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Endophytic microorganisms and nitrogen levels on rice plant growth

Micro-organismos endofíticos e níveis de nitrogênio no crescimento de plantas de arroz

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ABSTRACT

Endophytic microorganisms can stimulate growth depending on the interaction between the host plant and soil nutrients availability. This work aimed to evaluate the growth of rice BRS Tropical plants cultivated with endophytic microorganisms and nitrogen levels in greenhouse. The experimental design was 4 x 4 factorial scheme completely randomized, corresponding to four treatments (control - without fungal inoculum and three mycorrhizal isolates: UFLA351 - *Rhizoglomus clarum*, UFLA372 - *Claroideoglomus etunicatum* and UFLA401 - *Acaulospora morrowiae*) and four doses of urea (0, 100, 300 and 600 mg Kg^{-1} N), with four replications. Rice BRS Tropical plants were colonized by arbuscular mycorrhizal fungi (AMF) and endophytic fungi dark septate (DSE). Nitrogen fertilization based on urea does not influenced colonization by UFLAs and by endophytic fungi DSE in rice plants. Nitrogen fertilization inhibited the formation of hyphae, but does not the production of vesicles and arbuscules of the UFLAs isolates. Mycorrhizal sporulation was inhibited by nitrogen fertilization, depending on the fungi isolate. The control treatment rice plants colonization by endophytic fungi DSE, without AMF, was inhibited by nitrogen fertilization. The colonization by endophytic fungi DSE do not interfered in the mycorrhizal colonization by UFLA isolates. Rice BRS Tropical plants were responsive to the inoculation of isolates UFLA351 and UFLA401 with 600 mg Kg^{-1} N. Rice BRS Tropical plants cultivated without AMF and with 600 mg Kg^{-1} N were responsive to nitrogen fertilization.

Index terms: Symbiosis; nitrogen fertilization; microbial interaction.

RESUMO

Os micro-organismos endofíticos podem estimular o crescimento a depender da interação com a planta hospedeira e da disponibilidade de nutrientes no solo. O objetivo deste trabalho foi avaliar o crescimento de plantas de arroz BRS Tropical cultivadas com fungos endofíticos e níveis de adubo nitrogenado em estufa agrícola. O delineamento experimental utilizado foi inteiramente ao acaso no esquema fatorial de 4 x 4, correspondentes a quatro tratamentos (controle - sem inóculo fúngico e três isolados micorrízicos: UFLA351 - *Rhizoglomus clarum,* UFLA372 - *Claroideoglomus etunicatum* e UFLA401 - *Acaulospora morrowiae*) e quatro níveis de ureia (0, 100, 300 e 600 mg Kg^{-1} de N) com quatro repeticões. O arroz BRS Tropical foi colonizado por fungos micorrízicos arbusculares (FMA) e por fungos endofíticos "dark septate" (DSE). A adubação nitrogenada à base de ureia não influenciou na colonização por isolados UFLAs e por fungos endofíticos DSE em plantas de arroz. A adubação nitrogenada inibiu a formação de hifas, mas não a produção de vesículas e de arbúsculos dos isoladods UFLAs. A esporulação micorrízica foi inibida por adubação nitrogenada, a depender do isolado fúngico. No controle, sem FMA, a colonização das plantas de arroz por fungos endofíticos DSE foi inibida pela adubação nitrogenada. Os fungos endofíticos DSE não interferiram na colonização micorrízica pelos isolados UFLAs. As plantas de arroz BRS Tropical foram responsivas a inoculação dos isolados UFLA351 e UFLA401 com 600 mg Kg^{-1} N. As plantas de arroz BRS Tropical cultivadas sem AMF e 600 mg Kg^{-1} N foram responsivas à adubação nitrogenada.

Termos para indexação: Simbiose; adubação nitrogenada; interação microbiana.

INTRODUCTION

Fungi and bacteria that develop internally in plant tissue without causing apparent damage can be considered as endophytic microorganisms (Yan et al., 2015), such as: arbuscular mycorrhizal fungi (AMF); endophytic fungi of hyaline hyphae and/or melanized called dark septate (DSE); rhizobacteria; and nitrogen fixing bacteria (Lasudee et al., 2018; Zhang et al., 2017; Hoseinzade et al., 2016; Ribeiro et al., 2011; Detman et al., 2008; Azevedo, 1998).

In microbial interaction with host plants, these organisms can: promote plant growth, depending on the interaction (Santos et al., 2018); to induce the plant defense system against pathogens and/or pests (Volpe et al., 2018, Van Heidjen et al., 2015); increase water and nutrient uptake by plants (Atama et al., 2018); reduce the consumption of

mineral fertilizers (Ziane et al., 2017); reduce loss of the nitrogen and phosphorus in the soil (Teutscherova et al., 2019; Okonji et al., 2018); and promote tolerance to drought and salinity (Rivero et al., 2015).

Arbuscular mycorrhizal fungi (AMF) are biotrophic endophytic microorganisms belonging to the Phylum Glomeromycota, capable of symbiosis with more than 80% of plant species (Van Heidjen et al., 2015). In the mycorrhizal colonization of plants, the AMF forms structures such as spores, hyphae, vesicles, and arbuscules. The mycorrhizal spores are responsible for dissemination and survival of AMF, being predominantly external to the roots. Spores on germination produce hyphae that increase the absorption of water and nutrients, which are made available directly to the plants through the arbuscules. In contrast, the plants transfer photoassimilates in the arbuscules. The vesicles are responsible for storing energy in the form of lipids for the fungi (Van Heidjen et al., 2015).

The high relation arbuscules: vesicle, with a higher percentage of arbuscules characterizes the mutual transport of nutrients among the symbionts, which favors the development of the AMF and the host plant. On the other hand, the greater percentage of vesicles in relation to that arbuscules demonstrates a competition interaction between the AMF and the plant (Jalonen et al., 2013). Thus, the higher rate of mycorrhizal colonization does not always guarantee an increase in plant biomass (Santos et al., 2018).

Among the crops of economic interest, rice (*Oryza sativa* L.) is one of the most produced and consumed cereals in the world, being considered the main food of the world population. In 2018, Brazil planted 1.877.822 hectares, the Northeast being the second in cultivated area (218.792 hectares) with rice plants, following the Southeast (1.235.024 hectares) (IBGE, 2018).

Atama et al. (2018) verified that rice plants were highly dependent on AMF and that the growth variables analyzed were positively correlated with the mycorrhizal colonization from 23.2% to 79.3%, which ensured an increase in the production and productivity of the rice plants. And Zhang et al. (2017) observed that the mycorrhizal colonization of rice by AMF *Rhizophagus intraradices* increased 7.4% in protein content in the grains. However, Bernaola et al. (2018) verified that colonization of rice plants may vary according to region and year of cultivation. Bhattacharjee and Sharma (2011) cite that: the season of the year; soil fertility; the use of organic and mineral fertilizers; soil pH; soil temperature and humidity; the susceptibility of the plant to root infection; the quality and the type of AMF propagules are also factors that may influence the mycorrhizal colonization and, consequently, the plant response to mycorrhization.

According to Zhang et al. (2016), nitrogen fertilizers can reduce the diversity of mycorrhizal species. Püschel et al. (2016) mentioned that nitrogen fertilizers stimulated mycorrhizal colonization of grass plants and promoted plant growth with immobilization of nitrogen to the biomass of mycorrhizal plants. Zhang et al. (2016) found that AMF colonization associated with nitrogen fertilization reduces the negative effect of high temperature on poaceaus biomass production. Teutscherova et al. (2019) verified that nitrogen absorption by mycorrhizal plants reduces the losses of this element to the atmosphere, mainly in nitrous oxide form, one of the gases of the greenhouse effect and stimulates the development by bacteria that oxide the ammonia, which decompose organic matter. And Hoseinzade et al. (2016) also observed that the interaction of AMF *Glomus mosseae* with free-living nitrogen-fixing bacteria *Herbaspirillum seropedicae* together with the urea (nitrogen source) and triple super phosphate (phosphorus source) fertilizers promoted the growth of rice plants.

The BRS Tropical rice cultivar is a plant adapted to the arid and semi-arid conditions of Brazil, with high productivity (Pereira et al., 2009), whose interaction with endophytic microorganisms and nitrogen fertilization has not yet been found in the literature. Thus, the objective of this work was to evaluate the initial growth of BRS Tropical rice cultivated with endophytic microorganisms and urea levels in greenhouse.

MATERIAL AND METHOD

The experimental design was a 4 x 4 factorial scheme completely randomized, corresponding to the rice (*Oryza sativa* L.) plants cultivation in four treatments (control - without AMF inoculation, and three mycorrhizal isolates: UFLA351 - *Rhizoglomus clarum* Nicolson & Schenck, UFLA372 - *Claroideoglomus etunicatum* Becker & Gerd.), UFLA401 - *Acaulospora morrowiae* Gerdemann and Spain & Schenck), and four urea levels (0, 100, 300, and 600 mg Kg^{-1} N) plus 100 mg dm^{-3} de P_2O_5 with four replications.

Mycorrhiza inoculum production

Mycorrhizal isolates were obtained by donation from Soil Microbiology Laboratory at the Universidade Federal de Lavras. The mycorrhizal propagules (AMF inoculum) were multiplied in autoclaved sandy soil and seeded with 'Marandú' (*Brachiaria brizantha*). Brachiaria 'Marandú' cultivation was carried out under greenhouse conditions for 150 days up until maturation of the plant

under sprinkler irrigation. After this period, the shoot was cut and suspended irrigation for 20 days, and the soil was dried at room temperature, crush, mixed, and used as mycorrhizal inoculant.

Initial rice plants growth in relation to urea levels and endophytic microorganisms

The soil used in the experiment was classified as sandy texture characterized by pH 6.9, organic matter content 4.7 g Kg^{-1}, cation exchange capacity 1.3 cmolc dm^{-3}, percentage saturation per base 76.5%, potassium 8.0 mg Kg^{-1}, phosphorous 8.0 mg Kg^{-1}, and nitrogen total 0.75 g Kg^{-1} (ammonium 26,7 mg Kg^{-1} and nitrate 0,22 mg Kg^{-1}).

In the autoclaved sandy soil were added 100 mg dm^{-3} of rock powder with 15% P_2O_5 and urea (45% N) levels 0, 100, 300, and 600 mg Kg^{-1} as treatments. The substrate (500 g) was distributed in 500 mL plastics cups at a rate of 2:1 (substrate: mycorrhizal inoculum). On control treatment (without AMF inoculum) was added only substrate composed by sandy soil with phosphorus and the levels of urea. It were added 50 g of mycorrhizal inoculum into the treatments with fungi isolates. That one was composed by sandy soil plus host plant's root fragments colonized by fungal isolate and, on average, 100 mycorrhizal spores per 50 grams of substrate. The number of the mycorrhizal spores present in the substrate was determined according to the methodology described by Gerdemann and Nicolson (1963). Afterward, two to three rice BRT Tropical seeds were sown per cup and randomly distributed at the greenhouse and conducted during 90 days with micro sprinkler irrigation.

After 15 days of germination, the nutritional deficiency was observed in the control treatment and were added 5.0 mL of a solution prepared with 5.0 g L^{-1} of the commercial fertilizer composed by 6% nitrogen (N), 18% (P_2O_5), 12% potassium (K_2O), 5% sulphur (S), 2% calcium (Ca), 0.08% magnesium (Mg), 2% iron (Fe), 0.2% zinc (Zn), 0.08% manganese (Mn), 0.06% boron (B), 0.05% copper (Cu), and 0.005% molybdenum (Mo), as described in the label. The fertilization was repeated weekly until the harvest.

Variables evaluated were: germination and identification of endophytic microorganisms in seeds, colonization and mycorrhizal structures (vesicles, arbuscules, and hyphae), number of mycorrhizal spores, colonization by dark septate endophytic (DSE) fungi, plant height, root length, dry shoot matter, dry root matter, and total dry matter after 90 days of inoculation.

For germination test and identification of endophytic microorganism were used 200 seeds of rice BRT Tropical. Seeds surface was disinfested with alcohol at 70%, 0.1% sodium hypochlorite, and subjected to triple washing in autoclaved distilled water for 1 minute at each solution (Alfenas; Mafia, 2007). Seeds were transferred to plastic box Gerbox type with autoclaved filter paper and autoclaved distilled water. The percentage of seed germination was evaluated after eight days of incubation to 25 ± 1 °C without photoperiod. In seeds, fungi have been identified based on reproductive structures; bacteria were isolated in culture medium PDA (potato-dextrose-agar; 39.0 g L^{-1} of commercial mixture) and identified by sequencing of 16S region of the Ribosome, the Embrapa Soja, Londrina, Brazil.

Colonization and mycorrhizal structures were evaluated by the method of intersection according to the methodology by Giovannetti and Mosse (1980) with modifications. For evaluation of mycorrhizal colonization and structures, root fragments with 1.0 cm in length were distributed over a checkered blade (5 x 5 mm) and analyzed the number of fragments colonized and not colonized by optical microscope. The percentage of mycorrhizal colonization (MC) was calculated by equation: MC (%) = ((TCRF/TRF) x 100, where TCRF: total number of colonized root fragments and TRF: total number of colonized root fragments and not colonized. Mycorrhizal colonization was classified according to Carneiro et al. (1998), in which values of 1 to 19%, 20 to 49%, and above 50% were considered as low, medium and high colonization rate, respectively. The percentage of mycorrhizal structures was determined by equation: STR (%) = (A/B) x 100, where STR: structure analyzed, A: total number of root fragments were colonized by the specific structure and B: total number of colonized root fragments.

The number of the mycorrhizal spores present in the substrate was determined according to the methodology described by Gerdemann and Nicolson (1963) in 50 g of soil.

Colonization by DSE was evaluated by the presence of the hyphae septate and melanized according to the classification by Ribeiro et al. (2011) and calculated by equation: DSE (%) = ((DSE/TRF) x 100, where DSE: number of colonized root fragments by DSE and TRF: total number of analyzed root fragments.

Rice plant height and root length were determined with a millimetric rule, being the measurement carried out from the soil surface to the apex of the plant. After drying plant material in an oven with forced circulation of air at 60 °C until constant weight, dry shoot and root matter were determined by a semi-analytical scale. Total dry matter was assessed by the sum of the shoot and root dry matter.

Mycorrhizal dependence (DM), in percentage, was calculated on the dry shoot mass by the equation: DM = [(PM - PC) / PM] x 100, where PM: value of the variable in the plant cultivated with mycorrhizal inoculum and PC: value of the variable in the plant in the control treatment (without AMF inoculum) and cultivated with 0 mg Kg^{-1} N. Mycorrhizal dependence was classified according to Machineski, Balota and Souza (2011), where plants that presented dependency values> 75% were considered to be excessively dependent, from 50 to 75% with high dependence; from 25 to 50% with moderate dependence; and <25% as marginal dependence, which does not respond to mycorrhizal inoculation.

The results were submitted to Anova and Tukey test; F-test and t-test were applied to regression and correlation analysis, respectively to 1% and 5% of probability.

RESULTS AND DISCUSSION

Endophytic microorganisms in rice BRS Tropical seeds

Rice seeds presented 80% of germination, in which 2.8% of the germinated seeds without the presence of fungi and 8.3% of them without bacteria; 62.9% of the seeds had fungi and 26.0% of the seeds contained bacteria. In this result, it should be considered that in some seeds were colonized by fungi and bacteria at the same time.

On germinated seeds were observed the following fungi: *Rhizoctonia* sp. (28.7%), *Fusarium* sp. (13.9%), *Curvularia* sp. (11.1%), *Alternaria* sp. (7.4%), and *Microsporium* sp. (0.9%). Among bacteria observed, *Burkholderia vietnamiensis* (0.9%) was identified and one unidentified with purple colony (25.1%).

Rhizoctonia, Curvularia, Fusarium, and *Alternaria* can be classified as endophytic fungi growth promoters or phytopathogenic depending on the interaction with the plant (Azevedo, 1998). The presence of these fungi can characterize a beneficial relation, but the presence of endophytic fungi may interfere in the colonization by AMF, as described by Yan et al. (2015) with other endophytic fungi.

On non-germinated seeds (20%), the presence of fungi (54.1%) or bacteria (45.9%) was observed. Fungi identified were: *Rhizoctonia* sp. (46.0%), *Fusarium* sp. (5.4%), and *Curvularia* sp. (2.7%); and 5.4% of seeds infected with *Burkholderia vietnamiensis* bacteria and 40.5% seeds with purple colony of unidentified bacteria. The occurrence of these fungi and bacteria on non-germinated seeds can characterizes a pathogenic relationship. However, the bacteria *Burkholderia vietnamiensis*, identified in germinated and non-germinated seeds, was considered as an endophytic plant growth promoter and rice plants nitrogen-fixation by Coenve and Vandamme (2003), but reports were not found on the germination of seeds and its interaction with AMF.

For rice plants, Hoseinzade et al. (2016) observed that free nitrogen-fixing bacteria (*Herbaspirillum seropedicae*) associated to AMF and mineral fertilizers such as urea and triple super phosphate favored the growth of plants. Thus, the presence of the bacteria B. vietnamiensis in germinated seeds may influence in the interaction by UFLAs isolates on rice plant cultivation with urea. However, in the literature, the presence of microorganisms associated with seeds has been disregarded, which may influence mycorrhizal colonization and plant development.

Colonization of rice plants by endophytic microorganisms with nitrogen levels

Verzeaux et al. (2016) cited that the mycorrhizal colonization was reduced by addition of nitrogen, but Püschel et al. (2016) mentioned that the nitrogen fertilization stimulated mycorrhizal colonization and increased the absorption of phosphorus in grass plants. Wang et al. (2018) verified that low nitrogen availability may stimulate competition between AMF and the host plant for nutrients as well it reduced rice plants growth and symbiosis efficiency.

For BRS Tropical rice plants, the levels of 0 to 600 mg Kg^{-1} N did not influenced mycorrhizal colonization (Table 1), contrary to what was found by Püschel et al. (2016) with grass plant, but in this current result it should be considered that mycorrhizal colonization is a complex and dynamic process, which can be influenced by factors such as: plant species and cultivars; by mycorrhizal isolates; season of year as described by Bernaola et al. (2018).

Atama et al. (2018) verified that the rice plants presented mycorrhizal colonization from 23.2% to 79.3% with *Glomus* and *Acaulospora* isolates. Zhang et al. (2017) obtained mycorrhizal colonization from 5.7 to 33.9% with *Rhizophagus intraradices* in rice plants. Comparatively, BRS Tropical rice plants presented mycorrhizal colonization from 18.8% to 60.9% with UFLAs isolates, which can be considered as low to high, according to Carneiro et al. (1998), as also observed in the literature.

On control treatment (without AMF inoculum), the mycorrhizal colonization of 10.7% with 100 mg Kg^{-1} N will not influence the evaluation of the effect of nitrogen and UFLAs on plant growth, since the reference treatment will be the control without fertilization (0 mg Kg^{-1} N), which was not colonized by AMF.

Table 1: Mycorrhizal colonization (MC), hyphae (HYP), vesicles (VES), arbuscules (ARB), relation arbuscules: vesicle, number of mycorrhizal spores (NS) and colonization by endophytic fungi dark septate (DSE) in rice BRS Tropical plants cultivated with various urea levels after 90 days of inoculation[1,2].

Trat.	N (mg Kg^{-1})	MC (%)	HYP (%)	VES (%)	ARB (%)	ARB: VES	NS	DSE (%)
Control	0	0.0a	0.0a	0.0a	0.0a	0.00a	256.8a	261.1a
	100	10.7a	16.7a	1.7a	1.7a	0.25a	220.8ab	107.2b
	300	0.0a	0.0a	0.0a	0.0a	0.00a	255.8ab	25.1b
	600	0.0a	0.0a	0.0a	0.0a	0.00a	106.3b	23.9b
	Equation R^2	Linear 0.18 ns	Linear 0.18 ns	Linear 0.18 ns	Linear 0.18 ns	Linear 0,14 ns	Linear 0.70*	Linear 0.65 ns
UFLA351	0	34.0a	87.2a	12.8a	9.1a	0.43a	174.5a	2.4a
	100	60.9a	37.6b	13.9a	9.2a	0.42a	128.5a	13.4a
	300	48.2a	35.4b	12.8a	16.5a	1.17a	168.3a	31.8a
	600	50.0a	23.9b	23.2a	18.3a	0.41a	137.0a	2.1a
	Equation R^2	Linear 0,28 ns	Linear 0.60**	Linear 0.75 ns	Linear 0.87 ns	Linear 0.01 ns	Linear 0.14 ns	Linear 0.01 ns
UFLA372	0	49.4a	43.6ab	1.2a	3.5a	0.25a	161.8a	23.6a
	100	20.8a	76.7a	16.7a	8.4a	0.50a	244.5a	6.6a
	300	22.3a	19.2b	5.8a	1.9a	0.08a	192.3a	29.0a
	600	23.1a	42.2ab	12.9a	8.6a	0.17a	156.0a	14.6a
	Equation R^2	Linear 0,33 ns	Linear 0,21 ns	Linear 0.14 ns	Linear 0.29 ns	Linear 0.27 ns	Linear 0.47 ns	Linear 0.08 ns
UFLA401	0	35.0a	82.4a	36.0a	48.5a	0.78a	285.5a	54.1a
	100	30.6a	100.8a	19.2a	21.6a	0.30a	95.5b	3.9a
	300	50.3a	76.9a	44.7a	29.2a	0.51a	123.0b	6.2a
	600	18.8a	1.7b	23.3a	10.0a	0.11a	132.0b	1.7a
	Equation R^2	Linear 0.7 ns	Quadratic 0.99 **	Linear 0.21 ns	Linear 0.66 ns	Linear 0.59 ns	Linear 0.62 *	Linear 0.44 ns

[1] Means followed by the same letter in the column do not differ from each other by the Tukey test at 5% probability; [2] ns - not significant ($p \geq 0.05$); * significant at 5% probability ($0.01 \leq p < 0.05$); ** significant at 1% probability ($p < 0.01$).

Bernaola et al. (2018) verified that during mycorrhizal colonization of rice plants with native AMF, it presented higher percentage of hyphae, followed by vesicles and arbuscules, as observed with BRS Tropical rice plants cultivated with UFLAs of AMF (Table 1). Increasing nitrogen levels resulted in significant reduction of hyphae percentage on treatments UFLA351, UFLA372, and UFLA401. For vesicles and arbuscules nitrogen levels have not influenced in all UFLAs treatments (Table 1).

The arbuscules: vesicle (ARB: VES) relation has been used as an indicator of the relation between AMF and the host plant, where lower values characterize a competition and higher values indicate the benefit by the symbionts (Jalonen et al., 2013). For UFLAs treatments, the arbuscules: vesicles relation varied from 0.08 to 1.17, which characterizes from a competition relation to a favorable interaction to the development of the plant depending on the treatment. In the ARB: VES relation was observed maximum value in the treatments UFLA351, UFLA372, and UFLA401 with 300, 100, and 0 mg Kg^{-1} N, respectively, which characterizes a beneficial interaction between these UFLAs isolates and rice plants. However, the increase in nitrogen level did not influenced this variable, whose data were not adjusted for any regression model in all treatments (Table 1).

According to Zhang et al. (2016), the addition of nitrogen fertilizer reduced in 14% the AMF population in rice cultivation. At current work, in the control treatment (without AMF inoculum) with 600 mg Kg^{-1} N the number of mycorrhizal spores was reduced to 41.4% in relation to the control without nitrogen. However, for UFLA 401, reduction in the number of mycorrhizal spores ranged from 33.5% to 46.3% with 100 to 600 mg Kg^{-1} N compared to 0 mg Kg^{-1} N. The reduction of the number of spores in the control and in the UFLA401 was adjusted to linear regression with the increase of the nitrogen levels, but did not influence in the other UFLAs isolates (Table 1).

In the control treatment, mycorrhizal spores can belong to native AMF present in the sandy soil used in the bioassay, but the absence of mycorrhizal colonization with 0, 300, and 600 mg Kg^{-1} N in this treatment demonstrates that the autoclaving of the substrate must have reduced its native AMF spores viability.

Teutscherova et al. (2019) verified that the mycorrhizal colonization of rice plants stimulated the development of the native microorganisms, but Yan et al. (2015) emphasized that endophytic microorganisms do not always stimulate the growth of other organisms in the soil, since they may present antagonistic action depending on the microbial interaction.

In the evaluation of the mycorrhizal colonization of rice plants by UFLA isolates, it was verified colonization by endophytic fungi, which varied from 1.7% to 54.1% without influence of the nitrogen levels. In the control treatment (without AMF inoculum), the level of 100 to 600 mg Kg^{-1} N reduced from 58.9% to 90.8% colonization by endophytic fungi DSE in relation to control without nitrogen (Table 1). There was no correlation between the mycorrhizal colonization by the UFLAs isolates and the endophytic fungi DSE, in all treatments.

The presence of endophytic microorganisms in the control have originated from the seeds and/or from the sandy soil used as the cultivation substrate. Once, AMF-related studies should consider that heat treatment of substrates, and/or surface seed disinfection does not guarantee total elimination of endophytic microorganisms (Barrow; Osuma, 2002).

Besides that, was no correlation between colonization by endophytic fungi DSE and the number of mycorrhizal spores in all treatments, except for UFLA401 which presented a positive correlation ($r = 0.66$, $p < 0.01$), which shows that the colonization by endophytic fungi DSE stimulated the sporulation of mycorrhizal in the isolate UFLA401. Thus, the effect of colonization by endophytic fungi DSE depends on the interaction with isolates UFLAs, as mentioned by Yan et al. (2015) with other endophytic microorganisms.

In the evaluation of colonization by endophytic fungi DSE, the melanized hyphae, may belong to *Curvularia* and *Alternaria* fungi identified in the seeds. However, the absence of typical structures of these fungi did not allow the taxonomic identification, as described by Pereira et al. (2011) with endophytic fungi in rice plants.

In general, it is important to analyze the microbiota present in the seeds used in AMF bioassays, because the presence of fungi and/or endophytic bacteria may influence the interaction mycorrhizal isolates with host plant. Another aspect to be considered is that in the cultivation of rice plants with UFLAs isolates, the nitrogen fertilization did not favor mycorrhizal colonization, which can to represent a reduction of production costs, and also to reduce loss of nitrogen in the soil, as well as stimulate the microbiota present in the rhizosphere, which may assist in the cycling of nutrients in the soil, and promote plant growth as mentioned by Teutscherova et al. (2019).

Initial growth of rice plants with endophytic microorganisms and nitrogen levels

Nitrogen fertilization in poaceaus plants cultivated with AMF can promote growth (Püschel et al., 2016) and improve phosphorus absorption (Okonji et al., 2018). Lasudee et al. (2018) and Hoseinzade et al. (2016) also observed that the association of AMF with nitrogen fertilizers and rhizospheric bacteria stimulated the growth of rice plants in low fertility soils. And Bhattacharjee and Sharma (2011) mentioned that the rhizospheric bacteria increased the availability of nitrogen in the soil, which favored the mycorrhizal colonization of rice plants.

In the rice BRS Tropical, the nitrogen fertilization did not stimulate the growth evaluated by the height of the plants cultivated in the control treatment (without AMF inoculum) and colonized by the UFLAs isolates, except in the treatment with UFLA372 that presented a significant increase from 237.3%, 204.6% to 265.5% with 100, 300, and 600 mg Kg^{-1} N, respectively, in relation to the control treatment (without AMF inoculum) with 0 mg Kg^{-1} N. Nitrogen fertilization also did not increase the dry shoot matter rice plants for all treatments (control and UFLAs) (Table 2), which differ from results cited by Scandellari (2017) with grapevines and by Püschel et al. (2016) with *Andropogon gerardii,* a perennial grass.

At root length, nitrogen fertilization also did not influence all treatments (control and UFLAs), except for UFLA401 whose data were adjusted to linear regression

($p<0.01$). The rice plants cultivated with UFLA351 and 300 mg Kg^{-1} N showed a significant increase by 167.5% in root length, when compared to control treatment (without AMF) and with 0 mg Kg^{-1} N by ANOVA, but the data did not fit any regression model with increasing nitrogen levels. For UFLA401, the increase of the nitrogen levels reduced the root length, whose data were adjusted to linear regression, but without significant difference by the Tukey test (Table 2).

Nitrogen fertilization also did not influence the dry shoot matter and dry root matter, whose data were not adjusted to any regression model, in all treatments (control and UFLAs) (Table 2).

Teutscherova et al. (2019) observed that the higher increase in root biomass compared to the aerial part of the plant, with the addition of nitrogen fertilizers such as urea, may characterize a competition relationship between the AMF and the host plant.

In the rice BRS Tropical plants, the increase of the nitrogen did not influence the dry shoot matter: root dry matter relation, in all treatments (control and UFLAs), but this relation ranged from 1.1 to 3.1, indicating a beneficial interaction between UFLAs and rice plants, as well as nitrogen fertilization in the plants grown in the control (Table 2).

Table 2: Plant height (PH), dry shoot mass (DSM), root length (RL) and dry root mass (DRM) and relation dry shoot mass by dry root mass (DSM/DRM) of rice BRS Tropical plants cultivated with UFLAs isolated and with various nitrogen levels, after 90 days of inoculation[1,2].

Treatments	N (mg kg^{-1})	PH (cm)	DSM (g)	RL (cm)	DRM (g)	DSM/ DRM
Control	0	45.2a	0.33a	11.7a	0.16a	2.0a
	100	30.5a	0.15a	12.1a	0.15a	1.3a
	300	33.8a	0.23a	15.2a	0.14a	1.6a
	600	39.4a	0.45a	13.3a	0.35a	1.5a
	Equation R^2	Linear 0.01 ns	Linear 0.41 ns	Linear 0.29 ns	Linear 0.71 ns	Linear 0.16 ns
UFLA351	0	36.8a	0.29a	11.6b	0.13a	2.3a
	100	36.2a	0.28a	12.6b	0.22a	1.5a
	300	40.3a	0.30a	19.6a	0.19a	2.3a
	600	41.8a	0.35a	13.4b	0.25a	1.4a
	Equation R^2	Linear 0.87 ns	Linear 0.86 ns	Linear 0,09 ns	Linear 0.61 ns	Linear 0.27 ns
UFLA372	0	11.0b	0.13a	11.1a	0.06a	3.2a
	100	37.1a	0.26a	15.5a	0.26a	1.1a
	300	33.5a	0.18a	13.5a	0.11a	1.8a
	600	40.2a	0.38a	14.6a	0.26a	1.5a
	Equation R^2	Linear 0.50*	Linear 0.66 ns	Linear 0.21 ns	Linear 0.31 ns	Linear 0.21 ns
UFLA401	0	39.7a	0.35a	16.9a	0.17ab	2.4a
	100	37.0a	0.27a	14.3a	0.32ab	3.1a
	300	35.7a	0.24a	13.0a	0.10b	3.0a
	600	46.7a	0.48a	12.4a	0.43a	1.1a
	Equation R^2	Linear 0.47 ns	Linear 0.37 ns	Linear 0.75*	Linear 0.31 ns	Linear 0,51 ns

[1] Means followed by the same letter in the column does not differ from one another by the Tukey test at 5% probability; [2] (ns) - not significant ($p \geq 0.05$); (*) significant at 5% probability ($0.01 \leq p < 0.05$); and (**) significant at 1% probability ($p < 0.01$).

According to Atama et al. (2018), the growth of rice plants was correlated with mycorrhizal colonization. In rice BRS tropical plants there was no correlation between the growth variables analyzed (plant height, root length, dry shoot matter, dry root matter) of plants colonized by UFLAs isolates. Similarly, colonization by endophytic fungi DSE presented no correlation with the growth variables analyzed, except with UFLA351 that occured a positive correlation between colonization by endophytic fungi and root length ($r = 0.68$, $p < 0.01$).

Mackineski, Balota and Souza (2011) considered that plants colonized by AMF are responsive to inoculation, when present mycorrhizal dependence is higher than 25%. In the rice BRS Tropical plants presented 44.7%, 36.3%, and 63.3% increase in dry root matter of the plants cultivated in the control treatment, UFLA351, and UFLA401 with 600 mg Kg^{-1} N (Figures 1 and 2), which characterizes that BRS Tropical rice plants presents moderate to high mycorrhizal dependence, depending on the fungal isolate and nitrogen fertilization the according by classification and Mackineski, Balota and Souza (2011).

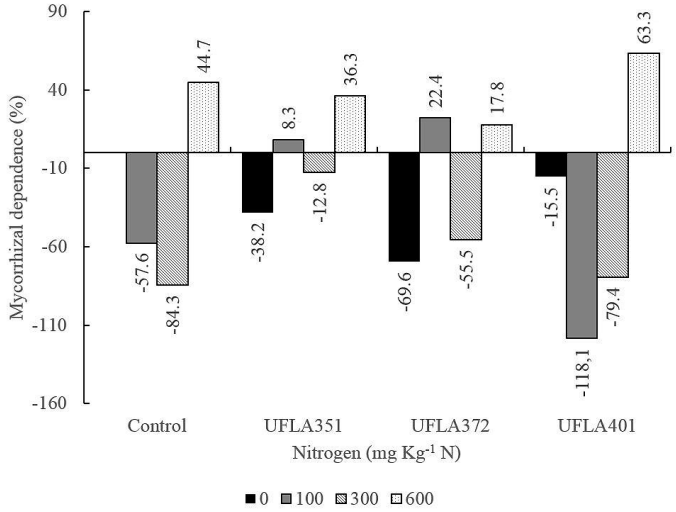

Figure 1: Mycorrhizal dependence of BRS Tropical rice cultivated with UFLAs isolates and nitrogen levels, in relation to the control (without AMF inoculum) and with 0 mg Kg^{-1} N.

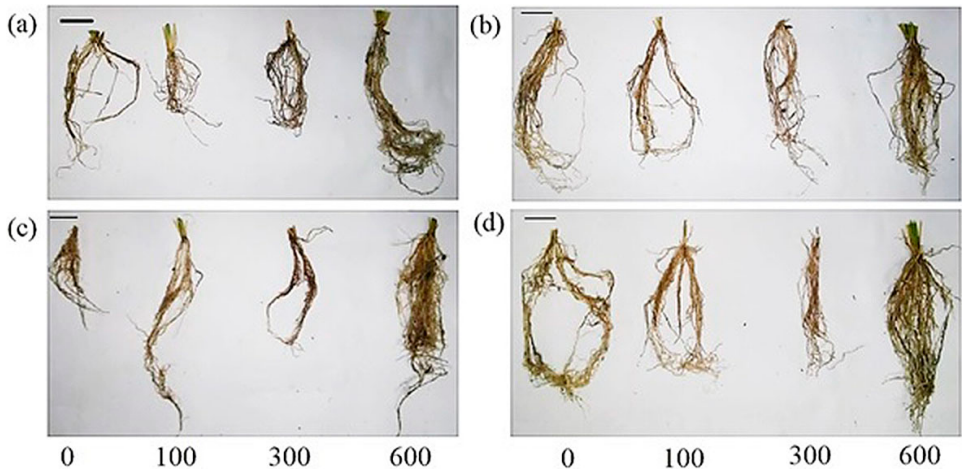

Figure 2: Roots of rice BRS-Tropical plants cultivated in the control (a), UFLA351 (b), UFLA372 (c) and UFLA401 (d) treatments with 0, 100, 300, and 600 mg Kg^{-1} N (ruler = 2 cm).

The increase in biomass with nitrogen fertilization in mycorrhizal and control plants with 600 mg Kg^{-1} N may be associated with improved nitrogen uptake (Hoseinzade et al. (2016), and by immobilization of nitrogen in biomass plant (Zhang et al., 2017). Lasudee et al. (2018) also verified that the mycorrhizal plants increased the solubilization of phosphates present in the soil, when associated to the rhizobacteria, which also may have influenced the growth of the roots.

In general, urea nitrogen fertilization favored rice growth considering the dry root matter of rice plants without AMF (control) and cultivated with isolates UFLA351 and UFLA401. Mycorrhizal colonization of rice RBS Tropical by UFLAs isolates may reduce nitrogen and phosphorus losses, as well as favor precocity in flowering and maturation, as cited by Okonji et al. (2018). Thus, it is important to continue this study, especially at different seasons, since Bernola et al. (2018) observed variation in mycorrhizal colonization depending on the year of cultivation.

CONCLUSIONS

The process of surface disinfestation of rice seeds does not eliminate endophytic microorganisms. Nitrogen fertilization based on urea does not influenced colonization by UFLAs and dark septate endophytic fungi of rice plants. Nitrogen fertilization inhibited the formation of hyphae, but does not influenced the production of vesicles and arbuscules of the UFLAs isolates. Mycorrhizal sporulation is influenced by nitrogen fertilization, depending on the fungi isolate. The colonization by endophytic fungi DSE does not interfered in the mycorrhizal colonization by UFLA isolates. In the control treatment, colonization of rice plants by dark septate endophytic fungi was inhibited by urea - based nitrogen fertilization. Rice BRS Tropical plants were responsive to the inoculation of isolates UFLA351 and UFLA401 with 600 mg Kg^{-1} N. Rice BRS Tropical plants cultivated without AMF and with 600 mg Kg^{-1} N were responsive to nitrogen fertilization.

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