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Inoculation of diazotrophic bacteria in chicory

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ABSTRACT

Inoculating crops with diazotrophic bacteria constitutes a feasible biotechnological tool to reduce or even avoid the use of nitrogen fertilizers and to improve the nutritional status of crops. However, the potential use of such technology is limited due to the lack of studies in diverse vegetable crops. We evaluated the production of chicory (*Cichorium intybus*) in response to inoculation with diazotrophic bacteria in two soil classes. The experiments were performed under greenhouse conditions and under a completely randomized design. The treatments were constituted by strains UFRB FA34C2-2, UFLA 03-84 and INPA 0311B, and two control treatments (devoid of bacterial inoculation and with and without nitrogen fertilization). Two experiments were carried out, one using a soil classified as a dystrophic yellow Latosol (Oxisoil) and one using a Quartsandy Neosol (Typic Quartzipsamment). Chlorophylls *a*, *b* and total; plant height; number of leaves; dry mass of the aerial portion; dry mass of roots; total dry mass; effectiveness; efficiency in addition to N and P gathering in the aerial portion, were evaluated 60 days after seeding. The inoculation with diazotrophic bacteria resulted in adequate production when compared to the control treatment without nitrogen fertilization and without inoculation. Diazotrophic bacteria showed potential supplying nitrogen to chicory plants and spare the use of nitrogen fertilization in both soil classes. The strain INPA 0311B, recommended for cowpea, promoted vegetal growth and increased the dry weight of the aerial portion in chicory, under both dystrophic yellow Latosol (Oxisoil) and Neossolo Quartzarênico (Typic Quartzipsamment), when cultivated under greenhouse conditions.

Keywords: *Cichorium intybus*, growth promoting rhizobacteria, organic production, sustainability.

RESUMO

Inoculação de bactérias diazotróficas na chicória

A inoculação de bactérias diazotróficas representa grande potencial biotecnológico por reduzir ou dispensar o uso de fertilizantes químicos nitrogenados e por melhorar as condições nutricionais da planta. No entanto, a potencialidade de aplicação desta tecnologia está limitada por carência de estudos em culturas diversas. Objetivou-se avaliar a produção do almeirão (*Cichorium intybus*) em resposta à inoculação com bactérias diazotróficas em duas classes de solos. Os experimentos foram realizados em casa de vegetação em delineamento inteiramente casualizado. Os tratamentos foram constituídos pelas estirpes UFRB FA34C2-2, UFLA 03-84 e INPA 0311B, e dois tratamentos controle (sem inoculação bacteriana e com e sem adubação nitrogenada). Foram realizados dois experimentos, um utilizando um solo classificado como Latossolo amarelo distrófico e outro utilizando Neossolo Quartzarênico. Aos 60 dias após a semeadura foram avaliados em ambos os experimentos: clorofila *a*, *b* e total; altura; número de folhas; massa seca da parte aérea; da raiz e total, eficácia, eficiência e acúmulo de N e P na parte aérea. A inoculação com bactérias diazotróficas resulta em maior rendimento da produção, quando comparada ao tratamento controle sem adubação nitrogenada e sem inoculação. As bactérias diazotróficas apresentam potencial no fornecimento de nitrogênio às plantas de almeirão e dispensam o uso da fertilização nitrogenada nas duas classes de solo. A estirpe INPA 0311B, recomendada para o feijão-caupi, promove o crescimento vegetal e incrementa a parte aérea do almeirão, quando cultivado em Latossolo amarelo distrófico e Neossolo Quartzarênico em casa de vegetação.

Palavras-chave: *Cichorium intybus*, rizobactérias promotoras de crescimento, produção orgânica, sustentabilidade.

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Chicory (*Cichorium intybus*) is a herbaceous vegetable belonging to the Asteraceae family. It is originated in the Mediterranean Europe and stands out as a nutritive and medicinal vegetable. It differs from cichorium (*Cichorium pumilum*) by its bitter

taste and more elongated and narrower leaves covered with hairs (Filgueira, 2013), being commonly known as bitter chicory. Chicory produces better under moderate temperatures around 14 to 24°C (Pereira *et al.*, 2012).

Chicory is a leafy vegetable and

nitrogen fertilization is extremely important for its development. However, the excessive or inadequate use of fertilizers in crop production, especially nitrogen fertilizers, has environmental implications (Biscaro *et al.*, 2012) such as soil and water contamination and

increase of nitrous oxide emissions, one of the greenhouse effect gases.

In addition to crop yield increment and food nutritional quality, modern society demands the consumption of products originated from a sustainable agriculture. Under such context, in leguminous crops such as soya (*Glicine max*) (Costa *et al.*, 2014) and cowpea (*Vigna unguiculata*) (Ferreira *et al.*, 2013; Costa *et al.*, 2016), biological nitrogen fixation (BNF) mediated by selected symbiotic diazotrophic bacteria may substitute nitrogen fertilization, once it is considered an efficient nitrogen nutrition process in these crops. In non-leguminous crops diazotrophic bacteria may also contribute with associative BNF, reducing nitrogen fertilization, as verified in corn (*Zea mays*) (Martins *et al.*, 2018; Coelho *et al.*, 2019) and sugar cane (Girio *et al.*, 2015).

Biological nitrogen fixation through rhizobacteria in non-nodulating plants occurs by means of an association between plants and these microorganisms, which promote plant growth and supply nitrogen for these microorganisms which in turn benefit from carbohydrates delivered by the plant. Although this form of interaction is not as efficient as are leguminous plants, diazotrophic bacteria may promote plant growth through the solubilization of phosphates, production of exopolysaccharides and synthesis of phytohormones (Silva *et al.*, 2016), such as auxins (indole-3-acetic acid, AIA) (Gopalakrishnan *et al.*, 2015). Thus, the use of inoculants with associative bacteria may represent an alternative to develop vegetable cultivation systems, aiming to increase production through bio-control and growth promotion, which may reduce or avoid the use of mineral fertilization (Costa *et al.*, 2014; Silva *et al.*, 2016), in addition to the reduction of production cost and provide potential winnings for the farmer.

The use of inoculant with N fixing bacteria in leguminous plants is widely spread, their effects are verified (Costa *et al.*, 2014; Girio *et al.*, 2015; Gopalakrishnan *et al.*, 2015; Silva *et al.*, 2016; Lima *et al.*, 2017; Costa *et al.*, 2019), but their use in vegetables is incipient, once there is not yet an

authorized inoculant by the Ministry of Agriculture, Livestock and Supply (MAPA) for this group of plants.

Information available concerning chicory is scarce, being one of the less studied vegetables in Brazil, when compared to the most commercialized vegetables (Biscaro *et al.*, 2012). There are few studies regarding the use of inoculation with plant growth promoting rhizobacteria in vegetables. Production of tomato (*Lycopersicon esculentum*) in organic substrate inoculated with streptomyces promoted increased growth of seedlings (Sousa *et al.*, 2009). In lettuce (*Lactuca sativa*), the efficiency of *Azospirillum brasilense* inoculation was verified when observing higher tolerance against virus and positive influence in plant height, stem diameter and fresh and dry weight of the aerial portion of the plant (Lima *et al.*, 2017). Higher growth of the aerial portion of the plant and rooting was also observed in lettuce plantlets (Mangmang *et al.*, 2015).

Studies have shown responsiveness of vegetal species which do not have authorized inoculants by the Ministry of Agriculture Livestock and Supply (MAPA), when inoculated with growth promoting diazotrophic bacteria (Mangmang *et al.*, 2015; Lima *et al.*, 2017; Braulio *et al.*, 2019). Thus, it is important to increase the use of this technology as well as to select suitable strains for non-nodulating species, evaluating plant growth promotion, crop production and the potential of these microorganisms in diverse applications. Knowing the aptitudes of diazotrophic bacteria for fixing nitrogen and production of growth promoting plant hormones, we hypothesize that plants of chicory inoculated with diazotrophic bacteria show higher production of plant biomass. Thus, the present work had the objective to evaluate the production of chicory in response to inoculation with diazotrophic bacteria.

MATERIAL AND METHODS

Two experiments were performed in plastic pots filled with two soil classes: dystrophic yellow Latosol (Oxisol)

(LAd) and Typic Quartzipsamment (NQ) (Santos *et al.*, 2018).

The experiments were performed in greenhouse at the Agrarian, Environmental and Biological Sciences Center (CCAAB) at Federal University of Recôncavo da Bahia (UFRB), located in the county of Cruz das Almas, Bahia, Brazil (12°40'19"S, 39°06'23"W). During the experimental period in Lad (from November 2017 to January 2019), the temperature in the greenhouse varied from 31.1 to 29.5°C with 30.3°C in average and, during the experimental period in NQ (from March to April 2019), the temperature varied from 30.6 to 28.4°C with 29.5°C in average. Both, LAd and NQ were previously used under conventional system being planted with corn (*Zea mays*). Seeking to maintain the conditions of crop management in the region.

The first experiment was completed with LAd, sampled at the UFRB campus at 0.20 m depth. The chemical and physical characteristics of the soil were described as: pH (H₂O)= 6.0; organic matter = 12.8 g dm⁻³, P (Mehlich 1)= 10.5 mg dm⁻³, K⁺= 67.7 mg dm⁻³, Ca²⁺= 0.7 cmol_c dm⁻³, Mg²⁺= 0.3 cmol_c dm⁻³, (H+Al)= 2.0 cmol_c dm⁻³, SB= 1.2 cmol_c dm⁻³, effective CEC= 1.2 cmol_c dm⁻³, potential CEC= 3.2 cmol_c dm⁻³, V= 37.5%, Zn²⁺= 5.05 mg dm⁻³, Fe²⁺= 49.05 mg dm⁻³, Mn²⁺= 9.1 mg dm⁻³, Cu²⁺= 1.6 mg dm⁻³ and B= 0.28 mg dm⁻³; Sand = 533 g kg⁻¹; Silt = 280 g kg⁻¹; Clay = 182 g kg⁻¹.

In this first experiment the treatments were constituted by a strain under selection stage for cowpea inoculation: UFRB FA34C2-2 (Sousa, 2017), two strains already recommended and authorized by MAPA (Brasil, 2011) as inoculants for cowpea: INPA 0311B - SEMIA 6462 (*Bradyrhizobium elkanni*) and UFLA 03-84 - SEMIA 6461 (*Bradyrhizobium viridifuturi*) (Costa *et al.*, 2019), and two control treatments: one with nitrogen fertilization using urea (135 mg dm⁻³ N per plastic pot) (Steiner *et al.*, 2010) and a second one without nitrogen fertilization. The experiment was set in a completely randomized design with four replicates, and one plant per replicate.

The second experiment was

completed with samples of NQ collected at the campus of Federal Institute of Bahia (IF Baiano), in the county of Catu, Bahia, Brazil, at 0.20 m depth. The chemical and physical characteristics of the soil were described as: pH (H₂O)= 5.15; organic matter = 1.04 g dm⁻³, P (Mehlich 1)= 6.4 mg dm⁻³, K⁺= 14 mg dm⁻³, Ca²⁺= 1.50 cmol_c dm⁻³, Mg²⁺= 0.34 cmol_c dm⁻³, (H+Al)= 2.2 cmol_c dm⁻³, SB= 1.88 cmol_c dm⁻³, effective CEC= 1.88 cmol_c dm⁻³, potential CEC= 4.08 cmol_c dm⁻³, V= 46.1%, Zn²⁺= 3.14 mg dm⁻³, Fe²⁺= 114.5 mg dm⁻³, Mn²⁺= 13.7 mg dm⁻³, Cu²⁺= 0.89 mg dm⁻³ and B= 0.17 mg dm⁻³; Sand = 575g kg⁻¹; Silt = 0.030 g kg⁻¹; Clay = 0.118 g kg⁻¹.

Treatments were constituted as described for the first experiment under a completely randomized design, with five treatments and ten replicates. Soil samples were dried and sieved in a 4 mm mesh, homogenized and conditioned in 3 L plastic pots.

In order to obtain the inoculum, bacteria were grown in semi-solid culture medium 79 inside a BOD (*Biochemical Oxygen Demand*) chamber for 4 days, at 25°C, achieving the log growing phase (10⁸ UFC mL). Chicory seeds of green leaves' spadona Radiche® were used in the experiments. Seeds were disinfected

with alcohol 70% for 30 seconds and then, washed three consecutive times with sterilized distilled water. For each replicate 1 mL of the inoculant was added to the seeds. In control treatments without inoculation 1 mL of the semi-solid culture medium 79 without inoculum, was added.

Seeding was performed in polystyrene trays with 128 cells, with 18.5 x 19.0 x 11.0 cm length, width and depth, respectively. Each tray cell had a volume of 50 mL, with two seeds being distributed on each cell. Seedlings were transplanted into 3.0 L pots 14 days after seeding. Plants were irrigated daily.

Plants were collected 60 days after seeding and the following variables were evaluated: chlorophyll *a* (CLA), chlorophyll *b* (CLB), determined with an electronic chlorophyll meter (clorofiLOG CFL 1030) Falker®, the resulting dimensionless unit expressed as Index of Chlorophyll Falker (ICF) and the number of leaves (NL). Total chlorophyll (CLT) was obtained by adding the values from CLA + CLB. Then, plants were segmented in aerial portion and roots, conditioned in paper bags and set in a forced air oven at 60°C, until attaining a constant weight in order to determine the dry

mass, and to evaluate: dry mass of the aerial portion (DMAP), dry mass of roots (DMR), total dry mass (TDM), effectiveness of inoculation compared to the absolute control (EFSN) and efficiency of inoculation compared to fertilization with mineral N (EFCN).

Data of DMAP was used to calculate the effectiveness of the strains when compared to the control without EFSN and EFCN. Effectiveness was calculated using the formula: Effectiveness = (DMAP inoculated)*100/ (DMAP of the absolute control). Efficiency (EFCN) was calculated using the formula: Efficiency = (DMAP inoculated)*100/ (DMAP of plants fertilized with mineral N).

Samples of the dry aerial portion of the plants cultivated in LAd were used to determine contents (%) of nitrogen (N) (GN) and phosphorus (P) (Gp). Samples of approximately 0.1 g DMAP were digested in a mixture of 3.0 mL concentrated sulfuric acid (H₂SO₄) and 3 mL 30% hydrogen peroxide (H₂O₂). Then, the digested was diluted to 100 mL in distilled water, obtaining the extract to perform the analyses of N and P. Contents of N and P (%) were determined by the spectrophotometric method of the phenol-hypochlorite

Table 1. Mean values for chlorophyll *a* (CLA), chlorophyll *b* (CLB), total chlorophyll (CLT), number of leaves (NL), dry mass of the aerial portion (DMAP), dry mass of roots (DMR) and total dry mass (TDM) from chicory, in response to inoculation with diazotrophic bacteria when cultivated in dystrophic yellow Latosol (Oxisoil) (LAd) and Quartsandy Neosol (Typic Quartzipsamment) (NQ). Cruz das Almas, UFRB, 2017-2019.

Treatment	CLA	CLB	CLT	NL (leaves/plant)	DMAP	DMR	TDM
					(g/plant)		
Lad							
INPA 0311B	30.85a	8.75a	39.06a	17.75a	1.88a	1.48a	3.35a
UFLA 03-84	27.86b	6.73bc	34.60b	15.00b	1.23c	0.52b	1.74c
UFRB FA34C2-2	29.03ab	8.16ab	35.48ab	16.00ab	1.54b	0.72b	2.26b
S/N	21.10c	5.03d	29.13c	8.50c	1.12c	0.51b	1.63c
C/N	22.78c	6.35cd	29.13c	6.75c	1.76c	0.51b	1.69c
CV (%)	4.85	10.62.0	4.19.0	9.57.0	7.15.0	17.90	6.21
NQ							
INPA 0311B	30.59a	8.65a	39.24a	11.00a	0.71a	0.20a	0.91a
UFLA 03-84	25.35c	6.84b	39.19c	9.00bc	0.43b	0.14b	0.57b
UFRB FA34C2-2	29.23ab	8.12a	37.35ab	9.40b	0.47b	0.15b	0.62b
S/N	24.28c	5.46c	29.74c	7.70c	0.16d	0.04d	0.21d
C/N	27.14c	6.39bc	33.53bc	8.10c	0.35c	0.10c	0.44c
CV (%)	9.66	10.78	9.36	13.74	11.28	24.28	24.28

*Means followed by the same letter within the column are not statistically different, Tukey test at 5% probability.

Table 2. Means of inoculation efficiency when compared with the control treatment without nitrogen fertilization and without inoculation (EFSN), efficiency of inoculation compared with fertilization with mineral nitrogen (EFCN), cultivated in dystrophic yellow Latosol (Oxisoil) (LAd) and Quartsandy Neosol (Typic Quartzipsamment) (NQ) and gathering of nitrogen (GN) and phosphorus (GP) in the aerial portion of chicory cultivated in LAd in response to inoculation with diazotrophic bacteria. Cruz das Almas, UFRB, 2017-2019.

Treatment	NQ		LAd			
	EFSN	EFCN	EFSN	EFCN	GN	GP
			(%)		(mg/plant)	
INPA 0311B	460.2a	203.1a	169.03a	159.90a	34.51a	6.60a
UFLA 03-84	285.6b	122.8bc	109.61c	103.82c	23.13c	4.36c
UFRB FA34C2-2	319.9b	134.3b	137.96b	130.82b	27.68b	5.28b
C/N	100.0c	45.86d	100.00c	94.87c	19.01d	3.67c
S/N	232.3b	100.00c	105.49c	100.00c	20.33cd	3.93c
CV (%)	9.66	10.78	9.6	7.96	7.09	7.06

*Means followed by the same letter in the column are not statistically different, Tukey test at 5% probability.

and the vanadium-molybdate for P (Faithfull, 2002). The N and P gathering in the aerial portion was calculated through $\text{g} = \text{mass of the aerial portion (g)} * (\% \text{ de N})$.

Data were submitted to variance analysis and F test. Means were compared by the Tukey test at 5% probability, using the statistical software "R" (R Development Core Team, 2018). Variables EFSN and EFCN were transformed in square root of $(x+0.5)$.

RESULTS AND DISCUSSION

Sources of nitrogen influenced production components of chicory cultivated in (LAd) and (NQ) ($p < 0.05$). Sources of nitrogen influenced all variables analyzed.

Plants cultivated in both LAd and NQ, inoculated with strains INPA 0311B, UFRB FA34C2-2 and UFLA 03-84 showed superior indices of chlorophyll *a*, *b* and total when compared to the control with mineral N and without N fertilization and without inoculation (Table 1).

The increase in chlorophyll may be related to the gathering of N in leaves (Ferreira *et al.*, 2006), evidencing the results obtained in the present work (Table 2), where chicory plants with higher indices of chlorophylls *a*, *b* and total, resulted in higher increments in the

number of leaves (NL), thus promoting higher biomass (Table 1). Previous studies demonstrated that the index of chlorophyll may be used to evaluate the nutritional status of N in plants, signaling higher production crops (Ferreira *et al.*, 2006; Silva *et al.*, 2014).

Supplying nitrogen, through inoculation with rhizobium strains, had a positive effect in the synthesis of chlorophylls and consequently in the photosynthetic potential of plants in both soils tested. The applied dosage of 135 mg N per pot had an inferior effect when compared to the inoculation with bacterial strains, therefore showing these strains are efficient in fixing nitrogen in chicory plants (Table 1).

Chicory plants, inoculated with strains INPA 0311B and UFRB showed higher mean values for NL in plants cultivated in LAd. In NQ, a higher value for NL was verified for strain INPA 0311B (Table 1). Such results reflect the effect of these rhizobia in the provision of nutrients and consequently in the increment of the chicory biomass. Production of active growth regulators such as phytohormones is part of the metabolism of many bacterial species associated with plants and may be considered causal agents of growth and development modifications (Patil *et al.*, 2011; Girio *et al.*, 2015).

Control treatment with mineral N had no difference from the control

without nitrogen fertilization and without inoculation for NL, in both studied soils (Table 1). The absence of differences between these treatments may be attributed to the capacity of native population of diazotrophic bacteria to fix nitrogen or promote growth in response to diverse mechanisms, such as the production of hormones for chicory plants. This species showed as being non-responsive to nitrogen fertilization for production of leaves, when cultivated in dystrophic red-yellow Latosol (Oxisol) under increasing dosages of N fertilization, without inoculation (Luz *et al.*, 2017).

The number of leaves and production of dry weight are linked to the contents of P in the soil. This element is fundamental during photosynthesis, respiration and transfer of energy, cell division, cell growth and many other plant processes (Marschner, 2012). In this regard LAd showed 10.5 mg dm⁻³ of P, this may also have contributed for a higher biomass production. Diazotrophic bacteria perform the solubilization of inorganic phosphates, solubilizing low solubility phosphate sources such as calcium phosphate, making those nutrients available for plant absorption (Costa *et al.*, 2016). The higher quantity of leaves per plant results, in a general way, in higher dry mass of the aerial portion (DMAP) and consequently, higher crop production, as was observed in the present work (Table 1).

Similar to previous results observed by Ferreira *et al.* (2013), nutrients were not supplied to the plants, with the exception of the treatment with mineral N. In addition to nitrogen fixation, bacteria perform diverse processes such as the solubilization of inorganic phosphates, influencing plant growth and nutrient gathering, as verified by Costa *et al.* (2015), when phosphate solubilizing bacteria improved plant growth in *Oryza sativa*, with increments of dry weight of the aerial portion and roots and nutrient gathering.

Concerning DMAP, DMR and TDM, the strain INPA 0311B showed higher biomass production in LAd than in NQ (Table 1).

An increment of 59.57% of DMAP and 34.46% of DMR was observed

in LAd and increment of 22.54% of DMAP and 20% of DMR in NQ, when compared to the control treatment without nitrogen fertilization and without inoculation. Strain UFRB FA34C2-2 showed superior mean values than the strain UFLA 03-84 and the control treatments, and those did not differ between themselves for DMAP and TDM in LAd (Table 1). Treatments did not differ for DMR with exception of the strain INPA 0311B in LAd ($p < 0.01$).

Increments of 22.54% of DMAP and 20% of DMR were observed in NQ, when compared to the control treatment without nitrogen fertilization and without inoculation. In plants cultivated in NQ, the control with mineral N was superior only to the control without mineral fertilization and without inoculation for DMAP and DMR. In plants cultivated in NQ, strains UFRB FA34C2-2 and UFLA 03-84 did not differ between themselves for DMAP, DMR and TDM ($p < 0.01$) (Table 1).

Inoculation with diazotrophic bacteria resulted in higher harnessing in the transport of N and P, promoting increase in chicory DMAP. Increase in DMAP was also verified in tomato plants, inoculated with isolates of streptomyces (AC-29, AC-92 and AC-103) (Sousa *et al.*, 2009) as well as in lettuce plants inoculated with *Azospirillum brasilense* (Lima *et al.*, 2017), which may indicate the potential for promoting vegetal growth from rhizobacteria in vegetable crops.

The increment in DMAP corresponds to the NL and the gathering on N in the aerial portion of chicory (Crusciol *et al.*, 2007). Inoculation with diazotrophic bacteria resulted in satisfactory production when compared to the control treatment without N fertilization and without inoculation (Table 1). Chicory, being a leafy vegetable, is very demanding in nitrogen fertilization (Steiner *et al.*, 2010). The results observed in the present work show the potential of the strains tested to supply nutrients and produce mass increments in chicory.

Inoculation with diazotrophic bacteria provided nutrients for root absorption, supporting the nutritional

status of chicory plants (Table 2). The increment of DMAP production on inoculated chicory plants, when compared to the control, may also be attributed to the production of growth regulating substances such as auxins, gibberellins and cytokinins (Gopalakrishnan *et al.*, 2015), which participate in the processes of growth, development, nutrient mobilization and cell division in the plant, thus promoting higher production.

Inoculation promoted increment of the dry weight of roots enhancing absorption of available nutrients from the soil. Modifications in root morphology, promoted by phytohormones produced by microorganisms in the rhizosphere, supports the development of the radicular system and better exploration of the soil, which makes plants less susceptible to water and nutrient deficits (Silva *et al.*, 2016). Amongst the effects produced by the indoleacetic acid synthesized by the bacteria, the development of lateral roots and primary root elongation was evidenced (Gopalakrishnan *et al.*, 2015). The effects of inoculation on DMR were also verified by Sousa *et al.* (2009), in tomato plants inoculated with streptomyces AC-92.

The control treatment with mineral N did not differ from the control without fertilization and without inoculation for DMAP, DMR and TDM in chicory plants cultivated in LAd (Table 1). Lettuce (*Lactuca sativa*) plants cultivated in eutrophic red Latosol (Oxisol) under different dosages of N did not show significant influence for the production of DMAP and DMR (Nascimento *et al.*, 2017). The absence of differences between these treatments may also be related with the capacity of native rhizobia to supply N to chicory plants, once this soil has no previous inoculation reported.

In respect to the efficiency of inoculation in the treatment control without N fertilization and without inoculation (EFSN) and inoculation efficiency in the treatment with N fertilization (EFCN), the strain INPA 0311B was superior to all other strains in plants cultivated in LAd and NQ (Table 2). In LAd, strains UFRB FA34C2-2 and UFLA 03-84 and the control without

N fertilization and without inoculation were not different between themselves, with higher EFSN mean values when compared to the control with mineral N. Plants cultivated in NQ, inoculated with strains UFRB FA34C2-2, UFLA 03-84 and the control treatment without N fertilization and without inoculation did not differ from the treatment with mineral N. Strain UFRB FA34C2-2 showed higher EFCN when compared to the strain UFLA 03-84 and the controls, which did not differ between themselves, in LAd. Plants cultivated in NQ and inoculated with the strains UFRB FA34C2-2 and UFLA 03-84, did not differ among themselves. The control treatment without N fertilization and without inoculation showed lower mean value than the control treatment with mineral N.

The higher EFSN and EFCN observed in strain INPA 0311B, followed by UFRB FA34C2-2 in both soil classes (Table 2), may be related with the probable mechanisms performed by the diazotrophic bacteria which influenced the development and production of foliar mass in chicory (Table 2), influencing the production of growth promoting substances (Silva *et al.*, 2016) as well as in the absorption of nutrients. Altogether evidencing the capacity for assimilation of atmospheric nitrogen by the inoculated rhizobium strains, and the consequent increase of DMAP.

Plants cultivated in LAd and inoculated with the strain INPA 0311B, followed by strain UFRB FA34C2-2, showed higher gathering of N and P in the aerial portion of the plant, when compared to all other treatments. Control treatments with mineral N and without N fertilization and without inoculation did not differ between themselves for GN and GP (Table 2). Crusciol *et al.* (2007) affirmed that the increase in dry weight of the aerial portion may be associated with a higher gathering of total N in plant leaves, once this element works increasing the index of chlorophyll, foliar area and photosynthesis. Such result was evidenced by the values of DMAP inoculated with rhizobia (Table 1), due to the Biological Nitrogen Fixation

(BNF). Some other influences due to biological process not evaluated in the present work may also occur, such as solubilization of phosphates, synthesis of phytohormones and production of exopolysaccharides, as previously evaluated in previous studies using the same strains as in the present work (Gopalakrishnan *et al.*, 2015; Silva *et al.*, 2016; Sousa, 2017).

Inoculated plants showed higher mean values for GN, when compared to values observed by Almeida *et al.* (2013), in chicory plants under nutritive solutions supplemented with micro-nutrients (20.1 mg plant⁻¹), in the absence of inoculation. However, the values of GP were lower (Table 2) than those found by these authors (23.1 mg plant⁻¹), when compared with all treatments evaluated in the present work. In a general manner, low values for GP observed in plants cultivated in LAd, independently of the treatments, may be related with the availability of nutrients in the soil, once plants were cultivated without the addition of mineral or organic fertilization, except the control treatment with mineral N. The absence of mineral fertilization (P and K) or organic fertilization may have caused a reduction in nutrient absorption, limiting vegetative growth of plants and consequently diminishing dry weight production and nutrient gathering, when compared with plants fertilized with mineral or organic sources (Biscaro *et al.*, 2012; Pereira *et al.*, 2012).

Another issue that may have limited the DMAP in both soils was the temperature, once this species produces better under moderate temperatures from 14 to 24°C (Pereira *et al.*, 2012), while the mean temperatures registered in the greenhouse during the present work were of 30.3°C in LAd and 29.5°C in NQ.

In both soil classes, evaluating the production of chicory inoculated with diazotrophic bacteria, strains INPA 0311B and UFRB FA34C2-2, when compared to the control without N fertilization and without inoculation, presented mean values near or higher than the control treatment with mineral N (Table 1). The strain INPA 0311B,

reacted positively for production of NL, DMAP, DMR and TDM in both soil classes studied, without the need for N fertilization, which besides representing an extra cost may pollute the environment if incorrectly used. It is important to mention the need for further investigation in order to verify the effect of inoculation under different soil and climatic conditions.

The strain INPA 0311B, authorized and recommended for cowpea, was more efficient to increase the production of aerial portion in plants of chicory while plants inoculated with the strain INPA 0311B showed the highest concentrations of N and P in the aerial portion. Chicory yield in soils without amendments and fertilization increased with the inoculation of diazotrophic bacteria. Diazotrophic bacteria showed potential to supply nitrogen to chicory plants.

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