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Growth and yield of carrot inoculated with *Bacillus subtilis* and *Pseudomonas fluorescens*

Crecimiento y rendimiento de zanahoria inoculada con *Bacillus subtilis* y *Pseudomonas fluorescens*

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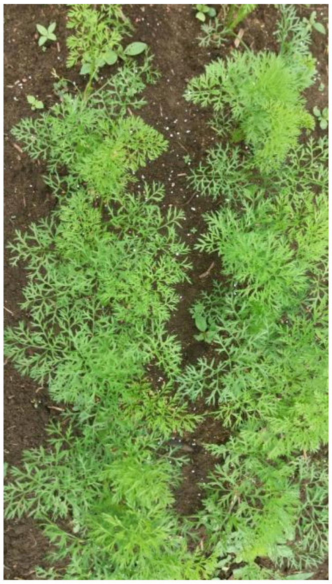

View of carrots in the field experiment.
Photo: A.R.S. França

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ABSTRACT

The objective of this study was to evaluate the effects of inoculation and co-inoculation of carrot with *Bacillus subtilis* and *Pseudomonas fluorescens* under reduced use of nitrogen. A field experiment was carried on, with five treatments and five replicates, evaluating growth and yield responses of carrot to inoculation. Responses in height were observed only two weeks before harvesting, but number of leaves was mostly affected from the beginning to the middle of cultivation cycle. Although mass of fresh shoot was negatively affected by inoculation treatments, mass of dry shoot did not respond to rhizobacteria. Length of roots inoculated with *P. fluorescens* or co-inoculated with both bacteria increased from 21 cm, without inoculation, to 25 cm. Values of root volume and dry mass were the same across treatments. However, mass of fresh roots was improved between 70 and 81.8% by *P. fluorescens* and co-inoculation, respectively. Most positive responses to inoculation were observed with reduced levels of nitrogen, suggesting the potential of these rhizobacteria strains to be used in reduced-input agricultural practices. Moreover, our findings support recommendation of *Pseudomonas fluorescens* as a plant growth-promoting microorganism used for carrot cultivation.

Additional key words: *Daucus carota*; co-inoculation; plant growth promoting rhizobacteria; biofertilizers.

RESUMEN

El objetivo de este estudio fue evaluar los efectos de inoculación y co-inoculación de *Bacillus subtilis* y *Pseudomonas fluorescens* en plantas de zanahoria con uso reducido de nitrógeno. Se realizó un experimento de campo, con cinco tratamientos y cinco repeticiones, evaluando las respuestas de crecimiento y rendimiento de la zanahoria a la inoculación. Las respuestas sobre la altura se observaron solo dos semanas antes de la cosecha, pero el número de hojas se vio afectado principalmente desde el comienzo hasta la mitad del ciclo de cultivo. Aunque la masa de brotes frescos se vio afectada negativamente por los tratamientos de inoculación, la masa de brotes secos no respondió a las rizobacterias. La longitud de las raíces inoculadas con *P. fluorescens* o co-inoculadas con ambas bacterias aumentó de 21 cm, sin inoculación, a 25 cm. Los valores del volumen de la raíz y la masa seca fueron los mismos en todos los tratamientos. Sin embargo, la masa de raíces frescas mejoró entre 70 y 81.8% por *P. fluorescens* y co-inoculación, respectivamente. La mayoría de las respuestas positivas a la inoculación se observaron con niveles reducidos de nitrógeno, lo que sugiere el potencial de estas cepas de rizobacterias para ser utilizadas en prácticas agrícolas de insumos reducidos. Además, nuestros hallazgos respaldan la recomendación de *Pseudomonas fluorescens* como microorganismo promotor del crecimiento de las plantas utilizado para el cultivo de zanahorias.

Palabras clave adicionales: *Daucus carota*, co-inoculación, rizobacterias; biofertilizantes.

INTRODUCTION

Carrot (*Daucus carota* L.) is an important crop, grown and consumed in most countries worldwide. China and United States are the world top producers. In Brazil, carrot is the fifth most cultivated crop and its average yield ranges from 30 to 35 t ha^{-1} (Paula, 2019).

Yield and quality of carrot rely on many factors related to soil fertility and irrigation. Inadequate growth conditions result in physiological anomalies and reduced root mass, which decrease market value or even cause product loss up to 30% (Nick and Borém, 2016). Inoculation with plant growth-promoting rhizobacteria (PGPR) rises as a promising alternative to improve environmental conditions for cropping of carrot, as previously reported for other agricultural species. Benefits of PGPR in other crops include higher germination rates, increased root and shoot biomass, improved nutrition and resistance to biotic and abiotic stresses (Kloepper *et al*., 1980; Vessey, 2003; Aloo *et al*., 2019; Fioreze *et al*., 2020; Lin *et al.*, 2020).

Studies about benefits of inoculation of carrots are scarce and focus on a reduced number of measured parameters. Evaluations by Kozusny-Andreani *et al*. (2014), Matsuoka *et al*. (2016) and Clemente *et al*. (2016) suggest benefits of *Pseudomonas* and *Bacillus* species such as increased root length, as well as mass of fresh and dry roots.

Other possibility is combining different species of PGPR in a process known as co-inoculation. Benefits of co-inoculation are well reported for cultures such as soybean, common bean and corn (Molla *et al*., 2001; Fukami *et al*., 2018; Kumawat *et al*., 2019; Zeffa *et al*., 2020). The inoculation of soybean with *Bradyrhizobium japonicum* resulted in yield of 2,200 kg ha^{-1}, while co-inoculation with this bacteria and *Azospirillum brasilense* increased this value by 12% (2,496 kg ha^{-1}) Hungria *et al*. (2013). Common bean yield is also benefited from co-inoculation, since seed production with the use of *Rhizobium tropici* associated to *Azospirillum brasilense* was 1,866kg/ha compared to the inoculation with only *Rhizobium tropici*, which resulted in 1,543 kg ha^{-1}.

Information regarding benefits of co-inoculation of carrot is provided by a single study by Merriman *et al*. (1974). Authors described benefits of *Bacillus subtilis* and *Streptomyces griseus*, resulting in 17% more root mass of carrots compared to absence of inoculation. However, all studies regarding carrot either lack bacterial species designation or do not include evaluations through the entire crop cycle, which limits understanding of inoculation and co-inoculation benefits. Moreover, implication of PGPR in reduced levels of N has not been evaluated in any study published so far, an aspect that deserves to be investigated considering the worldwide dependence on nitrogen fertilizers. Benefits of rhizobacteria to crops such as corn and soybean are often expressed or optimized under reduced nitrogen fertilization, what decreases costs of crop production (Mendes *et al*., 2003; Lana *et al*., 2012). Therefore, this study tested the hypothesis that inoculation with two PGPR (*Bacillus subtilis* and *Pseudomonas fluorescens*) in reduced levels of nitrogen improves growth and yield of carrot.

MATERIALS AND METHODS

The experiment was established in Ponte Alta do Norte, Santa Catarina State, Brazil (27º13'29'' S and 50º25'28' W) at 990 m of altitude. Climate is classified as Cfb (Köppen's classification) with average temperatures between 12.4 and 22.0ºC. Prior to establishing the experiment, soybean and corn were cultivated in the area used for the experiment for several years.

Soil samples (0-20cm) were collected to analyse chemical properties and soil granulometry. Results were as follows: pH in H_2O 6.60; Ca 9.65 cmolc dm^{-3}; Mg 5.35 cmolc dm^{-3}; H+Al 2.00 cmolc dm^{-3}; K 0.15 cmolc dm^{-3}; P 6.00 mg dm^{-3}, Na 1.00 mg dm^{-3} and K 1.57 mg dm^{-3}. The CTC at pH 7.0 was 13.99 cmolc dm^{-3}, effective CTC 19.59 cmolc dm^{-3}, Saturation of bases 90.74%, organic matter content 3.30%, soil C content 1.91% C, and 39% clay.

Correction of soil pH was not necessary because its value was within adequate range of requirements by the crop. Fertilization with N, P and K was performed as recommended for carrot culture in Brazil. A dose of 20 kg ha^{-1} of N (as urea) were applied in furrow before sowing in the control treatment (100% N). This value was reduced to 20% in all other inoculation treatments. 80 kg of N/ha were split-applied at 15, 30 and 40 days after sowing-DAS (16, 24 and 40 kg) in all treatments as recommended for the crop in South of Brazil (CQFS, 2016). Fertilization with P and K (as P_2O_5 and K_2O) was performed in all experimental units. 240 kg ha^{-1} of P_2O_5 were applied only at sowing, and 190 kg/ha of K_2O were split-applied at 15, 30 and 40 DAS (38, 57 and 95 kg, respectively).

Carrot was cultivated between 2018 and 2019 in the summer season. Seeds of Livia cultivar (Brasilia group) were used, with 90-100 days required for complete development. Distance between lines was of 20 cm, while distance between seeds was 8 cm. The experiment was a completely randomized design with five treatments and five replicates. Size of each experimental plot was 1 m^2, spaced each other by 0.2 m.

Five treatments were tested: 100% N; 20% N; 20% N and inoculation with *Bacillus subtilis* (20% N+*B. subtilis*); 20% N and inoculation with *Pseudomonas fluorescens* (20% N+*P. fluorescens*); 20% N and co-inoculation with *B. subtilis* and *P. fluorescens* (20% N+co-inoculation). All inoculants were provided by Total Biotecnologia (Curitiba, Brazil) and developed in liquid form. Both inoculants had a concentration of 1.0×10^8 CFU/mL and have commercial registration for soybean and maize at Ministry of Agriculture, Livestock, and Supply - MAPA (*B. subtilis*: PR 9392310112-8; *P. fluorescens*: PR 9392310104-7;). However, there is no register or recommendation of these inoculants for carrot.

In 20% N+*B. subtilis* and 20% N+*P. fluorescens*, inoculants were applied in furrow at sowing. We diluted inoculant in water (1L:499L per hectare) and this mixture was sprayed-applied inside the furrow before sowing. In 20% N+co-inoculation, this ratio was adjusted to 1 L of each inoculant, diluted in 498 L of water. Inoculants were also applied in furrow at sowing.

After emergence, thinning and control of weeds were performed manually throughout the entire experiment as recommended by Vieira and Pessoa (2008) and no chemical product was used at any stage.

Several parameters were evaluated throughout plant growth and also at harvest, as recommended for carrot by CEAGESP (2000). Plant height and number of green leaves were evaluated at 38, 45, 52, 66, 80, 95 and 100 DAS.

At harvest, roots and shoots were separated and fresh mass of both components was determined immediately. Root length was measured with a scale. Root volume was estimated by placing carrots in a flask with water and measuring the volume of liquid displaced (Novoselov, 1960). Plant material was dried in an air-forced dryer at 65°C until constant weight, when mass of dry roots and shoots was determined.

Presence of defects in each root was evaluated (split roots, cracks, purple shoulder, green shoulder and adventitious roots) and used to calculate the percentage of abnormal roots.

Tests of normality and homogeneity of variances were applied to all data collected. If the assumptions of ANOVA were fulfilled, then the F test was used. When a significant p value was obtained ($P \leq 0.05$), means were separated by Scott-Knott test, using the software R Core Team (2020). Data regarding root defects did not satisfy any statistical conditions of ANOVA or non-parametric procedures, even when submitted to several forms of transformation. Therefore only mean and average values were presented.

RESULTS AND DISCUSSION

Plant height was increased by all inoculation treatments compared to 100% N and 20% N from 95 DAS on. Average increase in growth ranged from five to eleven centimeters (Tab. 1). The highest values were observed when carrots were co-inoculated with *B. subtilis* and *P. fluorescens* (73.22 cm) compared to 100% N, with 100% of nitrogen dose (62.26 cm).

Table 1. Mean values of height of carrot plants (cm) from 38 to 100 days after sowing (DAS) grown under different inoculation treatments. Ponte Alta do Norte-SC, Brazil.

Treatment	38 DAS	45 DAS	52 DAS	66 DAS	80 DAS	95 DAS	100 DAS
100% N	9.78	13.04	21.82	43.74	51.08	58.34 b	62.26 b
20% N	9.36	12.88	22.98	42.86	50.90	61.36 b	65.10 b
20% N+*B. subtilis*	7.86	11.34	21.30	42.34	51.90	66.46 a	69.02 a
20% N+*P. fluorescens*	7.48	10.42	20.44	43.82	53.40	65.58 a	68.74 a
20% N+co-inoculation	6.26	9.16	20.62	41,50	51.92	67.42 a	73.22 a
CV (%)	25.29	23.40	9.24	7.19	6.06	6.04	5.85
Pr>Fc	0.0910	0.1566	0.2981	0.7348	0.7400	0.0085	0.0053

Means followed by the same letters and numbers are not different according to the Scott-Knott test ($P<0.05$). CV: Coefficient of variation; 20% N+co-inoculation: *B. subtilis* and *P. fluorescens*.

Effects of inoculation on number of green leaves were observed only until 66 DAS (Tab. 2). Inoculation with *P. fluorescens* was the only treatment that promoted the same number of leaves observed with 100% nitrogen across all evalutions where statistical differences were observed, namely up to 66 DAS (Tab. 2).

Table 2. Mean values of number of green leaves of carrot plants from 38 to 100 days after sowing (DAS) grown under different inoculation treatments. Ponte Alta do Norte-SC, Brazil.

Treatment	38 DAS	45 DAS	52 DAS	66 DAS	80 DAS	95 DAS	100 DAS
100% N	4.84 a	6.48 a	8.22 a	9.20 a	10.74	12.60	13.16
20% N	4.48 a	5.88 b	7.46 b	8.48 b	9.78	10.24	11.06
20% N+*B. subtilis*	3.72b	5.64 b	8.20 a	8.82 b	10.62	11.52	13.08
20% N+*P. fluorescens*	4.48 a	6.68 a	8.64 a	9.40 a	10.54	10.32	11.64
20% N+co-inoculation	4.60 a	5.72 b	7.98 ab	9.06 a	10.32	11.40	12.34
CV (%)	11.13	7.99	6.57	4.67	11.94	15.50	18.85
Pr>Fc	0.0272	0.0108	0.0387	0.0287	0.7602	0.2300	0.5567

Means followed by the same letters and numbers are not different according to the Scott-Knott test ($P<0.05$). CV: Coefficient of variation; 20% N+co-inoculation: *B. subtilis* and *P. fluorescens*.

The highest means of fresh shoot mass were detected in 100% N, with use of nitrogen, and all inoculation treatments associated to reduced levels of nitrogen decreased production of shoot biomass (Tab. 3). On the other hand, root length was increased by inoculation with *P. fluorescens* and co-inoculation, compared to 100% N, 20% N and 20% N+*B. subtilis*. Plants inoculated with both bacteria produced roots that were, in average, four to five centimeters longer than plants that were inoculated only with *B. subtilis* or not inoculated, regardless of the N supply (Tab. 3).

Table 3. Mean values of mass of fresh and dry shoot, root length and volume, mass of fresh and dry roots of carrot plants grown under different inoculation treatments. Ponte Alta do Norte-SC, Brazil.

Treatment	Mass of fresh shoot (g)	Mass of dry shoot (g)	Root length (cm)	Root volume (mL)	Mass of fresh roots (g)	Mass of dry roots (g)
100% N	177.04 a	18.98	21.24 b	296.80	164.97 b	16.72
20% N	135.54 b	15.78	21.30 b	302.56	172.62 b	16.79
20% N+*B. subtilis*	81.54 c	11.98	19.78 b	202.40	194.02 b	19.60
20% N+*P. fluorescens*	67.18 c	12.76	25.08 a	216.80	281.05 a	21.48
20% N+co-inoculation	66.66 c	12.44	25.30 a	217.60	300.38 a	23.17
CV (%)	27.14	34.17	10.94	29.70	29.21	31.28
Pr>Fc	0.0001	0.1735	0.0075	0.1199	0.0100	0.3975

Means followed by the same letters and numbers are not different according to the Scott-Knott test ($P<0.05$). CV: Coefficient of variation; 20% N+co-inoculation: *B. subtilis* and *P. fluorescens*.

Mass of fresh roots was increased when plants were inoculated with *P. fluorescens* and co-inoculated with *P. fluorescens* and *B. subtilis* (Tab. 3). Compared to 100% N, co-inoculation promoted an increase of 81.8% in root mass, whereas inoculation with only *P. fluorescens* improved mass of fresh roots by 70%.

More than 50% of roots were considered normal (Tab. 4). The only treatment in which split roots were not observed was 20% N+co-inoculation. Purple and green shoulders were detected in all treatments but 100% N (Tab. 4).

Table 4. Mean percentages of normal, split and cracked roots, occurrence of purple and green shoulder, as well as adventitious roots and other deffects of carrot plants grown under different inoculation treatments. Ponte Alta do Norte-SC, Brazil.

Treatment	Normal roots	Split roots	Cracked roots	Purple shoulder	Green shoulder	Adventitious roots	Other deffects
100% N	56.57	16.67	13.33	0.00	0.00	3.33	10.1
20% N	56.67	16.67	6.66	16.67	3.33	0.00	0.00
20% N+*B. subtilis*	56.67	13.33	10.00	6.67	10.00	0.00	3.33
20% N+*P. fluorescens*	53.33	6.67	13.33	20.00	3.33	3.33	0.01
20% N+co-inoculation	76.68	0.00	6.66	13.33	3.33	0.00	0.00
CV (%)	40.19	117.44	120.07	207.19	214.48	364.43	276.39

CV: Coefficient of variation; 20% N+co-inoculation: *B. subtilis* and *P. fluorescens*.

This is the first study evaluating effects of inoculation of carrot on developmental parameters throughout its entire cycle of cultivation. We also report, for the first time, benefits of co-inoculation on growth and yield of carrot. Our findings contribute to more sustainable agricultural practices linked with economical gains, as well as to widen the range of crop species known to benefit from co-inoculation.

There are no previous records about measurement of plant height throughout the entire development cycle in response to inoculation of carrot. Our data suggest that effects of bacteria on this variable are detected towards physiological maturity of carrots. The opposite pattern was observed regarding number of green leaves. Effects of any treatment were not observed after 80 DAS. *P. fluorescens* was the most efficient bacteria to increase number of leaves. Reports regarding other plant species support beneficial effects or *Pseudomonas* on this parameter. Corrêa *et al*.

(2010), for example, analysed response of lettuce to *Pseudomonas chlororaphis*, and observed 4 to 7% more leaves as a result of inoculation.

All inoculation treatments were associated to smaller values of shoot mass. Other studies, however, have shown no significant differences when inoculation was performed. Matsuoka *et al*. (2016) inoculated carrots with ACC (1-aminocyclopropane-1-carboxylate) deaminase-producing bacteria isolated from carrots, turnips and sweet pepper (named as OFT2 OFT5 e RH7). They observed no effect of inoculation on mass of fresh leaves, with values ranging from 20 to 24 g per plant. Kozusny-Andreani *et al*. (2014) tested the effects of 14 unidentified rhizobacteria isolated from *Crotalaria spectabilis* on carrot, and also found no difference between control and inoculation treatments regarding shoot mass.

Increases of root length in response to inoculation with PGPR have been reported by other researchers. Kozusny-Andreani *et al*. (2014) observed large variation in root length between different isolates (14 - 22 cm) among treatments, but showed that ten out of 14 bacterial isolates increased root length compared to absence of inoculation. Positive responses of root length are relevant for carrot, because this trait is commercially valuable. Five commercial classes are established in Brazil according to Nick and Borém (2016): 10 (>10 and <14cm), 14 (>14 and <18cm), 18 (>18 and <22cm), 22 (>22 and <26cm) and 26 (>26cm). Treatments 1, 2 and 3 resulted in values belonging to Class 18. However, only treatments of inoculation with *P. fluorescens* and co-inoculation with *P. fluorescens* and *B. subtilis* resulted in longer roots, which belong to class 22. Therefore, inoculation with these two species of bacteria may be an alternative to improve commercial quality of carrots grown in decreased N supply.

Yield of carrot was improved by inoculation with *P. fluorescens* and co-inoculation with *P. fluorescens* and *B. subtilis*. Merriman *et al*. (1974) first reported benefits of *Bacillus subtilis* associated with *Streptomyces griseus* in carrot, increasing root mass by 17%. Kozusny-Andreani *et al*. (2014) tested 14 bacteria strains and half of them were effective in promoting fresh root biomass. Authors showed, that while treatment with only N resulted in 28 g of roots, inoculation with the strain UCCBj-CE1 resulted in more than 60 g of roots produced. Clemente *et al*. (2016) studied the response of carrot to *Bacillus subtilis* and *B. methylotrophicus* and observed increased mass of fresh roots of carrots. Without inoculation, mean value was of 5.9 kg m^2. Inoculation with *B. subtilis* increased fresh root mass (6.5 kg m^2), as well as inoculation with *B. methylotrophicus*

(7.26 kg m^2). On the other hand, Matsuoka *et al*. (2016) found no differences on mass of fresh roots inoculated with two strains of bacteria. One of them (RH7) actually reduced mass of roots to 37 g, compared to control, with 48.7 g. Phylogenetic analyses revealed that all strains are related to species that belong to the genus *Pseudomonas.*

We observed no difference between treatments on mass of dry roots. However, Kozusny-Andreani *et al*. (2014) quantified more dry roots in carrots inoculated with four strains of unidentified rhizobacteria (mean of 5,5 g) when compared to nitrogen control (3 g).

Statistical analysis of data regarding carrot problems (such as split root, cracked roots, purple shoulder, green shoulder or adventitious roots) was not possible. However, co-inoculation was the only condition in which no plants presented split roots. Both biotic and abiotic stresses are known to promote split and cracked roots, often associated with inadequate humidity. Rhizobacteria are known to produce exopolysaccharides that help retaining water in the rhizosphere and reduce physical damage of roots (Chang *et al*., 2007). It is also possible that rhizobacteria decrease occurrence of purple and green shoulder, however analysis of data related to this hypothesis were compromised. According to Patten and Glick (1996), rhizobacteria are known to produce indole acetic acid, which would promote root elongation and deeper penetration in soil, causing less exposure to soil surface and sunlight. Further studies should be carried on to better understand the effects of inoculation on reducing carrot defects.

Our study´s hypothesis was not confirmed, since not all inoculation treatments associated to reduced N levels were effective in promoting plant development. Benefits of *B. subtilis* were also less pronounced than *P. fluorescens*. Although co-inoculation was as efficient as single inoculation with *P. fluorescens*, both treatments were revealed as outstanding methods to increase growth and yield of carrot. Reduced N fertilization, associated to obtaining carrots classified in commercial classes with more aggregate value, may motivate farmers to introduce inoculation as a routine practice when growing carrots.

CONCLUSION

Bacillus subtilis and *Pseudomonas fluorescens* improve growth and yield of carrot, and are indicated as plant growth-promoting microorganisms under reduced levels of nitrogen fertilization.

Conflict of interests: The manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

BIBLIOGRAPHIC REFERENCES

Aloo, B.N., B.A. Makumba, and E.R. Mbega. 2019. The potential of Bacilli rhizobacteria for sustainable crop production and environmental sustainability. Microbiol. Res. 219, 26-39. Doi: https://doi.org/10.1016/j.micres.2018.10.011

CEAGESP, Companhia de Entrepostos e Armazéns Gerais de São Paulo. 2000. Normas de classificação da cenoura. CQH/CEAGESP, São Paulo, Brazil.

Chang, W.S., M. Van de Mortel, L. Nielsen, G.N. Guzman, X. Li, and L.J. Halverson. 2007. Alginate production by *Pseudomonas putida* creates a hydrated 133 microenvironment and contributes to biofilm architecture and stress tolerance under water-limiting conditions. J. Bacteriol. 189(22), 8290-8290. Doi: https://doi.org/10.1128/JB.00727-07

Clemente, J.M., C.R. Cardoso, B.S. Vieira, I.M. Flor, and R.L. Costa. 2016. Use of *Bacillus* spp. as growth promoter in carrot crop. Afr. J. Agric. Res. 11(35), 3355-3359. Doi: https://doi.org/10.5897/AJAR2016.11316

Corrêa, E.B., W. Bettiol, and J.C. Sutton. 2010. Controle biológico da podridão radicular (*Pythium aphanidermatum)* e promoção de crescimento por *Pseudomonas chlororaphis 63-28* e *Bacillus subtilis* GB03 em alface hidropônica. Summa Phytopathol. 36(4), 275-281. Doi: https://doi.org/10.1590/S0100-54052010000400001

CQFS, Comissão de Química e Fertilidade do Solo. 2016. Manual de adubação e de calagem para os Estados do Rio Grande do Sul e de Santa Catarina. Sociedade Brasileira de Ciência do Solo, Porto Alegre, Brazil.

Fioreze, S.L., M.G. Pinheiro, Y.D. Pereira, and S.P. Cruz. 2020. Inoculation of wheat plants with *Pseudomonas* spp. and *Azospirillum brasilense* under drought stress. J. Exp. Agric 42(2), 1-7. Doi: https://doi.org/10.9734/jeai/2020/v42i230461

Fukami, J., C. de la Osa, F. Javier Ollero, M. Megías, and M. Hungria. 2018. Co-inoculation of maize with *Azospirillum brasilense* and *Rhizobium tropici* as a strategy to mitigate salinity stress. Funct. Plant Biol. 45, 328-339. Doi: https://doi.org/10.1071/FP17167

Hungria, M., M.A. Nogueira, and R.S. Araujo. 2013. Co-inoculation of soybeans and common beans with rhizobia and azospirilla: strategies to improve sustainability. Biol. Fertil. Soils 49(7), 791-801. Doi: https://doi.org/10.1007/s00374-012-0771-5

Kloepper, J.W., J. Leong, M. Teintze, and M.N. Schroth. 1980. Enhanced plant-growth by siderophores produced by plant growth-promoting rhizobacteria. Nature 286, 885-886. Doi: https://doi.org/10.1038/286885a0

Kozusny-Andreani, D.I., J.C. Agiado, and R. Andreani Junior. 2014. Efeito de bactérias rizosféricas sobre o desenvolvimento da cenoura. Rev. Univ. Vale Rio Verde 12, 211-220. Doi: https://doi.org/10.5892/ruvrd.v12i1.1368

Kumawat, K.C., P. Sharma, A. Sirari, I. Singh, B.S. Gill, U. Singh, and K. Saharan. 2019. Synergism of *Pseudomonas aeruginosa* (LSE-2) nodule endophyte with *Bradyrhizobium* sp. (LSBR-3) for improving plant growth, nutrient acquisition and soil health in soybean. World J. Microbiol. Biotechnol. 35, 47. Doi: https://doi.org/10.1007/s11274-019-2622-0

Lana, M.C., J. Dartora, D. Marini, and J.E. Hann. 2012. Inoculation with *Azospirillum*, associated with nitrogen fertilization in maize. Rev Ceres 59(3), 399-405. Doi: https://doi.org/10.1590/S0034-737X2012000300016

Lin, Y., D.B. Watts, J.W. Kloepper, Y. Feng, and H.A. Torbert. 2020. Influence of plant growth-promoting rhizobacteria on corn growth under drought stress. Commun Soil Sci. Plant Anal. 51(2), 250-264. Doi: https://doi.org/10.1080/00103624.2019.1705329

Matsuoka, H., Y. Ohwaki, J. Terakado-Tonooka, and F. Tanaka. 2016. Changes in volatiles in carrots inoculated with ACC deaminase-producing bacteria isolated from organic crops. Plant Soil 407, 173-186. Doi: https://doi.org/10.1007/s11104-015-2769-x

Mendes, I.C., M. Hungria, and M.A.T. Vargas. 2003. Soybean response to starter nitrogen and *Bradyrhizobium* inoculation on a cerrado oxisol under no-tillage and conventional tillage systems. R. Bras. Ci. Solo 27(1), 81-87. Doi: https://doi.org/10.1590/S0100-06832003000100009

Merriman, P.R., R.D. Price, J.F. Kollmorgen, T. Piggott, and E.H. Ridge. 1974. Effect of seed inoculation with *Bacillus subtilis* and *Streptomyces griseus* on the growth of cereals and carrots. Aust. J. Agric Res. 25(2), 219-226. Doi: https://doi.org/10.1071/AR9740219

Molla, A.H., Z.H. Shamsuddin, M.S. Halimi, M. Morziah, and A.B. Puteh. 2001. Potential for enhancement of root growth and nodulation of soybean coinoculated with *Azospirillum* and *Bradyrhizobium* in laboratory systems. Soil Biol. Biochem. 33(4), 457-463. Doi: https://doi.org/10.1016/S0038-0717(00)00186-3

Nick, C. and A. Borém. 2016. Cenoura do plantio à colheita. Editora UFV, Viçosa, Brazil.

Novoselov, V.S. 1960. A closed volumeter for plant root systems. Fiziologiya Rastenii 7, 243-244.

Patten, C.L. and B.R. Glick. 1996. Bacterial biosynthesis of indole-3-acetic acid. Can. J. Microbiol. 42(3), 207-220. Doi: https://doi.org/10.1139/m96-032

Paula, L.B. 2019. Cenoura: área menor, produtividade maior. Campo & Negócio, Piracicaba, Brazil.

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Vessey, J.K. 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255(2), 571-586. Doi: https://doi.org/10.1023/A:1026037216893

Vieira, J.V. and H.B.S.V. Pessoa. 2008. Cenoura (*Daucus carota*): Clima. Embrapa Hortaliças, Brasília.

Zeffa, D.M., L.H. Fantin, A. Koltun, A.L.M. de Oliveira, M.P.B.A. Nunes, M.G. Canteri, and L.S.A. Goncalves. 2020. Effects of plant growth-promoting rhizobacteria on co-inoculation with *Bradyrhizobium* in soybean crop: a meta-analysis of studies from 1987 to 2018. Peer J. 8, e7905. Doi: https://doi.org/10.7717/peerj.7905