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# Efficient microorganisms: an alternative tool for drought management in bean plants?

## *Microrganismos eficientes: uma ferramenta alternativa para o manejo da seca em plantas de feijoeiro?*

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ABSTRACT

The objective was to evaluate the effect of efficient microorganisms (EM) on common bean plants subjected to water stress. Bean plants were cultivated in a greenhouse which were subjected to treatments with different levels pot capacity (CP) (100; 75 and 50% CP) and/or efficient microorganism (EM) in the dilution of 1:20 or 1:100. The variables analyzed were gas exchange, water use efficiency (WUE), fluorescence and chlorophyll concentration, as well as biometric analysis. The data obtained were subjected to analysis of variance and means were compared by Tukey test ($p < 0.05$). Under severe water stress (50% CP) the photosynthetic rate (*A*) reduced 77% as well as the stomatal conductance. The treatment with EM 1:100 showed transpiration about 3 times higher than the treatment without EM, carboxylative efficiency 3 times higher and initial fluorescence ($F_0$) 1.2 times higher than the other treatments. Under moderate water stress (75% CP) the *A* reduced by 64%, plants treated with EM 1:100 increased the unregulated energy (compared to plants without EM incorporation and the values of EM were maintained, WUE by these treatments. At both levels of water stress the non-photochemical regulated energy dissipation in plants increased with the application of EM 1:20 and EM 1:100 compared to the control, both treatments with EM increased the $F_0$ by 1,7 (EM 1:100) and 1.33 (EM 1:20). Plants had reduced height, stem diameter, fresh biomass and dry for all treatments compared to the control (100% CP). The treatments with EM reduced the leaf area and EM 1:20 was superior. Efficient microorganisms promoted possible resistance to water deficit, indicating the need for further studies. It was concluded that the use of EM appears to be promising, requiring further studies regarding its effects.

*Palavras-chave:*
Agroecologia
Estresse hídrico
Feijão comum
Microrganismos benéficos

RESUMO

Objetivou-se avaliar o efeito dos microrganismos eficientes (ME) em plantas de feijoeiro submetidas ao estresse hídrico. As plantas de feijão foram cultivadas em estufa as quais foram submetidas aos tratamentos com diferentes níveis de capacidade de pote (CP) (100; 75 e 50% CP) e/ou microrganismo eficiente (ME) na diluição de 1:20 ou 1:100. As variáveis analisadas foram as trocas gasosas, eficiência no uso da água (EUA), fluorescência e concentração de clorofila, além de análises biométricas. Em estresse hídrico severo (50% CP) a taxa fotossintética (*A*) reduziu 77% bem como a condutância estomática. O tratamento com ME 1:100 apresentou transpiração cerca de 3 vezes maior que o tratamento sem ME, eficiência carboxilativa 3 vezes maior e fluorescência inicial ($F_0$) 1,2 vezes maior que os demais tratamentos. Em estresse hídrico moderado (75% CP) a *A* reduziu 64%, as plantas tratadas com ME 1:100 aumentaram a energia não regulada em relação às plantas sem incorporação de ME e houve a manutenção dos valores de EUA por esses tratamentos. Em ambos níveis de estresse hídrico a dissipação não-fotoquímica regulada de energia nas plantas aumentou com a aplicação de ME 1:20 e 1:100 em comparação ao controle, ambos os tratamentos com ME aumentaram a $F_0$ em 1,7 (ME 1:100) e 1,33 (ME 1:20). As plantas tiveram redução da altura, diâmetro de haste, biomassa fresca e seca para todos os tratamentos em comparação ao controle (100% CP). Os tratamentos com ME reduziram a área foliar e ME 1:20 foi superior.

## INTRODUTION

Beans are an extremely important crop in Brazil and in the world as they are part of the staple diet of most of the population due to their protein content among other nutrients and minerals that make up the grains (CTSBF, 2012). In this sense, it is also one of the main sources of income for family farmers, since the beans used in public school lunches come mainly from family farming (FERNANDES et al., 2020). In addition to its nutritional importance, it has socioeconomic importance for small producers (SILVA et al. 2019).

The average productivity of beans (*Phaseolus vulgaris* L.) can be affected by several factors such as the incidence of diseases, insects and the climate, with the climate being the main factor (KESHAVARZ; KHODABIN, 2019). Like many cultures, beans are very sensitive to stress caused by excess or lack of water, the latter being the most common one that leads to imbalances in biochemical and physiological processes, causing drastic reductions in productivity (KAZAI et al., 2019). The 2019 harvest of beans in Rio Grande do Sul was heavily affected by the drought, closing production at 18 thousand tons, representing a reduction of 33.3% compared to the previous season (CONAB, 2019). When under situations of low water availability, plants reduce stomatal conductance, which ends up increasing leaf temperature, increasing the chances of leaf burning, decreasing photosynthesis because the stomata closure prevents the entry of $CO_2$ (OLIVEIRA et al. 2012) Thus, plants end up producing smaller amounts of photoassimilates, essential compounds for grain filling and for its general development throughout the cycle (DIPP et al., 2017). Water also acts on cellular turgor and plant growth, translocation of solutes in xylem and phloem vessels respectively, in addition to being fundamental for the germination process (TAIZ; ZAIGER, 2016).

Recent studies indicate a trend of increasing temperatures (hot climates), although there are large variations for the daily thermal range (DTR - Tmax-Tmin) projections for the next 20 years indicate an increase in Tmax and Tmin of 1.33 ℃ and 1.34 ℃ (ZHUANG; ZHANG, 2020). Other studies have shown a global trend of rising temperatures on the Earth's surface between 1.1 and 6.4 °C between 1990 and 2100 (SILVA and PAULA, 2009). These events could lead to an acceleration of desertification due to the decrease in the frequency of precipitation and prolonged droughts by causing vulnerability to vegetation (XU et al., 2020). In this sense, not only the great environmental impact is seen, but also significant socioeconomic destabilization (LEITE et al. 2012).

In Brazil, the average annual temperature has risen by 0.5 °C in the last hundred years and there are signs of changes in rainfall in the Amazon and the Northeast, with increased rainfall and long periods of drought associated with the El Niño phenomenon (MARENGO, 2001). In the state of Rio Grande Sul, in recent years there have been repetitive droughts and droughts that directly affect the local economy, with family farming being one of the activities directly affected (PADILHA et al., 2019). In some regions of the USA and Canada, heat waves hit a record 49 °C and caused fires in some houses in the so-called "Heat Dome", phenomena that, according to the researchers, tend to become more and more frequent and more intense (PHILIP et al., 2021).

Creole beans provide accessibility, as they are low-cost in addition to having a range of varieties, free from biotechnological changes and may even be more productive (MATTAR et al., 2016). Thus, it is the main type of bean used in agroecological management, mainly in the Alto Uruguai region, where family farming prevails, defended by the so-called “seed guardians” (WINCKLER et al., 2018).

HIGA and PARR (1994) reported the first use of Efficient Microorganisms (EM) in plants, presenting excellent arguments for several possible uses of EM. This technology has spread to several locations due to its effectiveness for various managements as sustainable and, in Brazil, they are known as efficient microorganisms or beneficial microorganisms (HARMAN; UPHOFF, 2019). The product is a microbial inoculant, distributed in Brazil by the Mokiti Okada Foundation, whose constitution takes several microorganisms, with more than 10 genera and 80 microbial species such as photosynthetic bacteria, lactic acid bacteria, yeasts, actinomycetes and fermenting fungi that can stimulate plant growth and improve soil fertility (HU; QI, 2013). Several studies using EM have shown its efficiency in increasing crop growth and productivity under stress conditions (RANI et al., 2018). More recently, researchers in Egypt evaluated the effect of applying EM in *Phaseolus vulgaris* L. under salt stress, in which growth and productivity were promoted by the application of homemade EM (TALAAT, 2019). EM act in the modulation of protein synthesis, increase the removal of hydrogen peroxide through the activation of enzymes related to the removal of toxic peroxides, produced in plants when exposed to stress conditions by activating the secondary metabolism of plants (JOSHI et al., 2019). Thus, the application of EM can be promising and an environmentally friendly approach to obtain higher crop yields when they are exposed to stress situations, such as water stress (ASGHARI et al. 2020). This study aimed to evaluate the effect of efficient microorganisms on bean plants subjected to water stress.

## MATERIAL AND METHODS

### Development and conditions of the experimente

The experiment was carried out in a greenhouse and in the Biochemistry and Entomology laboratory of the Universidade Federal da Fronteira Sul in Brazil, RS-135, 200 - Zona Rural, Erechim.

Creole bean seeds were purchased from a producer affiliated with the Center for Support and Promotion of Agroecology (CAPA) and placed in pots containing 5 L of substrate plus soil (soil + organic compost + sand, in a 1:1:1), six seeds per pot were placed and after thinning, two seedlings were left per pot. The experimental design was in a completely randomized factorial scheme, with four replications. The treatments consisted of three doses of EM (0, 1:20 and 1:100) and three levels of irrigation (100, 75 and 50% CP). The plants were subjected to water deficit 5 days after sowing and remained under this condition until sample collection. The pot capacity (CP) was determined by admitting the weight of the

soil saturated with water by capillary action plus the pot weight and 25% (average stress 75% CP) and 50% (severe stress) were omitted for treatments with water restriction 50% CP) of this amount of water in each pot. For the control, 0% was omitted in the amount of water, represented by 100% CP. The pots without the plants were weighed at an interval of two days, to determine the amount of evapotranspiration water, the weight of the pots containing plants and those without plants being previously known. The replacement of evapotranspiration water in the period was carried out using a manual watering can.

From the EM stock prepared at home in accordance with the Technical Standard available at MAPA (Ministry of Agriculture, Livestock and Supply) it was diluted in the following proportions: 1:100 and 1:20 (in distilled water). These diluted solutions were used separately (according to the treatment scheme described below), pouring 4 mL per pot into the soil near the plant's root with the aid of a graduated pipette, every 7 days and at sowing, at the time of irrigation. In the control treatment, only distilled water was poured (TALAAT et al., 2019). The treatments consisted of the following combinations: 100% CP + distilled water; 100% CP + EM (1:20); 100% CP + EM (1:100); 75% CP + distilled water; 75% CP + EM (1:20); 75% CP + EM (1:100); 50% CP + distilled water; 50% CP + EM (1:20); 50% CP + EM (1:100).

**Collection and activation of EM**

The collection of efficient microorganisms was performed according to the MAPA capture methodology protocol: 700 g of organic rice was cooked in unsalted water, after cooling the rice was distributed in pet bottles that were cut in half and previously disinfected. They were placed in a bamboo forest on the premises of the Universidade Federal da Fronteira Sul campus Erechim and covered with litter, where they remained for about 15 days. After this period, it was collected and taken to the laboratory where it was carefully selected according to the ideal colors (orange, pink, blue and yellow) and transferred to new pet bottles in which 200 g of brown sugar was placed and completed with non-chlorine water. These bottles remained in a shaded place at room temperature and were opened every day for 12 days.

**Biometric and biochemical analysis**

The evaluations were made 28 days after sowing in plants at the V3 stage, in fully expanded leaves with approximately the same height. The analyzed variables were transpiration (*E*), stomatal conductance (*gs*), photosynthesis (*A*), and internal $CO_2$ (*Ci*), obtained with an Infrared Gas Analyzer (IRGA model ADC BioScientific (LCpro-SD System Serial No.33961)) from 8am to 11am. In addition, initial fluorescence ($F_0$), quantum yield, electron transport exchange (ETR) and non-photochemical quenching (qN and NPQ) were evaluated with the aid of an Opti-Sciences model fluorometer (OS5P) in dark and light. And the chlorophyll with a chlorophyll fluorometer (SPAD 502 PLUS) in four fully expanded sheets of each repetition, obtaining an average value. These analyzes were all done on the same day. The water use efficiency (USA) was calculated through the relationship between photosynthesis and transpiration = *A*/*E*. The calculations of the quantum yields YNPQ = (F/Fm') - (F/Fm) and unregulated energy dissipation, YNO = F/Fm were made according to Genty et al. (1989).

After 30 days of sowing, one plant was collected from each pot, which were separated into root and shoot, properly stored on ice and taken to the freezer for further protein analysis. After 33 days of sowing, a second collection was made for biometric analysis. Leaf area was analyzed using a Portable leaf area meter (Ci-203), plant height, root size and stem diameter were measured using a caliper, and fresh and dry biomass was measured by weighing in precision scale before and after drying in an oven at 60 ℃ for 48 h, discounting the storage container

Protein quantification was performed according to the method of Bradford (1976). The protein extract was diluted (1:6) in extraction buffer. To assess the protein content, 50 uL of the sample plus 2.5 mL of BG-250 dye reagent was used. After five minutes, readings were taken in a spectrophotometer at 595 nm. The absorbance values obtained for each sample were compared with a standard curve with known concentrations of bovine serum albumin (BSA). The results obtained for protein levels were expressed in mg $L^{-1}$.

*Statistical analysis*

The results were submitted to analysis of variance and the means of each factor were compared by Tukey's test ($p < 0.05$). Correlation analysis was also performed comparing the effect of factors on the analyzed variables. The statistical software plataform R® with used was the Experimental Desings package (Portuguese).

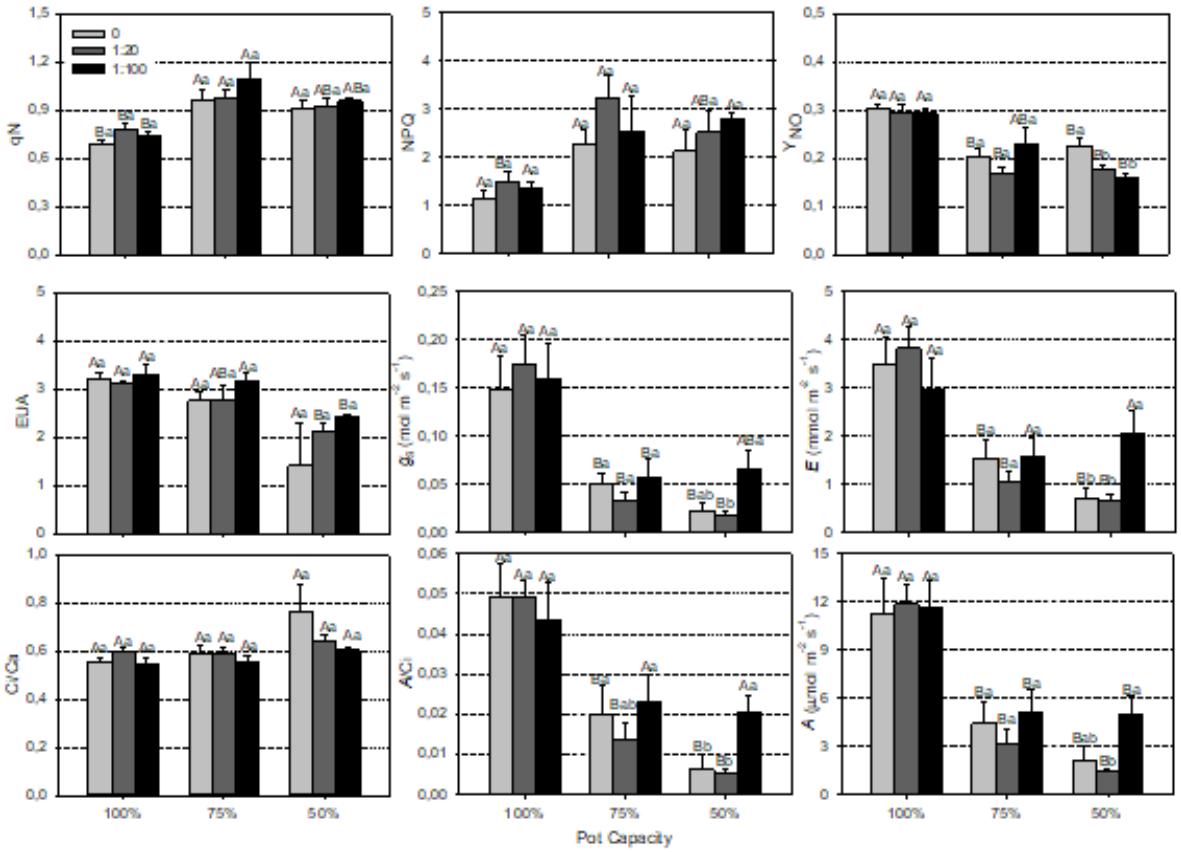
**RESULTS AND DISCUSSION**

The collection of EM according to the MAPA capture methodology was successful once the desired colors were obtained (orange, pink or blue) (Figure 1) and there were few unwanted colors (black, gray, brown). According to the literature (LEITE; MEIRA, 2010) these dark colors are indicative of the presence of microorganisms capable of causing diseases in plants and unwanted for treatment, on the other hand, the more colorful the more diversified the beneficial colonies. g

The treatment with EM 1:20 showed unregulated energy dissipation (Yno) 1.2 times lower than the control treatment at medium stress (75% CP) and the treatment with EM 1:100 showed Yno 1.4 times lower than the control treatment in severe stress (50% CP) (Figure 2) The expected behavior of plants under water deficit conditions is the inversion of the energy dissipation mechanism, where non-photochemical energy dissipation (YNPQ and YNO) is favored in in relation to photochemical energy dissipation (YII), this causes less conversion of electrons into ATP and NADPH in chloroplasts for photosynthesis reaction, thus reducing the photosynthetic rate and consequently the plant development (PELOSO et al., 2017).

Taken together, the results indicate that the use of EM seems to be promising, requiring further studies regarding its effects on other metabolic pathways of plants under different irrigation conditions and different concentrations.

**Figure 2**. Photosynthetic rate (*A*), stomatal conductance (*gs*), transpiration (*E*), carboxylative efficiency (*A*/ *Ci*), relationship between internal and external $CO_2$ concentration (*Ci*/*Ca*), water use efficiency (WUE), unregulated (YNO) and regulated (NPQ) energy dissipation in bean plants treated with different dilutions of efficient microorganisms (EM; 0, 1:20 and 1:100) subjected to different irrigation levels (100, 75 and 50% of pot capacity). Bars represent means (n = 4) ± SE. Means followed by the same lowercase letter, between treatments with EM and uppercase each level of irrigation, do not differ by the Tukey test ($p<0.05$).




The increase in YNO, even when used as a photochemical defense mechanism in plants under stress, confirms the occurrence of photodamage in the transfer of energy to Qa (FIGUEROA et al., 2019). More significantly, the regulated non-photochemical energy dissipation (NPQ) in plants exposed to water stress increased with the application of EM 1:20 (75.4% under moderate stress and 36.1% under severe water stress) and ME 1:100 (37.1% under moderate stress and 50.9% under severe water stress), in relation to the average value of plants grown under 100% CP, regardless of the addition of ME (Figure 2). The increase in NPQ represents the activation of the thermal dissipation pathway related to the xantholipha cycle (NIYOGI et al., 1997), as a mechanism to protect photosystem II from excess energy caused by water deficit. This activation of energy dissipation is used to prevent electrons from bonding to oxygen forming oxigen-reactive species (free radicals) that damage membranes, thus energy dissipation reduces thermal and oxidative stress (KROMDIJK et al., 2016).

The 1:20 treatment was more efficient in medium stress by disfavoring unregulated energy dissipation (Yno) (Figure 2), while the 1:100 EM treatment was more effective in severe stress and the highest average of chlorophyll was also observed for this treatment. agreeing with Fracasso, et al. (2020). Chlorophylls are responsible for absorbing light energy, which is then dissipated in three ways: photochemical dissipation (converted to chemical energy during the photosynthesis process producing NADP and ATP), fluorescence (radiation in the visible range emitted by PSII - red and red distant) and non-photochemical dissipation (represented by non-photochemical quenching) (ZAYOU et al., 2020).

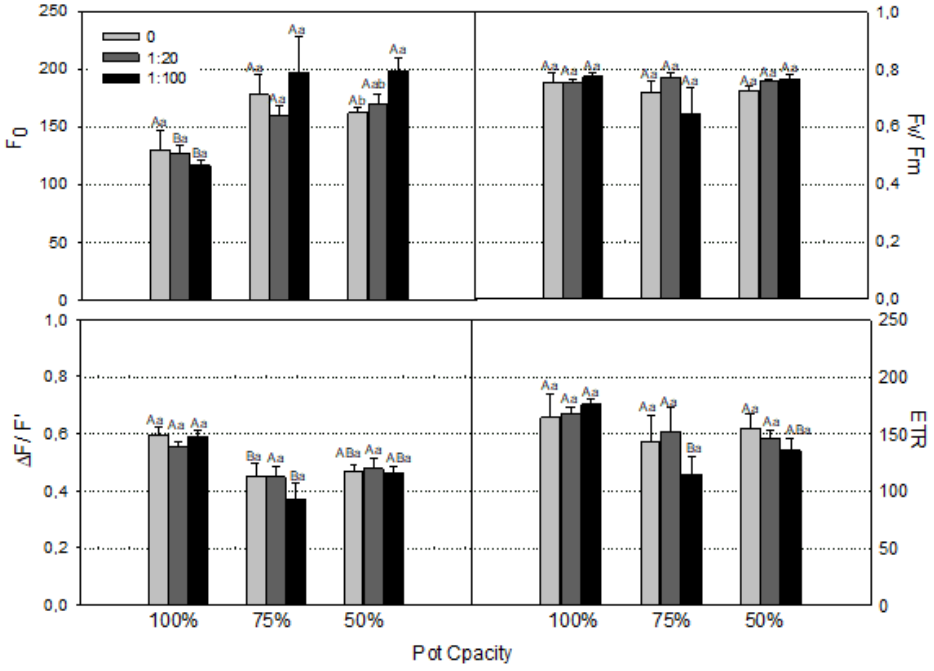
There was a reduction in transpiration (E) for all treatments compared to plants kept in pot capacity (100 % BW) (Figure 2). Plants under water stress reduce transpiration to reduce water loss since there is little availability in the soil (OLIVEIRA et al., 2012). However, under severe stress (50% CP) the plants treated with EM 1:100 transpired 2.84 times more than the control plants, which indicates a possible resistance to stress allowing the plants to maintain transpiration and thus the rate larger photosynthetics. This demonstrates that the reductions in photosynthesis are due to a non-stomatic limitation, possibly due to the restriction of the synthesis of ribulose bisphosphate (RuBP), caused by the inhibition of the synthesis of ATP (LAWLOR, 2002).

There was a reduction in carboxylative efficiency for all treatments, which is a consequence of reduced photosynthesis and increased internal $CO_2$ (CAMPOSTRINI, 2001), but the treatment with EM 1:100 in severe stress was about 3 times higher than the control, indicating resistance to this reduction and therefore a photosynthesis greater than the control. The reduction in water use efficiency can be explained by the

reduction in stomatal conductance, which also tends to reduce leaf area (VELASCO et al., 2019). However, the treatment with EM 1:100 presented the highest average of stomatal conductance (*gs*) and water use efficiency (USA) in relation to the control in severe stress (50% CP) (Figure 2). Plants tend to reduce stomatal conductance in situations of water stress as a way to prevent water loss by closing their stomata (OLIVEIRA et al., 2005). But, because they presented larger WUE, the plants treated with EM 1:100 were able to maintain a larger organ than the other treatments.

MS may have acted in a beneficial way in water regulation according to Ganjeali et al. (2017) through its action on the antioxidant system (enzymatic and non-enzymatic) of plants as well as in carbon assimilation, water use and transpiration rate. This can be explained by the fact that the microorganisms present in the mixture release phytohormones and other substances that activate these metabolic pathways (KHAN et al., 2009).

**Figure 4**. Initial fluorescence parameters ($F_0$), electron transport rate (ETR), fluorescence ratios (ΔF/F' and Fv/Fm) of bean plants treated with different dilutions of efficient microorganisms (EM; 0, 1 :20 and 1:100) subjected to different irrigation levels (100, 75 and 50% of the pot capacity). Bars represent means (n = 4) ± SE. Means followed by the same lowercase letter, between treatments with EM and uppercase each level of irrigation, do not differ by the Tukey test (p<0.05).




The average protein concentration (mL $L^{-1}$) of shoots under pot capacity conditions was the same for all treatments (Figure 3), indicating healthier plants according to the trophobiosis theory (SANTOS; SILVA, 2020). For the root protein concentration averages, the plants treated with EM 1:100 presented root protein amount about 2 times lower than the control under severe stress condition (Figure 3).

Plants treated with EM 1:100 had the lowest average leaf area ($cm^2$) under severe stress representing a reduction of 58% compared to the control (100% CP) and plants treated with EM 1:20 had leaf area of 1,5 times greater than EM 1:100 (Figure 5), which shows the need for further studies with higher concentrations. The stem diameter of plants subjected to water stress was on average 1.24 times smaller than that of plants kept in pot capacity (100% CP) for all treatments (Figure 5). Fresh biomass also reduced for all treatments: 51% (without ME), 53% (EM 1:20) and 56% (EM 1:100) (Figure 5). Just as dry biomass reduced 85% (without ME), 92% (EM 1:20) and 82% (EM 1:100) compared to the control.

Plants subjected to water stress and treated with EM increased the initial fluorescence ($F_0$) being 1.7 (EM 1:100) and 1.33 (EM 1:20) times greater than their respective controls (100% CP) (Figure 4). Under severe stress, plants treated with EM 1:100 had 1.22 times higher $F_0$ than plants without EM application (Figure 4). The increase in $F_0$ may indicate damage to the reaction center or energy dissipation via the antenna complex as a protective measure against excess energy in photosystem II (ALVES et al., 2020).

**Figure 3**. Protein contents of shoot and root of common bean treated with different dilutions of efficient microorganisms (EM; 0, 1:20 and 1:100) subjected to different irrigation levels (100, 75 and 50% CP). Bars represent means (n = 4) ± SE. Means followed by the same lowercase letter, between treatments with EM and uppercase each irrigation level, do not differ by Tukey's test (p<0.05).

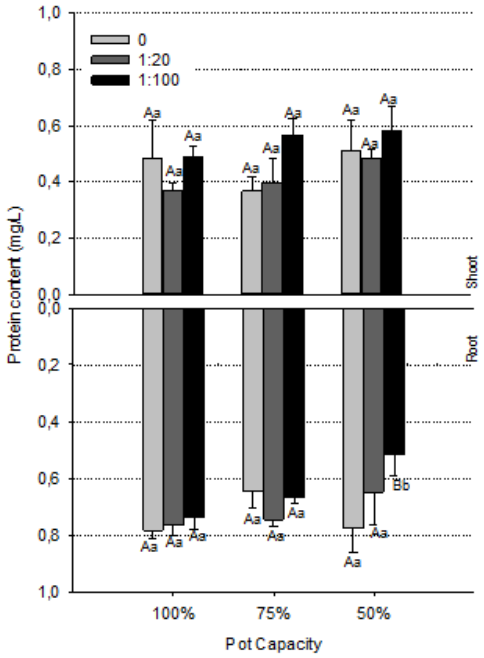
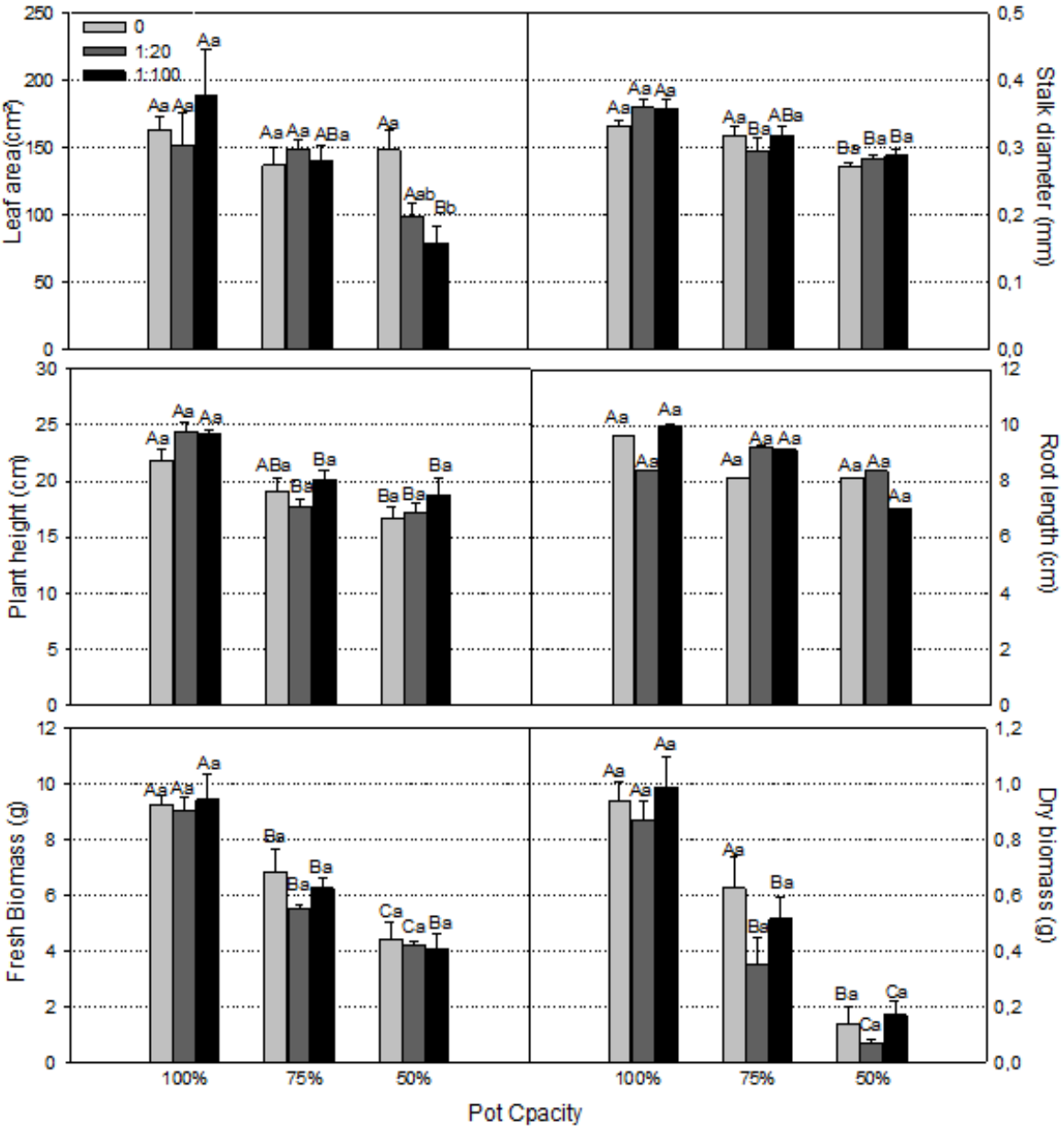


**Figure 5**. Biometric analysis of bean plants treated with different dilutions of efficient microorganisms (EM; 0, 1:20 and 1:100) subjected to different irrigation levels (100, 75 and 50% of the pot capacity). Bars represent means (n = 4) ± SE. Means followed by the same lowercase letter, between treatments with EM and uppercase each level of irrigation, do not differ by Tukey test ($p < 0.05$).




This dissipation was confirmed by the increase in YNO. Plants treated with EM 1:100 also had a reduction in electron transport exchange (ETR) at medium stress (75% PC) which indicates a conversion of the photochemical pathway from photosynthesis to the non-photochemical pathway (FLEXAS; MEDRANO, 2002).

Water plays a fundamental role in several metabolic stages of plants such as stomatal conductance, solute translocation, cell turgor and in photosynthesis it is an initial step (photolysis) without which it is not possible to carry out photosynthes, therefore in the absence of water the plants reduce the photosynthetic rate and this results in smaller stem diameter, plant size, leaf area, fresh and dry biomass (ANDROCIOLI et al., 2020; ARTEAGA et al., 2020).

The correlation analysis of the EM and CP factors with the analyzed variables (Figures 6 and 7) indicates that water stress (independent of the use of EM) had a greater influence on the abnormal behavior of the plants. This indicates the possibility of greater use of the beneficial effects of EM by plants under optimal irrigation conditions. Furthermore, the observed differences caused by both doses indicate the need for further studies.

**Figure 6.** Correlation between the morphological variables analyzed in common bean plants subjected to different degrees of water deficit (100, 75 and 50% of the pot capacity) and treated with different dilutions of efficient microorganisms (EM; 0, 1:20 and 1:100).

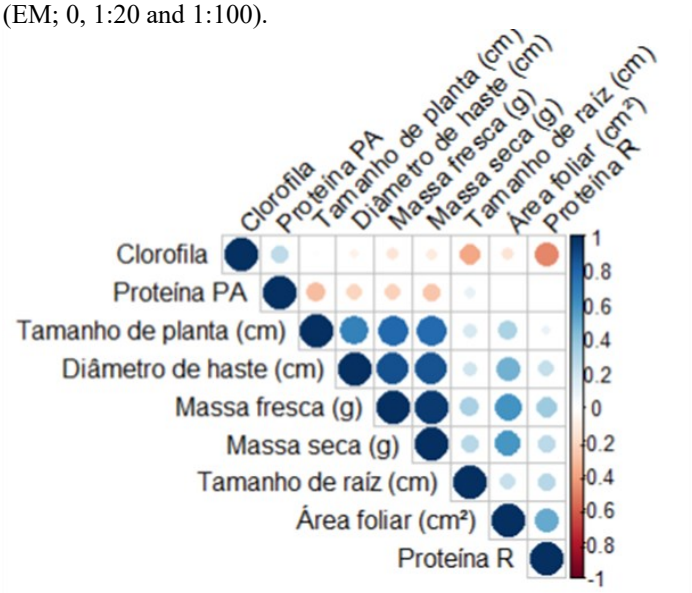
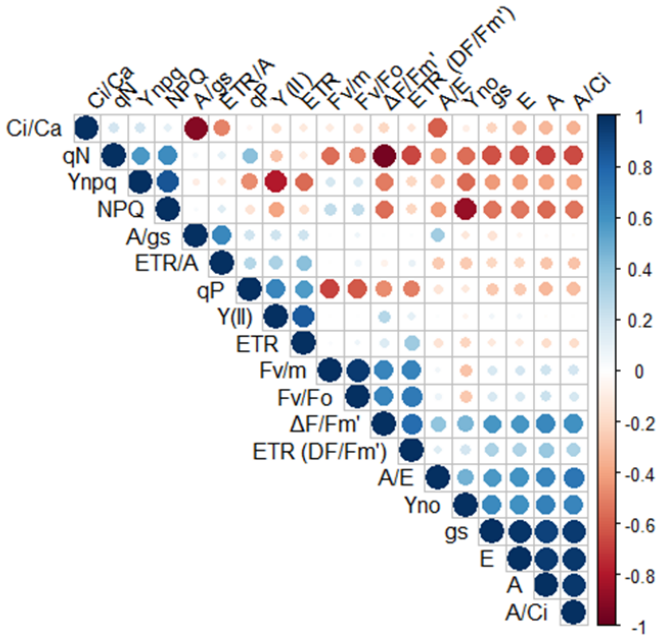



**Figure 7.** Correlation between non-photochemical extinction parameters (qN and NPQ), unregulated energy dissipation (Yno), initial fluorescence ($F_0$), potential quantum yield (Fv/Fm) and electron transport rate (ETR) and gas exchange: transpiration (*E*), stomatal conductance (*gs*), photosynthesis (*A*), relationship between internal and external $CO_2$ concentration (*Ci*/*Ca*), water use efficiency (WUA) and carboxylative efficiency (*A*/*Ci*), analyzed in submitted bean at different degrees of water deficit (100, 75 and 50% of the pot capacity) and treated with different dilutions of efficient microorganisms (EM; 0, 1:20 and 1:100).




## CONCLUSION

There was resistance in reducing transpiration, carboxylative efficiency and photosynthetic rate promoted by EM treatments, in addition to an increase in unregulated energy and non-photochemical regulated energy dissipation helping to protect the photosystem II.

The application of EM did not promote positive effects on leaf area and root protein content.

Water stress affected plant height, fresh and dry biomass, stem diameter and leaf area.

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