GROWTH OF *Kielmeyera rubriflora* INOCULATED WITH ARBUSCULAR MYCHORRIZAL FUNGI ON IRON MINING SUBSTRATES

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Received for publication: 12/04/2018 – Accepted for publication: 15/08/2019

Resumo

Crescimento de Kielmeyera rubriflora inoculada com fungos micorrizicos arbusculares em substratos de mineração de ferro. A restauração de áreas após a exploração dos recursos naturais, pode ser realizada através da adoção de técnicas usadas com a finalidade de otimizar o processo de restauração. Dentre as técnicas destaca-se o plantio de mudas de espécies adaptadas às condições edafoclimáticas adversas e que possuam associações interespecíficas com micro-organismos. Dessa forma, o objetivo desse trabalho foi avaliar a influência da inoculação de fungos micorrízicos arbusculares na sobrevivência e crescimento de *Kielmeyera rubriflora* (Spreng) Mart., em dois substratos oriundos da mineração de ferro. O experimento foi conduzido em viveiro do Centro de Propagação de Espécies Vegetais (CIPEF) da UFVJM, em delineamento de blocos casualizados e os tratamentos dispostos em fatorial 2 x 2, sendo dois substratos oriundos da mineração de ferro e as plantas inoculadas e não inoculadas, com cinco repetições. No momento do transplantio para vasos com 17 dm^3, as plântulas receberam junto às raízes 25 g da mistura do solo-inóculo dos fungos *Paraglomus ocultum* (C. Walker) J.B. Morton & D. Redecker e *Claroideoglomus etunicatum* (W.N. Becker & Gerd) C. Walker & A. Schüßler. O diâmetro do caule e número de folhas foi influenciado apenas pelos efeitos principais de substrato e inoculação, enquantoque a altura das plantas foi influenciada pela interação entre substrato e inoculação. A inoculação com fungos micorrízicos em *K. rubriflora* garante a sobrevivência, crescimento da espécie e desenvolvimento radicular em ambos os substratos de mineração de ferro sendo uma estratégia sugerida nos plantios em áreas degradadas pela mineração de ferro.
Palavras-chave: restauração, micorrizas, Serra do Espinhaço Meridional

Abstract

Mining results in major landscape changes. Restoring areas after exploitation of natural resources can be accomplished through adopting techniques used to optimize the restoration process. Among such techniques, planting seedlings of species adapted to adverse edaphoclimatic conditions and which have interspecific associations with microorganisms stand out. One of these indigenous species which occur in the Southern Espinhaço Mountain Range with potential for use in restoring degraded areas is *Kielmeyera rubriflora* (Spreng) Mart. Thus, the objective of this study was to evaluate the influence of inoculating arbuscular mycorrhizal fungi on the survival and growth of *Kielmeyera rubriflora* Cambess. on two substrates from iron mining. The experiment was carried out in a nursery of the *UFVJM* Plant Species Propagation Center (*CIPEF*) in a randomized block design and treatments arranged in a 2 x 2 factorial design, using two iron mining substrates and inoculated and uninoculated plants with five repetitions. The seedlings received 25 g of the soil-inoculum mixture of *Paraglomus occultum* (C. Walker) JB Morton & D. Redecker and *Claroideoglomus etunicatum* (WN Becker & Gerd) C. Walker & A. Schüßler at the transplantation time to 17 dm^3 pots. Stem diameter and leaf number were influenced only by the main effects of substrate and inoculation, while plant height was influenced by the interaction between substrate and inoculation. Inoculation with mycorrhizal fungi in *Kielmeyera rubriflora* ensures survival, species growth and root development on both iron mining substrates and is a suggested strategy for planting in areas degraded by iron mining.
Keywords: restoration, mycorrhiza, Southern Espinhaço Mountain Range

INTRODUÇÃO

Mining is the most impactful of anthropic activities, as it results in major habitat change. This activity has existed since the colonial era in Brazil, but industrial mining started to grow to focus on various types of mineral resources such as gold, bauxite and iron ore from the 1930s with the advent of industrialization and the government's political investment to exploit these resources (REZENDE, 2016).

Revegetation is a fundamental conservation strategy because it provides necessary protection to reduce erosion sediment loss through vegetation cover by improving the physical and chemical attributes of soils. The implantation of tree species in degraded areas is a procedure which allows skipping the initial stages of natural succession (PEREIRA *et al.*, 2012). Planting seedlings is a widely used technique and contributes to advancing ecological succession processes. However, it is very important to know the vegetation in the region and the edaphoclimatic conditions for the restoration of degraded areas by planting seedlings in order to correctly choose which species should be used in planting (FELFILI *et al.*, 2013).

The diversity of forest species is of great importance for restoration of degraded areas, as it brings benefits such as improved soil quality due to the input of organic matter and nutrient redistribution, favors ecosystem balance, greater attractiveness to fauna, greater soil protection from erosion processes, and greater resistance to pests and diseases (PEREIRA *et al*., 2012). Among the native species occurring in the Southern Espinhaço Mountain Range with potential for use in restoring degraded areas, *Kielmeyera rubriflora* (Spreng) Mart. is a deciduous species typical of more open cerrado areas and popularly known as *pau santo*. It has wide geographical distribution in terms of floristic composition when it comes to the cerrado (JORGE, 2014).

Arbuscular mycorrhizal fungi (AMF) promote plant development in degraded environments and their inoculation in *K. rubriflora* species can guarantee their establishment in mined areas. Studies on mycorrhizal dependence in the field are important to understand plant responses to mycorrhization, as these may have symbiotic associations and responses which vary according to environmental conditions such as soil phosphorus concentration, water content, pH, salinity, temperature, quality and light intensity, among others (BRAGHIROLLI *et al*., 2012). There are reports of plants which depend on mycorrhizae to develop, as is the case with *Piptadenia gonoacantha* (Mart.) J.F. Macbr. (OLIVEIRA-JÚNIOR *et al*., 2017).

Understanding the efficiency of native AMF is another study objective and important for use and application in forest species. The efficiency of AMF in plants is based on inoculation techniques, among which there is the use of mixed inoculum, which is composed of different species. In these cases, the results may be better when compared to inoculation with only one species of mycorrhizal fungus (CALDEIRA *et al*., 1997).

Thus, the aim of this study was to evaluate the influence of inoculation with arbuscular mycorrhizal fungi on the survival and growth of *K. rubriflora* seedlings in two substrates from iron mining.

MATERIAL AND METHODS

The experiment was installed in a greenhouse at the Plant Species Propagation Center (*CIPEF*) of the Federal University of Jequitinhonha and Mucuri Valleys (*UFVJM*) at the JK campus in Diamantina, MG, Brazil. The climate of the region is tropical, characterized by mild and humid summers and cooler, drier winters (Cwb according to the Köppen classification - mesothermal with mild summers, and droughts in winter). Annual rainfall averages between 1250 and 1550 mm and temperature between 18 and 19 ºC. Relative humidity is around 75.6%.

Two types of substrates representative of the AngloA merican Iron Ore Brazil mining area to be recovered were selected. The first is a dystrophic, dark-colored, sandy-colored substrate called DARK (FIGURE 1A). The other substrate is sandy, medium texture, dystrophic with light color, showing fragments of quartz and yoke called BROWN (FIGURE 1B). The substrates underwent solarization for 15 days to avoid interference from native mycorrhizal fungi (SILVA *et al*., 2018), a disinfestation method which consists of covering the ground with a transparent polyethylene film during the intense solar radiation period to increase the ground temperature (RITZINGER and ROCHA, 2010).

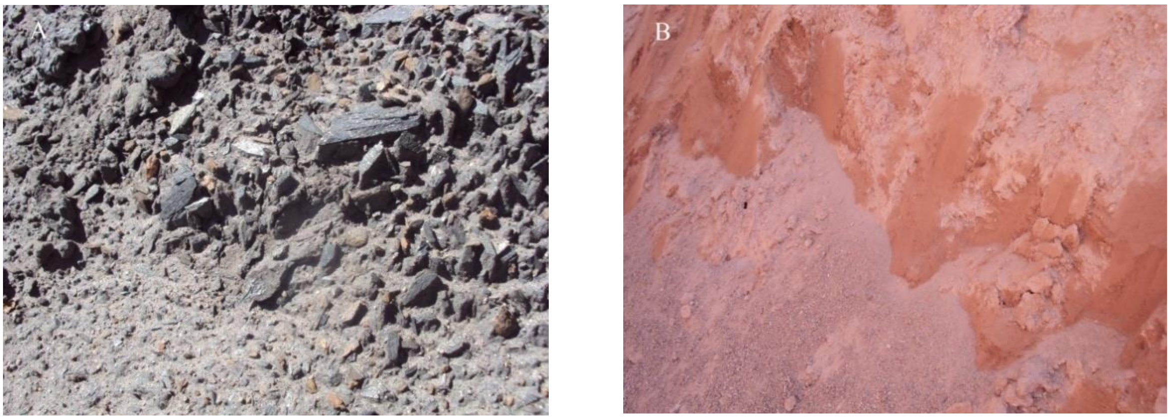

Figure 1. Overview of substrates used from iron mining in Conceição do Mato Dentro-MG. A = DARK and B = BROWN.

Figura 1. Visão geral dos substratos oriundos da mineração de ferro em Conceição do Mato Dentro-MG utilizados. A = ESCURO e B = MARROM.

Chemical analyzes were performed at the Fertility and Soil Analysis laboratory at *UFVJM*. To determine the pH, the ratio 1:2.5 of material:water was used to determine Ca^{2+}, Mg^{2+} and Al^{3+} (1 N KCl); K^{+} and H+Al (1 N calcium acetate pH 7.0); while the methodology proposed by Embrapa (1997) was used to determine P, Fe, Mn, Zn, Cu and Pb (Mehlich^{-1}).

K. rubriflora seeds were selected and collected from the forest fragments of the Anglo American company areas based on the inventory data, respecting the minimum distance of 100 meters between one matrix and another. These seeds were stored in plastic bags after collection and taken to the JF campus of *UFVJM* where they were processed and kept in a cold chamber (5 °C). The seeds were submitted to dormancy breaking by water soaking for 24 h, followed by germination in a BOD chamber with temperature adjusted to 25 °C in trays containing washed and sterilized sand to obtain the seedlings.

The experiment was carried out in a completely randomized design (CRD) and the treatments arranged in a 2 x 2 factorial design constituting two substrates from iron mining and the inoculated and uninoculated plants, with five replications. The experimental unit consisted of three plants, totaling 15 plants per treatment. Eighty (80) black pots with 17 dm^3 capacity (previously cleaned) were used to install the experiment. Three holes were drilled at the bottom of each pot to aid drainage. Three days after radicle emission, three seedlings were transferred to the pots containing the two mining substrates.

At the time of transplantation 25 g of *Paraglomus ocultum* J.B. Morton & D. Redecker (UFLA-252) soil-inoculum mixed with *Claroideoglomus etunicatum* C. Walker & A. Schüßler (UFLA-707) were placed near the roots. These fungi come from the Collection of Mycorrhizal Fungus Cultures from the Federal University of Lavras - *UFLA*. The soil inoculum provided approximately 200 spores of the two fungi per seedling; this count was obtained using the cross-linked plate method (MELLO, 2012).

Irrigation was done manually 3 times a day, according to the field capacity results of each substrate at 70%. This calculation corresponded to 250 mL of water for each seedling within each pot.

Two cover fertilizers were performed at 10 and 30 days after seedling transplantation into the pots. These needs were calculated based on the book "The 5th approximation of the Recommendations for the use of concealers and fertilizers in Minas Gerais" (*5ª aproximação das Recomendações para uso de corretivos e fertilizantes*, *em Minas Gerais*), where the calculation of urea (45% N) and potassium chloride (K_2O) was performed for the low need for plant production; this means the calculation considered 10 kg/ha of N needed and 1mmol/cm^3 of exchangeable potassium, resulting in the final application of 20 mL of urea and chloride solution per pot.

Plant survival was assessed at 180 days after seedling transplantation into the pots. Survival rate was calculated according to the equation: T (%) = nx100/N, where: T% = survival rate; N = number of individuals planted; n = number of individuals from last evaluation.

Height growth, stem diameter, leaf number and plant crown area were evaluated 180 days after transplanting. The shoot and the remaining roots were dried at 60 °C until reaching constant weight to determine the dry mass. In relation to the roots, duplicates of the plants were made in each pot, where one of the duplicates of each repetition was used for staining and the other for oven drying; thus, the value of root weight was not underestimated.

Root samples were collected and placed in 10% KOH solution at room temperature for 24 h for depigmentation of the colonized root length. Then the roots were washed in running water and placed in 1% HCl solution for 5 min. The depigmented samples were placed in trypan blue solution in lactophenol (0.05%) to stain the fungal structures within the roots and kept for 20 min (PHILLIPS and HAYMAN, 1970). The reticulated plate method was used to determine the percentage of colonized root length, which consisted of using a magnifying glass and a grid-shaped Petri dish (0.5 x 0.5 cm). The roots were randomly distributed on this plate and the reading was performed vertically and horizontally, considering the presence and absence of AMFs on the grid line (GIOVANETTI and MOSSE, 1980).

The results of all evaluated variables were subjected to Shapiro-Wilk normality test at 5% significance and the number of spores and colonized root length percentages were respectively transformed into $(x+1)^{1/2}$ and arc sine $(x)^{1/2}$. Then, the analysis of variance (ANOVA) was performed and the means were compared by the Tukey test ($p < 0.05$).

RESULTS

Height was influenced by the interaction between substrates and inoculation of mycorrhizal fungi. Inoculation with arbuscular mycorrhizal fungi provided an increase in plant height in both substrates (TABLE 1).

Table 1. Height (cm) of *Kielmeyera rubriflora* plants grown for 180 days on two distinct substrates (brown and dark).
Tabela 1. Altura (cm) de plantas de *Kielmeyera rubriflora* crescidas por 180 dias em dois substratos distintos (marrom e escuro).

Substrate	Height (cm)	
	Non-inoculated	Inoculated
Dark	0,75 bB	2,50 aA
Brown	1,49 aB	2,23 aA

Means followed by equal uppercase letters in the row and lowercase equals in the column do not differ from each other by the Tukey test at 5% significance.

The stem diameter of the plants was not influenced by inoculation, but there was interaction between treatments. However, in analyzing the simple effects it is noted that the stem diameter of the seedlings did not vary, regardless of the treatment (**Erro! Fonte de referência não encontrada.** 2). The number of leaves and crown area were only influenced by AMF inoculation, presenting higher number of leaves and leaf area in inoculated plants compared to non-inoculated seedlings (**Erro! Fonte de referência não encontrada.**).

Table 2. Average per treatment for diameter, number of leaves and crown area of *Kielmeyera rubriflora* plants grown for 180 days on two different substrates (brown and dark).
Tabela 2. Média por tratamento para diâmetro, número de folhas e área de copa de plantas de *Kielmeyera rubriflora* crescidas por 180 dias em dois substratos distintos (marrom e escuro).

Treatment		**Diameter (mm)**	**Number of leaves**	**Crown area (cm²)**
Inoculation	Yes	3,58 ± 1,22 ^{ns}	4,42 ± 1,18 A	6,11 ± 2,13 A
	No	2,91 ± 0,50 ^{ns}	2,71 ± 0,76 B	1,25 ± 0,31 B
Substrate	Dark	3,38 ± 1,36 ^{ns}	4,20 ± 1,30 ^{ns}	5,40 ± 2,85 ^{ns}
	Brown	3,21 ± 0,83 ^{ns}	3,33 ± 1,28 ^{ns}	3,47 ± 1,93 ^{ns}

Means followed by equal letters do not differ from each other by the Tukey test at 5% significance. Means followed by ^{ns} do not differ from each other by the F-test at 5% significance.

There was survival of all *K. rubriflora* seedlings in the brown substrate, while survival was higher for inoculated plants in the dark substrate (Figure 2). The colonization percentage in the roots of *K. rubriflora* was higher in plants that received the soil-inoculum containing *P. occultum* and *C. etunicatum* mycorrhizae, spores and hyphae in both substrates.

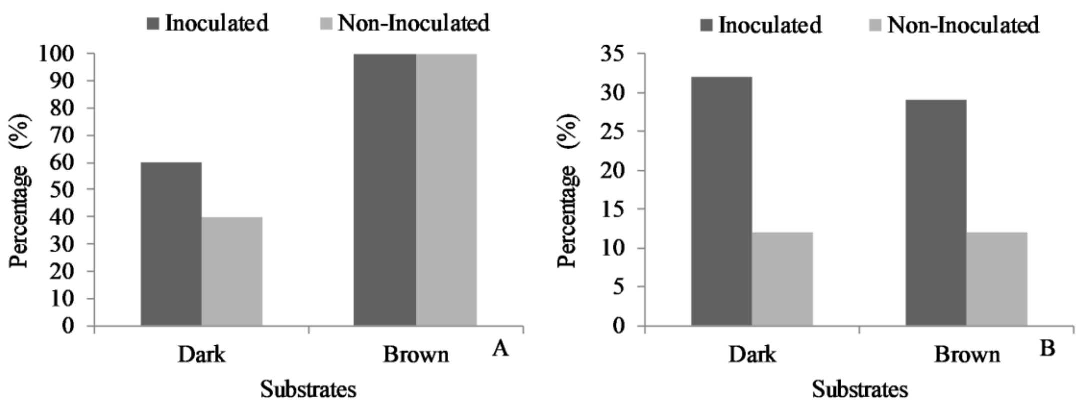

Figure 2. (A) Survival percentage and (B) colonization of *Kielmeyera rubriflora* roots with AMF in substrates of iron mining conducted in a greenhouse in Diamantina - MG.

Figura 2. (A) Porcentagem de sobrevivência e (B) colonização de raízes de *Kielmeyera rubriflora* com FMAs em substratos da mineração de ferro conduzido em casa de vegetação em Diamantina - MG.

DISCUSSION

The results for height showed the importance of using the AMF inoculation technique in forest species for reforestation, with heights of 2.50 cm for dark substrate and 2.23 cm for brown substrate, constituting superior data to the previously mentioned non-inoculated treatments which were 0.75 cm and 1.49 cm, respectively. Increased growth in height has also been observed in *Schinus terebinthifolius* Raddi seedlings also inoculated with *Claroideoglomus etunicatum* (SCHOEN *et al*., 2016).

The importance of mycorrhizal fungi inoculation in relation to plant growth in mining substrates which present poor, degraded and often contaminated environments becomes remarkable (STOFFEL *et al*., 2016). The inoculation of arbuscular mycorrhizal fungi generally promotes an increase in height of seedlings/plants in mining substrates, as observed for sabiá (*Mimosa caesalpinifolia*) seedlings in manganese mining substrate with the inoculation of *Glomus etunicatum* (GARCIA *et al*., 2016), in *Acacia mangium, Mimosa caesalpiniaefolia* and *Schinus terebinthifolius* forest species with the inoculation of mycorrhizal fungi of the *Glomus clarum, Glomus intraradices* and *Gigaspora rosea* species in an area degraded by cassiterite (MENDES *et al*., 2009), and in *Acacia mangium* seedlings with AMF inoculation in manganese contaminated substrate/soil (BECERRIL *et al*., 2013). However, the absence of the effect of mycorrhizal fungi inoculation was also observed in seedlings of *Copaifera martii* and *Dimorphandra macrotachya* (CALDEIRA *et al*., 1997), as well as *Peltogyne venosa* and *Sclerolobium paniculatum* (CALDEIRA *et al*., 1999).

With results close to those found in the present study in studying the response to inoculation in native tree species under greenhouse conditions, Silva *et al*. (2009) reported a very high root colonization for different forest species when inoculated with mycorrhizal fungi.

The plants were placed in the soil and substrate still small (seedlings) and the age of the plant may have influenced the growth response to inoculation, since mycorrhizal fungi, as well as promoting growth and/or nitrogen-fixing bacteria in the production phase of seedlings in nursery or even in the field make seedlings more responsive in development, quality and resistance to adverse conditions. The use of previously-inoculated larger seedlings could enable better development of the plants, since when seedlings are still in adaptation they can respond negatively to the soil or cultivation substrate, but when they are larger the probability of adaptation is greater (GÓES *et al*., 2015; DALANHOL *et al*., 2016).

All plant material of *K. rubriflora* exposed to iron in the present work showed typical symptoms of iron poisoning in the roots, which was observed by the dark brown coloration, brittle consistency and decreased root branches (SIQUEIRA-SILVA *et al*., 2012). Darkening of the root system may occur as a result of iron precipitation on the roots of cultivated plants and may lead to the formation of an iron plate over time which prevents the absorption of other nutrients and causes damage to the cell wall structure. At a more advanced stage, this iron plate formation is related to inhibitions in root and shoot growth (HOWELER, 1973). In fact, the survival of substrate seedlings suggests that inoculation favored seedlings under these conditions.

Studies on the effects of inoculation of arbuscular mycorrhizal fungi on plant species growth in mining areas are generally still quite incipient, especially when it comes to the iron element. In this sense, the knowledge of a certain AMF which provides better growth results and consequently tolerance of a cerrado species can be used to guide work which aims to recover environments with high iron availability.

CONCLUSION

- Inoculation with mycorrhizal fungi in *Kielmeyera rubriflora* plants had a positive effect on the growth of the species in both iron mining substrates and is a suggested strategy for plantations in areas degraded by iron mining.

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Electronic ISSN 1982-4688
DOI: 10.5380/rf.v50 i1. 58894