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Research Article

Use of *Bacillus aryabhattai*, in coffee seedlings to mitigate the effects of water deficit

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For coffee production to be successful, the stage of choosing, preparing and managing the seedlings is essential. In the present work, the objective was to study the influence of the application of doses of *Bacillus aryabhattai* in arabica coffee seedlings (*cultivar Araras*) recently planted, submitted to accentuated water deficit. The experiment was carried out in a greenhouse in plastic pots with a capacity of 5 dm^3 filled with a substrate and previously corrected to reach 70% base saturation and fertilized according to the needs of the culture. The dose used was 10-8 Colony Forming Units (CFU) of *B. aryabhattai* inoculated directly into the pots at the time of fertilization. Water deficit treatments consisted of two levels of soil moisture maintenance: at 80% of soil Field Capacity (FC) and at 60% CC. The plants were submitted to the following treatments: (i) Treatment 1-plants submitted to 80% of Soil Field Capacity, without fertilization and without inoculation; (ii) Trat 2-plants submitted to 80% of Soil Field Capacity, without fertilization and inoculated with 10-8 CFU of *B. aryabhattai* at a dose of 200 mL ha-1; (iii) Trat 3-plants submitted to 80% of Soil Field Capacity, with Conventional Fertilizer (MAP 11-52-00) and inoculated with 10-8 CFU of *B. aryabhattai* at a dose of 200 mL ha-1; (iv)Treat 4-plants submitted to 80% of Soil Field Capacity, with slow release fertilizer (Organomineral 06-32-00) and inoculated with 10-8 CFU of *B. aryabhattai* at a dose of 200 mL ha-1; (v) Trat 5-plants submitted to 80% of Soil Field Capacity, with controlled release fertilizer (Phusion 06-30-00) and inoculated with 10-8 CFU of *B. aryabhattai* at a dose of 200 mL ha-1; (vi) Treat 6-plants submitted to 60% of Soil Field Capacity, without fertilization and without inoculation; (vii) Trat 7-plants submitted to 60% of Soil Field Capacity, without fertilization and inoculated with 10-8 CFU of *B. aryabhattai* at a dose of 200 mL ha-1; (viii) Trat 8-plants submitted to 60% of Soil Field Capacity, with conventional fertilizer (MAP 11-52-00) and inoculated with 10-8 CFU of *B. aryabhattai* at a dose of 200 mL ha-1; (ix) Trat 9-plants submitted to 60% of Soil Field Capacity, with slow release fertilizer (Organomineral 06-32-00) and inoculated with 10-8 CFU of *B. aryabhattai* at a dose of 200 mL ha-1; (x) Trat 10-plants submitted to 60% of Soil Field Capacity, with controlled release fertilizer (Phusion 06-30-00) and inoculated with 10-8 CFU of *B.* aryabhattai at a dose of 200 mL ha-1. After transplanting, the vegetative growth of the coffee plant was measured monthly, through measurements of plant height and average crown diameter. At the end of the experiment, the number of leaves and the estimated leaf area were measured. In general, there was a difference between the treatments at 30 DAT (Days After Transplanting) and 60 DAT in relation to Crown Diameter (DCO), at 60 DAT the shoot height (APA) also differed, as well as the diameter of stem (DCA) at 75 DAT. More studies are needed to better elucidate the real effect of inoculation of *B. aryabhattai*.

Key words: Mitigation of water stress, arabica coffee, inoculation of seedlings

INTRODUCTION

The area cultivated with Arabica coffee in Brazil is currently 1.76 million hectares and 68% of this area corresponds to the state of Minas (CONAB, 2021). For coffee production to be successful, the stage of choosing, preparing and managing the seedlings is essential. The establishment of a coffee crop requires quality seedlings, which will result in desirable survival rates and rapid initial growth in the field, reducing replanting costs (Ferreira, et al. 2020). However, climate change has been a challenge due to cyclical variations in a short period of time (Dias, et al. 2020). In this sense, modern coffee

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farming needs to adapt to the use of new technologies to meet the consumer market, going through these climatic effects and still produce more at the lowest possible cost (Bravin, et al. 2020). Water restriction regions are the main limiting factor for coffee cultivation, in compliance with the current weather scenario. Climate change can be defined as local changes that have significant differences (Bravin, et al. 2020). Coffee is one of the most impacted grain crops because it is sensitive to high temperatures associated with water stress, affecting the country's economy (Tavares, et al. 2018). Authors such as (Martins, et al. 2010; Damatta, et al. 2019) pointed out the importance of this topic so that studies on strategies to mitigate deleterious climatic effects are developed, since there is a need to adapt agriculture to increasingly frequent climate changes. Impacts generated by weather phenomena on economic activities must be reduced (Foguesatto, et al. 2019). In the last thousands of years, variations in climate have been harming the agriculture sector and ecosystems (Coltri, et al. 2019). Different managements of new technologies and tools that favor the growth and development of plants can be manipulated in the field (Chen, et al. 1996). The applicability of the genetics of the species that most withstand extreme conditions of water scarcity and higher temperatures (Hoffmann, et al. 2011) and the association of plants with microorganisms that carry out processes that favor plant growth are examples of these (Andrade Neto, et al. 1999).

Early detection of crop changes that are not favorable to plant growth allows modifying the medium and maximizing initial growth (Jausoro, et al. 2010). Beneficial bacteria are able to allow greater resistance of plants to conditions of water stress, altering their physiological properties of production of hormones and secondary compounds (Melo, et al. 2007). Without a microbiota associated with the root system of plants, there can be a reduction in growth, vigor and also an increase in seedling mortality in the field. Arabica coffee is the most valued species in the domestic and foreign markets (Machado Filho, et al. 2020). In the present work, the objective was to study the influence of the application of doses of *Bacillus aryabhattai* in newly planted Arabica coffee seedlings, submitted to accentuated water deficit (Jorge, et al. 2008).

MATERIALS AND METHODS

The experiment was conducted in a greenhouse, located at the Centro Universitário do Cerrado Patrocínio-UNICERP in the city of Patrocínio-MG. The plants were subjected to irrigation according to their daily needs, in order to maintain a field capacity of 80% of the maximum water holding capacity in the soil, to avoid water stress before the determined period (Silva, et al. 2005). Soil field capacity was determined by the soil column method (Fernandes, et al. 1968). A crumpled paper towel was placed at the bottom of a 1000 mL plastic beaker with a small hole near the base adding soil (fine air-dried earth-TFSA) to a height of approximately 50 cm. Then the soil was added to the beaker so that the column presents regularity in the distribution of soil particles. At the top of the soil column, 100 ml of filtered water was added. In the opening of the test tube, it was necessary to place a plastic film, to avoid the evaporation of the water. After 48 hours, the top 5 cm of soil was removed with a spatula and discarded. Then, the soil present in the 5 cm to 7 cm layer of the column was removed and the determination of soil moisture (dry basis) of this portion was carried out. The determination of humidity was carried out in an oven at 65°C for 48 h, with the humidity value, expressed on a calculated dry basis, corresponding to the field capacity of the soil, expressed in % (g of water/100 g of dry soil). Soil water control was performed by weighing the pots daily.

In the experiment, the coffee cultivar Arara was used. The transplanting of the seedlings was carried out when they still had four pairs of leaves in plastic pots with a capacity of 5 dm^3, in August 2021. The pots were filled with a mixed substrate of clayey and sandy soil, which was sieved and previously corrected to reach 70% base saturation and fertilized according to the needs of the second crop (Nascimento, et al. 2006). The dose used was 10-8 Colony Forming Units (CFU) of *Bacillus aryabhattai* inoculated directly into the pots equivalent to 200 mL ha-1, at the time of fertilization. The water deficit treatments consisted of two levels of soil moisture maintenance (Pinto, et al. 2008). Plants were maintained at 80% of soil Field Capacity (FC) until 60 Days after Transplanting (DAT) and after this period the water was reduced to 60% CC, characterizing water stress, for 5 days. The plants were submitted to the following treatments (Table 1).

Table 1. Description of treatments.

Treatment/abbreviation[1]	water condition	Source of fertilization[2]	Inoculation with *B. aryabhattai*
1- 80-SA-SI	80% CC	Without fertilization	without
2- 80-SA-CI	80% CC	Without fertilization	with
3- 80-AC-CI	80% CC	Conventional fertilizer (MAP 11-52-00)	with
4- 80-ALL-CI	80% CC	Slow release fertilizer (Organomineral 06-3200)	with
5- 80-ALC-CI	80% CC	Controlled release fertilizer (Phusion 06-3000)	with
6- 60-SA-SI	60% CC	Without fertilization	without
7- 60-SA-CI	60% CC	Without fertilization	with
8- 60-AC-CI	60% CC	Conventional fertilizer (MAP 11-52-00)	with
9- 60-ALL-CI	60% CC	Slow release fertilizer (Organomineral 06-32-00)	with
10- 60-ALC-CI	60% CC	Controlled release fertilizer (Phusion 06-30-00)	with

Note: 1.CC: field capacity; SI: without inoculation; CI: with inoculation; SA: without fertilizer; AC: conventional fertilizer; ALL: slow release fertilizer; ALC: controlled release fertilizer.
2.Doses: 346 kg/ha of MAP, 600 kg/ha of Organomineral and 600 kg/ha of Phusion.

After transplanting, the vegetative growth of the coffee plant was measured monthly, through measurements of plant height and average crown diameter, with the aid of a graduated ruler and stem diameter taken at 2.0 cm from the ground, with the aid of of digital caliper. Since coffee growth is slow in the first year after transplantion, the number of leaves was counted at the end of the experiment and the estimated leaf area was measured (Ramiro, et al. 2004). The method used to estimate the leaf area was through a mathematical equation involving the measurement of the leaf blade length and greater leaf width, according to the equation determined by (Barros, et al. 1973).

AF=0.667.CL

Where: ÂF=estimated leaf area (cm²); C=greater length (cm) and L=greater width (cm).

The experiment was conducted in randomized blocks, with six replications (n=6). The means were submitted to analysis of variance (ANOVA), and later the means of the treatments were submitted to the multiple comparison analysis by the SNK test, and differences in $p<0.05$ were considered significant. For data analysis, the SPEED STAT statistical software was used (Carvalho, et al. 2017; Mendes, et al. 2017).

RESULTS AND DISCUSSION

In general, there was a difference between treatments at 30 DAT and 60 DAT in relation to crown diameter (DCO), at 60 DAT shoot height (APA) also differed, as well as stem diameter (DCA) at 75 DAT (Table 2). Regarding shoot height, a difference was observed only between treatments 80% CC-SA-SI and 80% CC-ALL-CI, in which the former was superior. With this result, it was not clear whether the positive influence on the greater height of the shoot was a joint or separate consequence of the fertilization with a slow release source to the detriment of the non-use of fertilization and/or by inoculation with *Bacillus aryabhattai*, since there was no water limitation in both treatments. Despite this, at 75 DAT this difference was no longer observed, which indicates that fertilization and inoculation had no significant effect on coffee seedlings compared to non-fertilized and non-inoculated plants. The same behavior was observed for the crown diameter, in which there was a difference between the treatments in the period of 60 DAT and at 75 DAT, there was no distinct effect between the treatments (Castro, et al. 2009).

Table 2. Aerial height (APA), stem diameter (DCA), crown diameter (DCO), and plant leaf area at 30, 60 and 75 days after transplanting (DAT). (Source: UNICERP Sponsorship, 2021).

	APA (cm)			DCA (mm)			OCD (cm)			AF (cm²)
Treatments	30 DAT	60 DAT	75 DAT	30 DAT	60 DAT	75 DAT	30 DAT	60 DAT	75 DAT	75 DAT
80% CC-SA-SI	8.92	12.50 b	19.00	14.92	17.83	21.33 d	3.00	3.33 ab	4.25	17.89
80%CC-SA-CI	10.08	14.08 a.m.	20.67	14.75	18.33	23.75 bcd	2.92	3.02 b	4.17	18.93
80% CC-AC-CI	8.33	13.33 ab	20.50	14.11	17.83	21.92 cd	2.67 ab	3.75 ab	4.67	19.16
80% CC-ALL-CI	8.58	15.17	22.33	11.25	18.02	28.08	2.58 ab	3.75 ab	4.50	20.43
80% CC-ALC-CI	8.50	14.17 a.m.	21.17 to	10.92	18.17 to	24.08 bcd	2.08 b	3.83 ab	4.83	23.00
60% CC-SA-SI	9.58	13.67 ab	21.17	11.83	17.67	25.33 abc	2.42 ab	4.08	4.50	24.96
60% CC-SA-CI	9.42	14.00 a.m.	21.45	12.92	18.08	21.25 d	2.58 ab	3.58 ab	4.50	21.55
60% CC-AC-CI	10.58	13.25 a.m.	22.25	13.75	18.25	27.92	2.33 ab	3.25 ab	4.50	25.37
60% CC-ALL-CI	9.92	14.33 ab	21.00	11.82	17.42	20.67 d	2.58 ab	3.50 ab	4.50	20.63
60% CC-ALC-CI	10.00	14.50 a.m.	22.17	12.50	18.58	27.17 a.m.	2.58 ab	3.83 ab	4.67	23.97
CV (%)	16.20	15.10	9.96	16.77	5.09	9.67	16.59	13.38	11.61	18.74

Note: Means followed by the same lowercase letter in the column do not differ from each other by the SNK test at 5% probability. Treatment abbreviations: CC: % of field capacity; SI: without inoculation; CI: with inoculation; SA: without fertilizer; AC: conventional fertilizer; ALL: slow release fertilizer; ALC: controlled release fertilizer.

Still regarding shoot height, as treatments with lower irrigation regime (60% of CC) were not different from treatments with good water supplementation (80% of CC), inoculation and fertilization were not able to increase the shoot height in plants in water deficit (Lecoeur, et al. 1996). The association between microorganism and plant may have been influenced by external factors that were not able to show the potential of the inoculation technology with *Bacillus aryabhattai* for mitigating water stress. This is due to the complexity of this interaction between organisms that we still do not know all the details and applications, requiring more research (Cardoso, et al. 2019).

In what regarding the stem diameter, in the last evaluation (75 DAT) there was a significant difference when fertilization and inoculation of coffee seedlings were managed, comparing treatments with the same water regime (Pinheiro, et al. 2005). When at 80% of CC, coffee seedlings that received inoculation and slow-release fertilizer had a larger stem diameter than those that were not fertilized or inoculated. On the other hand, when at 60% of the CC, the plants that were inoculated and fertilized with conventional fertilizer had a larger stem diameter than those that were also inoculated, but that received slow-release fertilizer. This result can be explained by the time taken to conduct the experiment. (Marana, et al. 2008) stated that slow-release fertilizer provides nutrients during the five-month period of coffee seedling formation. Therefore, this difference may have masked this positive effect of the gradual release of fertilization. This result does not corroborate with (Bachiao, et al. 2018) who used slow-release fertilizer and observed growth promotion of seedlings of different coffee cultivars. The leaf area was the same for all treatments.

CONCLUSION

It can be concluded that the results in this work were not concise to determine the real potential for mitigating water stress in coffee seedlings with the use of inoculation with *Bacillus aryabhattai* under the experimental conditions in which the study was conducted.

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