 330 DAT (a) and 660 DAT (b) of blueberry seedlings inoculated with different arbuscular mycorrhizal fungi and subjected to treatment with indole-3-butyric acid (IBA). *Means followed by the same letter do not differ from each other by Student's t-test for the factor indole-3-butyric acid (IBA) and Tukey's test for the factor mycorrhiza at a 5% probability level. Lowercase letters indicate differences between inoculation with different AMF species and uppercase letters between indole-3-butyric acid (IBA) treatments. Guarapuava, PR, 2021.

and could have a toxic effect on the fungus. Moreover, the increase in auxin contents may have generated stress on the fungus; consequently, activating its defense system, producing more spores for survival, and reducing interaction with the plant.

The way auxin is applied can also interfere with the plant's responses. IBA application in an alcoholic solution reduces the percentage of rooted cuttings. The reduction in seedling rooting and height may be related to the toxicity that the alcoholic solution can cause to the cuttings. The amount of IBA applied to the base of the cuttings should be sufficient to dissolve the cuticle and provide a tight seal at the basal end of the cutting to prevent decay, increasing the chances of survival. The inhibitory effect of some concentrations of IBA can be explained by the toxicity of potassium ions (K^+), which are free radicals. In this case, K^+ ions concentrate in the tissue when applying IBAand play a significant role in root initiation by dissolving the epidermal layer. However, they destroy the epidermal layer and adjacent cells when in excess instead of dissolving the epidermal layer (HIGUCHI et al., 2021). The results indicated that the use of an alcoholic solution and the IBAdose were toxic to cuttings of blueberry cv. Powder Blue.

The increase in dry matter in the shoots of plants inoculated with *G. clarum* suggested that AMF inoculation increased biomass allocation to the

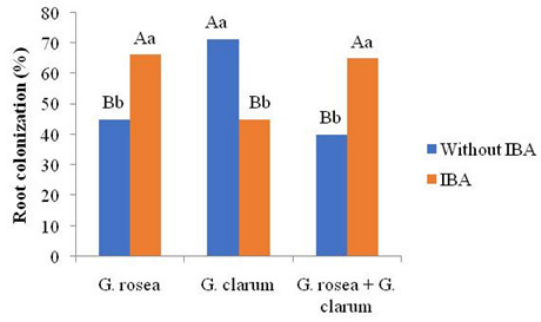

Figure 4 - Root colonization of blueberry seedlings inoculated with different arbuscular mycorrhizal fungi and subjected to treatment with indole-3-butyric acid (IBA). *Means followed by the same letter do not differ from each other by Student's t-test for the factor indole-3-butyric acid (IBA) and Tukey's test for the factor mycorrhiza at a 5% probability level. Lowercase letters indicate differences between inoculation with different AMF species and uppercase letters between indole-3-butyric acid (IBA) treatments. Guarapuava, PR, 2021.

shoots. The reduced dry mass accumulation in the host roots indicates that the fungus was a stronger carbon sink than the host roots. A shift in carbon allocation from root growth to fungal growth could explain the increased SDM and reduced RDM of mycorrhized plants (THORNLEY & PARSONS, 2014).

Furthermore, inoculation with *G. rosea* without IBA in blueberry seedlings was less effective, as they had a lower percentage of rooting, plant height, and root system length. The increase in root production observed with AMF is related to treatment with IBA, as this plant regulator plays a specific and direct role in the establishment of symbiosis between fungi and plant roots. In addition, it stimulates the fungus to form lateral roots in the host, leading to an improvement in the plant'snutritional status, thus reflecting dry massaccumulation. Auxin signaling is necessary for AMF infection, and the exchange of signals between plants and fungi is mediated by host auxin responses. Therefore, exogenous IBA application assists in the plant-AMF symbiotic association. The symbiotic association with AMF promotes faster and higher plant growth through higher efficiency in the absorption and translocation of macro-and micronutrients (FERNANDES et al, 2019).

SPAD index values indirectly quantify the relative chlorophyll content of leaves, which is closely related to leaf N content. Thus, higher SPAD values may be associated with better nutritional status of N and Mg, the central atom of the chlorophyll molecule. Also, the increase in the SPAD index is linked to the higher photosynthetic capacity of seedlings, which provides a higher accumulation of photoassimilates (OLIVEIRA et al., 2017).

Therefore, the increase in the SPAD index in seedlings inoculated with *G. rosea* and *G. rosea* + *G. clarum* associated with IBA shows the need to apply the plant regulator for the establishment of symbiosis with *G. rosea* and the plantspecies, thus mediating pre-symbiotic signaling essential for the establishment of AMF in the roots and absorption of macro-and micronutrients. Perennial plants in temperate climates remobilize nutritional reserves from leaves to perennial organs until complete leaf abscission. Therefore, lower SPAD values at 660 DAT represent the remobilization of leaf N to perennial plant organs (MUHAMMAD et al., 2020).

As demonstrated in this study, LIU et al. (2017) reported that the inoculation of blueberry seedlings with *G.mosseae*showed varying root colonization results according to the evaluated cultivar, with values ranging from 10 to 15% for the cultivars Misty and Brightwell. TU et al. (2019) observed that mycorrhizal colonization was higher in 'Premier'blueberryplants inoculated with *G. mosseae* (59%) than those inoculated with *G. etunicatum* (39%) or *G. intraradices* (29%). Different root colonization values were observed in the present study (40 to 71.25%), reaffirming that the establishment of symbiosis is dependent on the inoculated AMF species (SOARES et al., 2012; LANFRANCO et al., 2018). The genus *Glomus* was dominant in soil samples from the rhizosphere of species from the family Ericaceae, evidencing the compatibility between blueberry plants and the fungus *G. clarum* (CHAURASIA et al., 2005).

Still relative to root colonization, the results reaffirm the importance of auxin in signaling for the establishment of symbiosis, as an increase in colonization was observed in the association of the AMF *G. rosea* and *G. clarum* + *G. rosea* with exogenous IBA application. The external application of auxins modulates the symbiosis, promoting the formation of arbuscules at low concentrations, but repressing it at high concentrations. Furthermore, there is an ideal auxin dose for each fungus species (CHEN et al., 2021). Thus, the IBA dose may have partially inhibited the symbiosis of blueberry seedlings with the fungus *G. clarum*, thus reducing root colonization, but it was beneficial for treatments with the AMF *G. rosea*.

The treatment *G. clarum* + IBA showed higher spore density but lower root colonization. The

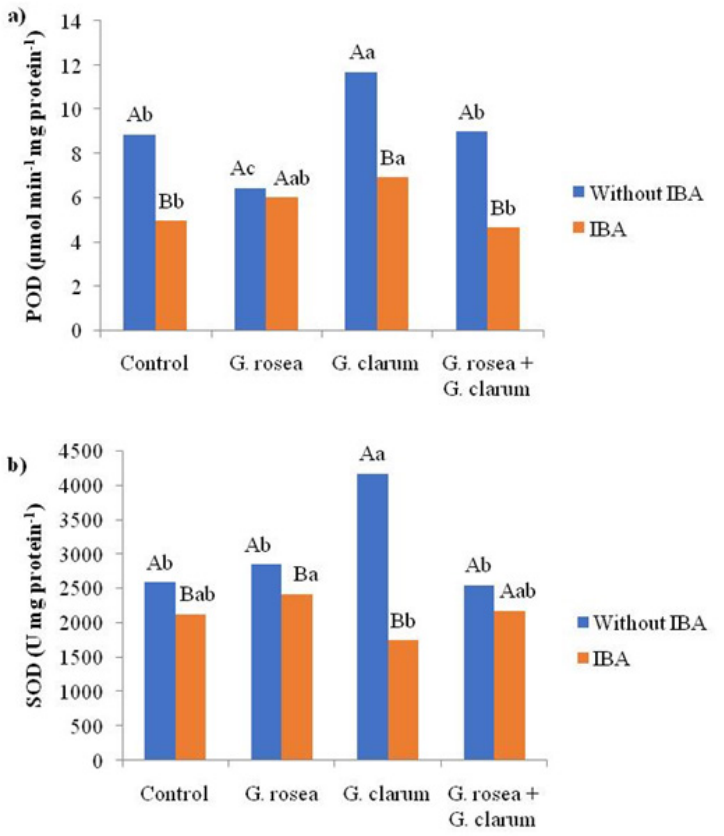

Figure 5 - Activity of the peroxidase (a) and superoxide dismutase enzymes(b) of blueberry seedlings inoculated with different arbuscular mycorrhizal fungi and subjected to treatment with indole-3-butyric acid (IBA). *Means followed by the same letter do not differ from each other by Student's t-test for the factor indole-3-butyric acid (IBA) and Tukey's test for the factor mycorrhiza at a 5% probability level. Lowercase letters indicate differences between inoculation with different AMF species and uppercase letters between indole-3-butyric acid (IBA) treatments. Guarapuava, PR, 2021.

function of spores is to spread the fungus to new areas and help the microorganism survive during periods of stress (DIJKSTERHUIS, 2019). JI et al. (2019) found higher AMF density under environmental stress. Therefore, we can conclude that IBA application at a dose of 2000 mg L^{-1} had negative effects on the AMF of the species *G. clarum*, generating stress on the fungus and promoting the multiplication of its spores.

In addition to the effects on the vegetative development of seedlings, mycorrhizae can act on the plant's oxidative stress pathways. One of the main responses against biotic and abiotic stresses is the elimination of reactive oxygen species (ROS). The mechanisms for ROS elimination involve enzymatic components and non-enzymatic antioxidants (MAHMOOD et al., 2020). Enzymatic components such as superoxide dismutase (SOD) and peroxidase (POD) enzymes participate in plant antioxidant systems and can convert harmful ROS such as hydrogen peroxide (H_2O_2) into water and O_2, thereby reducing oxidative damage induced by various sources of stresses on plants (STEPHENIE et al., 2020).

The increase in the activity of antioxidant enzymes with AMF inoculation has been described in several studies (LIU et al., 2017; AIT-EL-MOKHTAR et al., 2019; WANG et al., 2022), as observed in the present research. These results can be correlated with the data already presented in the present study. Plants inoculated with *G. clarum* or *G. rosea* + IBA hadhigher

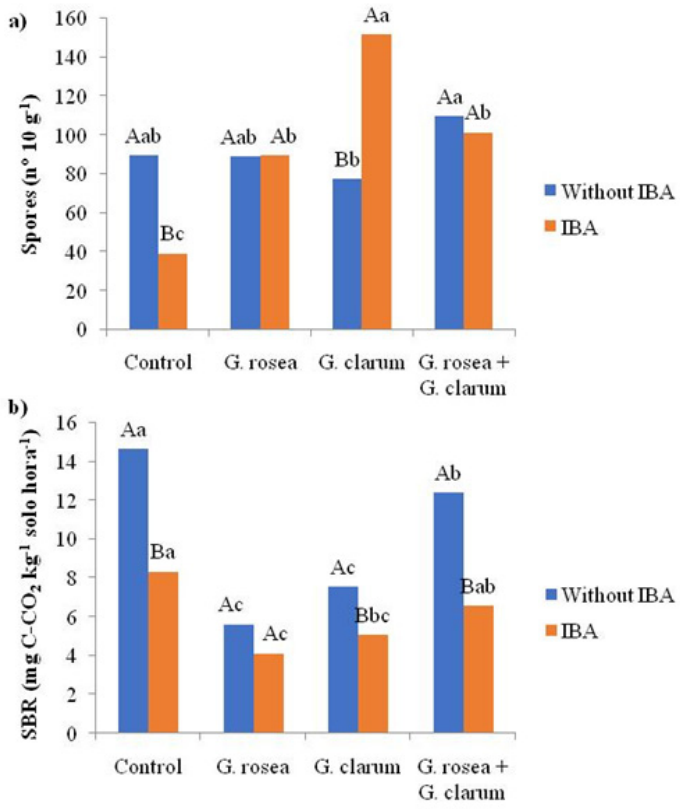

Figure 6 - Number of spores (a) and soil basal respiration (b) of blueberry seedlings inoculated with different arbuscular mycorrhizal fungi and subjected to treatment with indole-3-butyric acid (IBA). *Means followed by the same letter do not differ from each other by Student's t-test for the factor indole-3-butyric acid (IBA) and Tukey's test for the factor mycorrhiza at a 5% probability level. Lowercase letters indicate differences between inoculation with different AMF species and uppercase letters between indole-3-butyric acid (IBA) treatments. Guarapuava, PR, 2021.

colonization of their root segments, culminating in systemic plant responses that promote stress resistance, leading the seedlings to present higher dry matter accumulation, higher photosynthetic capacity, and better nutritional supply (SPAD index).

CONCLUSION

Mycorrhizal fungi of the species *G. clarum* without the use of IBA showed the highest affinity with blueberry cv. Powder Blue between species, resulting in higher root colonization. This symbiotic interaction resulted in increased dry matter accumulation and systemic plant defense responses. The IBA dose of 2000 mg L^{-1} had negative effects on the AMF *G. clarum*. The symbiosis between blueberry seedlings and the AMF *G. rosea* requires the use of the plant regulator IBA.

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DECLARATION OF CONFLICT OF INTEREST

I, Karla Siebert Sapelli, the author responsible for submitting the manuscript entitled "Growth and physiological attributes of blueberry seedlings inoculated with arbuscular mycorrhizal fungi" and all co-authors presented here, declare that we have no conflict of interest.

AUTHORS' CONTRIBUTION

The authors contributed equally to the manuscript.

REFERENCES

AIT-EL-MOKHTAR, M. et al. Use of mycorrhizal fungi in improving tolerance of the date palm (Phoenix dactylifera L.) seedlings to salt stress. **Scientia Horticulturae**, v.253, p.429-438, 2019. Available from: <https://doi.org/10.1016/j.scienta.2019.04.066>. Accessed: Mar. 18, 2022. doi: 10.1016/j.scienta.2019.04.066.

AN, H. et al. Rooting ability of hardwood cuttings in highbush blueberry (*Vaccinium corymbosum* L.) using different indole-butyric acid concentrations. **HortScience**, v.54, n.2, p.194-199, 2018. Available from: <https://doi.org/10.21273/HORTSCI13691-18>. Accessed: Mar. 18, 2022. doi: 10.21273/HORTSCI13691-18.

ANTONIOLLI, Z. I.; KAMINSKI, J. Micorrizas. **Ciência Rural**, 21, 441-455, 1991. Available from: <https://doi.org/10.21273/HORTSCI13691-18>. Accessed: Mar. 20, 2022. doi: 10.21273/HORTSCI13691-18.

ALEF, K. Enrichment, isolation and counting of soil microorganisms. In: **Methods in applied soil microbiology and biochemistry**. Academic press, 1995. p.123-191. Available from: <https://doi.org/10.1016/B978-012513840-6/50019-7>. Accessed: Apr. 20, 2022. doi: 10.1016/B978-012513840-6/50019-7.

BRADFORD, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical biochemistry**, v.72, n.1-2, p.248-254, 1976. Available from: <https://doi.org/10.1016/0003-2697(76)90527-3>. Accessed: Apr. 20, 2022. doi: 10.1016/0003-2697(76)90527-3.

BOIVIN, S. et al. How auxin and cytokinin phytohormones modulate root microbe interactions. **Frontiers in Plant Science**, v.7, p.1240, 2016. Available from: <https://doi.org/10.3389/fpls.2016.01240>. Accessed: Aug. 17, 2022. doi: 10.3389/fpls.2016.01240.

CHAURASIA, B. et al. Distribution, colonization and diversity of arbuscular mycorrhizal fungi associated with central Himalayan rhododendrons. **Forest Ecology and Management**, v.207, n.3, p.315-324, 2005. Available from: <https://doi.org/10.1016/j.foreco.2004.10.014>. Accessed: Apr. 20, 2022. doi: 10.1016/j.foreco.2004.10.014.

CHEN, X. et al. Auxin-mediated regulation of arbuscular mycorrhizal symbiosis: a role of SlGH3. 4 in tomato. **Plant, Cell & Environment**, v.45, n.3, p.955-968, 2021. Available from: <https://doi.org/10.1111/pce.14210>. Accessed: Jan. 20, 2002. doi: 10.1111/pce.14210.

COLOMBO, R. C. et al. Blueberry propagation by minicuttings in response to substrates and indolebutyric acid application methods. **Journal of Agricultural Science**, v.10, n.9, p.450-458, 2018. Available from: <https://doi.org/10.5539/jas.v10n9p450>. Accessed: Jan. 20, 2022. doi: 10.5539/jas.v10n9p450.

DIJKSTERHUIS, J. Fungal spores: Highly variable and stress-resistant vehicles for distribution and spoilage. **Food microbiology**, v.81, p.2-11, 2019. Available from: <https://doi.org/10.1016/j.fm.2018.11.006>. Accessed: Jan. 20, 2022. doi: 10.1016/j.fm.2018.11.006.

EOM, A.-H. et al. Host plant species effects on arbuscular mycorrhizal fungal communities in tallgrass prairie. **Oecologia**, v.122, n.3, p.435-444, 2000. Available from: <https://doi.org/10.1007/s004420050050>. Accessed: Jan. 20, 20222. doi: 10.1007/s004420050050.

FAN, S. et al. Detection of blueberry internal bruising over time using NIR hyperspectral reflectance imaging with optimum wavelengths. **Postharvest Biology and Technology**, v.134, p.55-66, 2017. Available from: <https://doi.org/10.1016/j.postharvbio.2017.08.012>. Accessed: Dec. 20, 2021. doi: 10.1016/j.postharvbio.2017.08.012.

FARIAS, D. da H. et al. Desenvolvimento de mudas de mirtileiro inoculadas com fungos micorrízicosarbusculares. **Revista Brasileira de Fruticultura**, v.36, p.655-663, 2014. Available from: <https://doi.org/10.1590/0100-2945-128/13>. Accessed: Dec. 20, 2021. doi: 10.1590/0100-2945-128/13.

FERNANDES, M. D. S da S. et al. Arbuscular mycorrhizal fungi and auxin associated with microelements in the development of cuttings of Varronialeucocephala. **Revista Brasileira de Engenharia Agrícola e Ambiental**, v.23, p.167-174, 2019. Available from: <https://doi.org/10.1590/1807-1929/agriambi.v23n3p167-174>. Accessed: Dec. 14, 2021. doi: 10.1590/1807-1929/agriambi.v23n3p167-174.

FERREIRA, D. F. SISVAR: A computer analysis system to fixed effects split plot type designs. **Brazilian Journal of Biometrics**, v.37, n.4, p.529-535, 2019. Available from: <https://doi.org/10.28951/rbb.v37i4.450>. Accessed: Dec. 14, 2021. doi: 10.28951/rbb.v37i4.450.

FISCHER, D. L. de O. et al. Efeito do ácido indolbutírico e da cultivar no enraizamento de estacas lenhosas de mirtilo. **Revista Brasileira de Fruticultura**, v.30, p.285-289, 2008. Available from: <https://doi.org/10.1590/S0100-29452008000200003>. Accessed: Nov. 14, 2021. doi: 10.1590/S0100-29452008000200003.

FU, S. F. et al. Indole-3-acetic acid: A widespread physiological code in interactions of fungi with other organisms. **Plant Signaling & Behavior**, v.10, n.8, p.e1048052, 2015. Available from: <https://doi.org/10.1080/15592324.2015.1048052>. Accessed: Nov. 14, 2021. doi: 10.1080/15592324.2015.1048052.

GERDEMANN, J. W.; NICOLSON, T. H. Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. **Transactions of the British Mycological society**, v.46, n.2, p.235-244, 1963. Available from: <http://dx.doi.org/10.1016/S0007-1536(63)80079-0>. Accessed: Oct. 14, 2021. doi: 10.1016/S0007-1536(63)80079-0.

GIANINAZZI, S.; GIANINAZZI-PEARSON, V. Cytology, histochemistry and immunocytochemistry as tools for studying structure and function in endomycorrhiza. **Methods in Microbiology**, Amsterdam, v.24, p.109-139, 1992. Available from: <https://doi.org/10.1016/S0580-9517(08)70090-4>. Accessed: Oct. 14, 2021. doi: 10.1016/S0580-9517(08)70090-4.

GIANNOPOLITIS, C. N.; RIES, S. K. Superoxide dismutases: II. Purification and quantitative relationship with water-soluble protein in seedlings. **Plant Physiology**, v.59, n.2, p.315-318, 1977. Available from: <https://doi.org/10.1104/pp.59.2.315>. Accessed: Sept. 14, 2021. doi: 10.1104/pp.59.2.315.

GILANI, S. A. Q. et al. Influence of indole butyric acid (IBA) concentrations on air layerage in guava (*Psidium guajava* L.) cv. Sufeda. **Pure and Applied Biology (PAB)**, v.8, n.1, p.355-362, 2019. Available from: <http://dx.doi.org/10.19045/bspab.2018.700194>. Accessed: Sept. 14, 2021. doi: 10.19045/bspab.2018.700194.

GOYALI, J. C. DNA methylation in lowbush blueberry (*Vaccinium angustifolium* Ait.) propagated by softwood cutting and tissue culture. **Canadian Journal of Plant Science**, v.98, n.5, p.1035-1044, 2018. Available from: <https://doi.org/10.1139/cjps-2017-029>. Accessed: Oct. 14, 2021. doi: 10.1139/cjps-2017-029.

HIGUCHI, M. T. et al. Methods of application of indolebutyric acid and basal lesion on 'Woodard'blueberry cuttings in different seasons. **Revista Brasileira de Fruticultura**, v.43, 2021. Available from: <https://doi.org/10.1590/0100-29452021022>. Accessed: Oct. 27, 2021. doi: 10.1590/0100-29452021022.

JI, L. et al. Arbuscular mycorrhizal mycelial networks and glomalin-related soil protein increase soil aggregation in CalcaricRegosol under well-watered and drought stress conditions. **Soil and Tillage Research**, v.185, p.1-8, 2019. Available from: <https://doi.org/10.1016/j.still.2018.08.010>. Accessed: Oct. 27, 2021. doi: 10.1016/j.still.2018.08.010.

KOSKE, R. E et al. Vesicular-arbuscular mycorrhizae in Hawaiian Ericales. American **Journal of Botany**, v.77, n.1, p.64-68, 1990. Available from: <https://doi.org/10.1002/j.1537-2197.1990.tb13528.x>. Accessed: Oct. 27, 2021. doi: 10.1002/j.1537-2197.1990.tb13528.x.

KUMAR, N. et al. Effect of arbuscular mycorrhiza fungi (AMF) on early seedling growth of some multipurpose tree species. **International Journal of Current Microbiology and Applied Sciences**, v.6, n.7, p.3885-3892, 2017. Available from: <https://doi.org/10.20546/ijcmas.2017.607.400>. Accessed: Oct. 27, 2021. doi: 10.20546/ijcmas.2017.607.400.

LANFRANCO, L. et al. Partner communication and role of nutrients in the arbuscular mycorrhizal symbiosis. **New Phytologist**, v.220, n.4, p.1031-1046, 2018. Available from: <https://doi.org/10.1111/nph.15230>. Accessed: Oct. 27, 2021. doi: 10.1111/nph.15230.

LIU, X. M. et al. Physiological responses of the two blueberry cultivars to inoculation with an arbuscular mycorrhizal fungus under low-temperature stress. **Journal of Plant Nutrition**, v.40, n.18, p.2562-2570, 2017. Available from: <https://doi.org/10.1080/01904167.2017.1380823>. Accessed: Oct. 27, 2021. doi: 10.1080/01904167.2017.1380823.

LUDWIG-MÜLLER, J.; GÜTHER, M. Auxins as signals in arbuscular mycorrhiza formation. **Plant Signaling & Behavior**, v.2, n.3, p.194-196, 2007. Available from: <https://doi.org/10.4161/psb.2.3.4152>. Accessed: Oct. 27, 2021. doi: 10.4161/psb.2.3.4152.

MACHINESKI, G. S. et al. Effects of arbuscular mycorrhizal fungi on early development of persimmon seedlings. **Folia Horticulturae**, v.30, n.1, p.39-46, 2018. Available from: <https://doi.org/10.2478/fhort-2018-0004>. Accessed: Oct. 27, 2021. doi: 10.2478/fhort-2018-0004.

MAHMOOD, T. et al. Insights into drought stress signaling in plants and the molecular genetic basis of cotton drought tolerance. **Cells**, v.9, n.1, p.105, 2020. Available from: <https://doi.org/10.3390/cells9010105>. Accessed: Oct. 27, 2021. doi: 10.3390/cells9010105.

MIHALJEVIĆ, S.; SALOPEK-SONDI, B. Alanine conjugate of indole-3-butyric acid improves rooting of highbush blueberries. **Plant, Soil and Environment**, v.58, n.5, p.236-241, 2012. Available from: <https://doi.org/10.17221/34/2012-PSE>. Accessed: Oct. 27, 2021. doi: 10.17221/34/2012-PSE.

OLIVEIRA, D. F. B. et al. Pre-colonized seedlings with arbuscular mycorrhizal fungi: an alternative for the cultivation of *Jatropha curcas* L. in salinized soils. **Theoretical and Experimental Plant Physiology**, v.29, n.3, p.129-142, 2017. Available from: <https://doi.org/10.1007/s40626-017-0089-7>. Accessed: Oct. 27, 2021. doi: 10.1007/s40626-017-0089-7.

PERTUZATTI, P. B. et al. Phenolics profiling by HPLC-DAD-ESI-MSn aided by principal component analysis to classify Rabbiteye and Highbush blueberries. **Food Chemistry**, v.340, p.127958, 2021. Available from: <https://doi.org/10.1016/j.foodchem.2020.127958>. Accessed: Oct. 23, 2021. doi: 10.1016/j.foodchem.2020.127958.

PHILLIPS, J. M.; HAYMAN, D. S. Improved procedures for clearing roots and staining parasitic and vesicular mycorrhizal fungi for rapid assessment of infection. **Transactions of the British Mycological Society**, Cambridge, v.55, p.158-161, 1970. Available from: <https://www.sciencedirect.com/science/article/pii/S0007153670801103>. Accessed: Oct. 23, 2021.

PLENCHETTE, C. et al. Growth responses of several plant species to mycorrhizae in a soil of moderate P-fertility. **Plant and Soil**, v.70, n.2, p.199-209, 1983. Available from: <https://doi.org/10.1007/BF02374780>. Accessed: Oct. 23, 2021. doi: 10.1007/BF02374780.

SILVA, F. C. et al. **Manual de análises químicas de solos, plantas e fertilizantes**. Brasília, DF: Embrapa Informação Tecnológica; Rio de Janeiro: Embrapa Solos, 2009. 627p.

SPINARDI, B.; AYUB, R. A. Desenvolvimento inicial de cultivares de mirtileiro na região de Ponta Grossa (PR) Initial Development of mirtileiro cultivars in the region of Ponta Grossa (PR). **Ambiência**, v.9, n.1, p.199-205, 2013. Available from: <https://doi.org/10.5777/ambiencia.2013.01.01rc>. Accessed: Oct. 23, 2021. doi: 10.5777/ambiencia.2013.01.01rc.

SOARES, A. C. F. et al. Fungos micorrízicosarbusculares no crescimento e nutrição de mudas de jenipapeiro. **Revista Ciência Agronômica**, v.43, n.1, p.47-54, 2012. Available from: <https://doi.org/https://doi.org/10.1590/S1806-66902012000100006>. Accessed: Oct. 13, 2021. doi: 10.1590/S1806-66902012000100006.

STEPHENIE, S. et al. An insight on superoxide dismutase (SOD) from plants for mammalian health enhancement. **Journal of Functional Foods**, v.68, p.103917, 2020. Available from: <https://doi.org/10.1016/j.jff.2020.103917>. Accessed: Oct. 13, 2021. doi: 10.1016/j.jff.2020.103917.

TEISSEIRE, H.; GUY, V. Copper-induced changes in antioxidant enzymes activities in fronds of duckweed (Lemna minor). **Plant Science**, v.153, n.1, p.65-72, 2000. Available from: <https://doi.org/10.1016/S0168-9452(99)00257-5>. Accessed: Oct. 10, 2021. doi: 10.1016/S0168-9452(99)00257-5.

THORNLEY, J. H.; PARSONS, A. J. Allocation of new growth between shoot, root and mycorrhiza in relation to carbon, nitrogen and phosphate supply: teleonomy with maximum growth rate. **Journal of Theoretical Biology**, v.342, p.1-14, 2014. Available from: <https://doi.org/10.1016/j.jtbi.2013.10.003>. Accessed: Oct. 10, 2021. doi: 10.1016/j.jtbi.2013.10.003.

TU, J. L. et al. Effects of arbuscular mycorrhizal inoculation on osmoregulation and antioxidant responses of blueberry plants. **Bangladesh Journal of Botany**, v.48, n.3, p.641-647, 2019. Available from: <https://doi.org/10.1038/s41598-021-97742-1>. Accessed: Oct. 10, 2021. doi: 10.1038/s41598-021-97742-1.

WANG, L. et al. Effect of arbuscular mycorrhizal fungi in roots on antioxidant enzyme activity in leaves of Robinia pseudoacacia L. seedlings under elevated CO2 and Cd exposure. **Environmental Pollution**, v.294, p.118652, 2022. Available from: <https://doi.org/10.1016/j.envpol.2021.118652>. Accessed: Oct. 10, 2021. doi: 10.1016/j.envpol.2021.118652.

YANG, Lu et al. Comparative transcriptome analysis reveals positive effects of arbuscular mycorrhizal fungi inoculation on photosynthesis and high-pH tolerance in blueberry seedlings. **Trees**, v.34, p.433-444, 2020. Available from: <https://doi.org/10.1007/s00468-019-01926-2>. Accessed: Oct. 10, 2021. doi: 10.1007/s00468-019-01926-2.