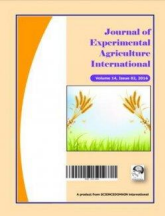

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Evaluation of Potential Vegetal Growth of Corn by Using Endophytic Bacteria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors DAP and TSB designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors RP and TSB managed the analyses of the study. Author EDC reviewed the literature and correct the mistakes. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The study aimed to verify the potential for growth promotion of five bacterial strains to determine the best period to verify the interaction between plant and bacteria.
Study Design: The experimental design was completely randomized and the analyzes were performed at seven and fourteen days after the inoculation.
Place and Duration of Study: The experiment was performed at the biotechnology lab of the Pontifical Catholic University of Paraná, Campus Toledo.
Methodology: The strains *Azospirillum brasiliense, Herbaspirillum sropedicae, Pantoea ananatis, Burkholderia ambifaria* and *Burkholdeira* sp., were tested to growing promotion on simple hybrid corn 30F53 YH being the inoculation made in pre-germinated seeds with 10^6 CFU mL^{-1} posteriorly the same seeds were kept on genobiotic conditions with N restriction. Were evaluated the

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parameters aerial and root fresh weight, aerial length, root length, N percentage on aerial part, root morphology and epiphytic and endophytic population
Results: It was observed that to the seven or even the fourteen days was possible to verify the interaction between plant and bacteria by means of the parameters evaluated in other words in both periods were significant differences between treatments.
Conclusion: Bacteria were not very efficient to promising grow on the hybrid 30F53 YH in vitro on N restriction conditions since no treatment statistically overcame the control to the evaluated parameters.

Keywords: Biological fixation of nitrogen; Zea mays L.; Nitrogen, Poaceae.

1. INTRODUCTION

Corn (*Zea mays* L.) is one of the oldest crops of the world, it also have a huge importance in the world economic scenario because, besides been a commodity, it also have an importance in the familiar and subisistence agriculture [1], nowadays the cultivated area of corn in the world suppras 184 million hectars, resulting in a yield of about one million tons, distributed among human and animal food, mainly animal in Brazil [2].

The production on Brazil is destined to different sectors of the productive chain it also have a tendency to increase since the end of the decade of 80 [3]. The rentability of the crop involves different factors, yet, physiological and nutritional parameters are responsible for a part of the important productivity and quality of the final product.

One of the most required elements of the corn crop is the nitrogen because, from this element, the basic processes of the metabolism of the plant are performed since it is a part of the proteins, nucleic acids, cell constituents and hormones [4]. However, this element is considered the most onerous of the culture in addition to the elevated costs for its production in the inorganic form, due to its elevated energy demand on its manufacture, the nitrogen is required in high quantities for the plants of the Poaceae family, as it is the corn [5].

This great supply of nitrogen in the inorganic form, with the use of chemical industrialized fertilizers, is a very common practice in the current production systems, due to it, many times this nutrient is used without an observation of its real necessity, resulting in high expenses besides damages to the natural characteristics of the soil [6].

Vegetal growth promoting bacteria have as its characteristic an association to plant roots and also to help in the production of important compounds to the vegetal development, among other factors highlight the availability of atmospheric nitrogen in the assimilable form for crops [7].

Since they are living organisms with different characteristics in the soil environment, it is essential more studies that characterize its action form of each identified bacteria as a potential growth promoter, since the association with plants can occur in a varied way.

The present work aimed to verify the potential of plant growth promotion of five bacterial strains in order to determine the best period of plant-bacterial interaction.

2. MATERIALS AND METHODS

2.1 Local, Hybrid and Inoculum Obtainment

The experiment was performed at the biotechnology lab of the Pontifical Catholic University of Paraná, Campus Toledo. To evaluate the growing potential were used five bacteria strains:

- *Azospirillum brasiliense* Ab-V5,
- *Herbaspirillum sropedicae,*
- *Pantoea ananatis,*
- *Burkholderia ambifaria* and
- *Burkholderia* sp
- Control – No inoculation.

Which strain was considered a treatment. All the strains were gentle provided by Parana Federal University, Campus Palotina, and also they were kept by successive replications on solid growth medium and kept at incubators set to 28ºC.

The strains were isolated from different crops of previous experiments being the *B. ambifaria* and

Burkholderia sp., isolated from corn roots and *P. ananatis* from wheat roots. The *A, brasiliense* Ab-V5 and *H. seropedicae* strains were used as positive control because they are standard strains. To choose the strains were followed some criteria being considered the results from the phosphate solubilizing and the test of IAA production (indoleacetic acid) both performed *in vitro*.

The hybrid utilized was the Pionner 30F53YH which is indicated for summer crops and second harvest, the material stands out due to its high productive potential, early cicle with stability, high grain quality and responsive to cultivation, in special to ferilization. Presents itself as an excellent option for silage, mostly indicated for altitudes between 400 and 700 meters, with a recommendation to avoid the seeding of corn over corn. Requires a preventive handling to fungal deseases it also have responsivity for the use of fungicides. In addition, monitoring is recommended for sucking insects and areas with history of attack [8].

2.2 Prepare of the Inoculum

To standardize the bacteria, grow exponential phase (*log)* were draw the growing curve on liquid medium DYGS to all the bacteria used in this experiment. This analysis was performed by spectrophotometry at 660 nm and the bacterial cell count from micro droplet methodology proposed by Romeiro [9].

To prepare the pre-inoculum one bacterial colony was collected from Petri dish and transferred to a 50 ml Falcon tube with 5 ml of liquid DYGS medium, those were kept in a shaker type incubator at 120 rotations per minute (rpm) and 28ºC overnight. After this time an aliquot of 1 ml was collected from the Falcon tube and transferred to a 250 ml Erlenmeyer filled with 20 ml of liquid DYGS medium then returned to the shaker incubator at 120 rpm and 28ºC until the inoculum reached the log phase of growth. To check the growth and the inoculation was necessary to adequate the equation to the concentration of 10^6 colony forming units (CFU) ml^{-1}. The necessary volume was arranged in microtubes and centrifuged at 9000 rpm at 20ºC the supernatant was discarded and the pellet resuspended in a 0.9% saline solution.

2.3 Experiment *in vitro*

In vitro seeds of the corn genotype (*Zea mays* L.) simple hybrid PIONEER® 30F53 YH were washed in acidified hypochlorite solution for 20 minutes at constant agitation followed by 3 washes in distilled and autoclaved water in a laminar flow chamber. Posteriorly at laminar flow chamber the seeds were transferred to a Petri dish on top of 2 sheets of germitest paper soaked with distilled and autoclaved water about 2,5 times its weight. The dish was enveloped with aluminum foil and kept on BOD incubator for 48 hours at 25ºC to pre-germinate. This hybrid was used due to its importance in the agriculture of the region.

On the pre-germinated seeds were inoculated the bacterial strains according to the respective treatment. Each treatment received the 0,9% saline solution + pellet, on the pre-germinated seeds, and they stayed in contact with the suspension for 2 hours. Posteriorly the already inoculated seeds were transferred to test tubes filled with 20 ml of liquid MS medium [10] suppressed of nitrogen (NH_4NO_3 and KNO_3) and pH adjusted to 5,8. Then were added polypropylene balls to the tube making a layer of 5 cm that worked as a support for the seeds in the medium so those did not submerge in the culture medium. Each tube received one pre-germinated seed, and each treatment had 25 repetitions. Those were kept into a growing room at the photoperiod of 16 hours of light and 8 hours of dark with temperature set to 25± 2ºC and evaluated on 2 periods at 7 and 14 days after inoculated, being the evaluations performed the fresh and dry weight of roots and aerial part, aerial part length, root length, aerial nitrogen content, epiphytic and endophytic bacterial population and root morphology.

After the growth period 10 plants of each treatment were taken from the tubes and the aerial parts were separated from the roots. Both were measured with a graduated tape (cm) to obtain the aerial and root length after that they were weighted (mg) with an analytical balance to determine aerial and root fresh weight. The same samples were left in hot air oven with forced circulation at 54ºC for 72 hours to obtain a constant aerial and root dry weight.

2.4 Determination of the Foliar Nitrogen Content

To determine nitrogen content of the aerial part were weighed 100 mg from the dry sample in triplicate grinded with a mortar and kept in test tubes adapted to a protein distiller Kjeldahl. Was added 1 g of the digestive mix previously made and 5 ml of concentrated sulfur acid. Posteriorly

the tubes were taken to protein digest plate where started the digestion at 50ºC and every 30 min was increased another 50ºC on the temperature until it reached 400ºC [11].

To the distillation were used 125 ml erlenmeyer filled with 10 ml of H_3BO_3 solution previously made. Then was added a solution of NaOH 50% and left until it distilled to the volume of 50 ml. In the titration was used a buret with HCl at 0,05 mol L^{-1} previously titrated with a known correction factor releasing HCl until the color changes from light green to pink [12].

The formula used to determine the nitrogen perceptual (N) was:

$$\%N = \frac{V \times N \times f \times 14 \times 100}{weight\ of\ the\ sample\ (mg)}$$

V = volume of HCl (mL); N = normality; f = correction factor.

2.5 Determination of the Epiphytic and Endophytic Root Population

To evaluate the epiphytical bacterial cell count was taken 1 plant to a laminar flow chamber than the roots were separated from its aerial part. The roots were etriple washed with autoclaved and distilled water and posteriorly transfered to microtube with 900 µl of saline solution and taken to ultrasound for 1 minute. Next was taken one aliquot of 100µl from the sample and transferred to microtube with 900 µl of saline solution at 0,9% and this was the dilution of 10^{-1}, from that dilution were taken another 100 µl which were transferred to microtube filled with saline solution at 0,9% that was the dilution of 10^{-2} this procedure was repeated until the 10^{-8} concentration and from the last one were taken 100 µl of solution and discarded to keep the volume of dilutions constant.

To the endophytic bacteria cell count was used the same root for the epiphytic count which was taken from the microtube with saline solution and transferred to Chloramine – T solution at 1% for 30s. Posteriorly the root was washed on distilled and autoclaved water. Next the same root was macerated in mortar filled with 900 µl of saline solution at 0,9% after that, all the content of the mortar was added to microtube and taken to ultrasound for 1 minute. The dilutions made for the endophytic count were the same performed for the epiphytic bacteria cell count.

All the 8 dilutions, separating epiphytic from endophytic were allocated into one Petri dish which was subdivided on 8 equal parts. The dishes had solid DYGS medium where 5µl micro drops were disposed on triplicate for each dilution on their respective division in the dish constituting 3 repetitions per dilution where each micro drop was considered a repetition [9]. The dishes were allocated on incubation oven at 28ºC and after 12 hours was performed the bacterial cell count and the population was estimated by the average of the 3 repetitions to every dilution that had shown signs of growth.

Another parameter evaluated was the morphological root analysis where those were submerged in methylene blue for one minute and posteriorly placed on a slide and coverslip with distilled water and observed in microscope with a 10-times increase, each treatment was observed in triplicate.

2.6 Statist Analisis

The experimental design was completely randomized and the results were submitted to variance analysis at 5% of probability and when they presented significant difference was performed an average comparison with the Tukey test at 5% of probability using the CoStat software [13].

3. RESULTS AND DISCUSSION

Observing the data present on Table 1 it is verified that on the control there was microbial population endophytically at 7 days after inoculation. That cannot be attributed to mistakes during asepsis because there was no microbial presence epiphytically. What can be inferred is that this microbial load was already inside the seed and also that the bacteria can survive for long periods of storage as reported for Moreira & Siqueira [14]. What reinforces this is the fact that 14 days after inoculation there was no microbial load epiphytically or endophytically.

Neiverth [15] working with the inoculation of *H. seropedicae* on wheat cultivars also obtained epiphytic and endophytic growth whereas tests without inoculation did not show any kind of contamination. That happened because during the experiment procedure the author removed the seed embryo rejecting the endosperm area that can have microorganisms on latent phase.

However, Dall'Óglio-Chaves [16] when performing the inoculation of some bacterial species on pre-germinated wheat seeds did not obtain epiphytic and endophytic bacterial load on the control treatment.

By evaluating the treatments with inoculation it is noticed that the bacteria *P. ananatis* was more aggressive on the colonization, once its population was in greater number on both epiphytic and endophytic ways on the first evaluation period. 14 days after the inoculation its endophytic population already becomes similar to the other treatments like *B. ambifaria* and *H. seropedicae* being that, on the epiphytic population case, the treatment with *P. ananatis* still superior to the other.

It is noted that after some time the population tended to increase due to the plant growth and its root development, propitiating an increase on its habitat.

It is difficult to affirm the exact colonization niche of each studied strain. Barbosa et al. [17] affirmed that the same bacteria can colonize rhizosphere, ripple and endosphere as a result of this, the bacteria classification according to its habitat have to be done with caution. This information corroborates with the data obtained on the microbial evaluations at 7 and 14 days after inoculation once the bacterial growth happened epiphytically and endophytically for all the tested strains despite the numeral variation among niches.

On the Table 2 are presented the referring data to the bacteria growth promotion on corn plants at 7 days after inoculation. To the parameter aerial fresh weight, it is possible to observe that the biggest average was for the treatment control, which had no inoculation, and the smallest average was in the treatment with *H. seropedicae* inoculated being those two treatments the only ones that differed statistically among themselves, the others did not differ from the control or the treatment with *H. seropedicae.* Neiverth [15] when evaluating dry aerial weight of eight wheat cultivars conducted *in vitro*, with no N restriction, detected that only for one cultivar the inoculation was beneficial, being that for two of the cultivars the inoculation was harmful and indifferent for the rest on this parameter.

Thebiggest averages of aerial dry weight were obtained on the treatments control and *A. brasiliense* and the smallest on the treatment inoculated with *H. seropedicae.*

The treatment inoculated with *H.* seropedicae showed the biggest average of root fresh weight, not differing from the treatment control, *A. brasilense* and *P. ananatis.* The smallest average was found in the treatment inoculated with *Burkholderia* sp. Neiverth [15] while working with *H. seropedicae* detected that different wheat genotypes answered distinctly to root fresh weigh being that, for the CD 104 cultivar, the inoculation was deleterious for this parameter. However, with respect to root dry weight no significant differences occurred between treatments.

Dall'Óglio-Chaves [16] performing a study with the inoculation of different bacteria on wheat cultivars *in vitro* for 5 days verified, for the parameter plant height, that the biggest averages were obtained on the plants without the inoculation with *A. brasilense.* For the parameter root length the biggest average was obtained on the treatment inoculated with *B. ambifaria*, for the present work the biggest averages of rot length were found in the treatment inoculated with *A. brasilense* and control, being that, they did not differ statistically from the treatments *H. seropedicae* and *B. ambifaria.*

Table 1. Bacterial population on the roots of corn plants cultivated *in vitro* at 7 and 14 days after the inoculation expressed in colony forming units (CFYU) per mL

Treatment	Population (cfu ml^{-1})			
	Epiphytic		Endophytic	
	7	14	7	14
Control	0	0	$8,7 . 10^4$	0
A. brasilense	$2 . 10^4$	$6,6 . 10^5$	$5,3 . 10^5$	$6 . 10^5$
H. seropedicae	$1,2 . 10^5$	$9,3 . 10^4$	$4 . 10^6$	$7,3 . 10^7$
P. ananatis	$3 . 10^8$	$1,1 . 10^8$	$6,2 . 10^8$	$7,3 . 10^7$
B. ambifaria	$6,7 . 10^4$	$2 . 10^5$	$4 . 10^6$	$8 . 10^7$
Burkholderia sp.	$2 . 10^5$	$1,4 . 10^6$	$6,6 . 10^4$	$6,6 . 10^5$

Table 2. Results obtained on growth promotion essay performed at 7 days after inoculation being evaluated aerial fresh weight (AFW) in mg, aerial dry weight (ADW) in mg, root fresh weight (RFW) in mg, root dry weight (RDW) in mg, aerial height (AH) in cm, root length (RL) in cm and aerial percentage of nitrogen. (%N)

Treatment	AFW	ADW	RFW	RDW	AH	RL	%N
Control	281,72 a	34,05 a	755,95 ab	233,33 a	8,87 a	9,87 a	4,48 a
A. brasilense	261,42 ab	32,03 a	745,75 ab	239,94 a	8,09 a	9,88 a	3,84 a
H. seropedicae	203,75 b	20,98 b	810,22 a	235,68 a	7,97 a	8,15 ab	3,42 a
B. ambifaria	218,03 ab	26,43 ab	699,04 b	248,00 a	7,56 a	7,59 ab	3,51 a
P. ananatis	268,35 ab	30,03 ab	711,21 ab	243,71 a	8,48 a	6,67 bc	3,76 a
Burkholderia sp.	228,64 ab	29,75 ab	593,35 c	228,66 a	6,76 a	4,12 c	3,45 a

**Distinct letters in the column represent statistic difference by the Tukey test p<0,05*

The aerial N percentage had no significant statistic differences on all evaluated treatments. Neiverth [15] evaluating the total N of 8 wheat cultivars conducted *in vitro* for 7 days, in the presence of the inoculant *H. seropedicae,* also did not detect differences among inoculated and not inoculated treatments. Dall'Óglio-Chaves [16] at 5 days after inoculating wheat pre-germinated seeds, obtained significant differences for the treatments with inoculation related to the N content on the foliar tissue, the inoculation was benefic for the wheat plants who received the species *Agrobacterium tumenfaciens, A. brasilense, H. seropedicae* and *Burkholderia* sp., and those had shown higher averages when compared to the treatment control, on environment with N restriction.

By observing the values obtained on the present research in relation to the N content in the foliar tissue it is noticed that they are higher values than the ones considered ideal for the crop from Coelho & França [18], which are 2,75 to 3,25%. However, those values are considered ideal on the appearance of the female inflorescence period of the plant, which is when it is performed the sampling for foliar analysis on commercial corn. Epstei & Bloom [6] emphasize that crops on the beginning of its development, while in fully vegetative stages accumulating biomass, can reach values of 5% of N on its weight and at the mature stage can be with less than 1% of N in its constitution. That justify the higher value obtained on bothtreatments control and inoculated, once the plants were at the beginning of the vegetative stage.

Araujo et al. [19] evaluating 35 corn genotypes, related to inoculation of *H. seropedicae* in greenhouse, obtained an increase on the growth and N accumulation, only on 9 hybrids being evident a low contribution from the bacteria with respect to the biological N fixation proper. The increase was related, probably, with the capacity of the bacteria to produce phytohormones. Also observed that the genetic variability of the used material influenced on the interaction with the microorganisms. This fact corroborates with the data presented on Table 2, once occurred differences on the plant development in relation to the treatments evidencing that the bacteria can act in distinctly ways on the same hybrid. Besides that, to the hybrid of this study, there was no contribution related to the N increase on the vegetative growth once the control had shown the biggest averages and, when did not show, it did not differ statistically from the treatment with higher average.

At Table 3 are presented the data related to the evaluation of vegetable growth promotion, provided by the bacteria 14 days after the inoculation. To the aerial dry and fresh weight parameters no significant difference occurred among the treatments.

There were statistically significant differences for the root fresh weight parameter, being the highest average for the treatment *H. seropedicae* and the only treatment that differed statistically from this was the *Burkholderia* sp, whereas to the parameter root dry weight, no significant differences occurred among the treatments.

For the plant height, the treatment *A. brasilense* showed a higher average, statistically differing from the treatments *B. ambifaria, P. ananatis* and *Burkholderia* sp., which averages were smaller for this parameter.

In the treatment *A. brasilense* was found the highest average of root length, which did not differ statistically from the treatment control. Sala et al. [20] by inoculating endophytic bacteria

Table 3. Results obtained on growth promotion essay performed at 14 days after inoculation being evaluated aerial fresh weight (AFW) in mg, aerial dry weight (ADW) in mg, root fresh weight (RFW) in mg, root dry weight (RDW) in mg, aerial height (AH) in cm, root length (RL) in cm and aerial percentage of nitrogen. (%N)

Treatment	AFW	ADW	RFW	RDW	AH	RL	%N
Control	349,44 a	40,30 a	864,17 ab	203,16 a	17,09 a	11,89 ab	4,34 a
A. brasilense	374,22 a	43,09 a	938,72 a	193,78 a	17,81 a	12,38 a	3,92 ab
H. seropedicae	334,15 a	40,76 a	942,44 a	193,34 a	14,53 ab	9,29 bc	3,91 ab
B. ambifaria	323,83 a	41,05 a	872,36 ab	199,31 a	11,80 bc	8,76 c	4,14 a
P. ananatis	306,86 a	34,22 a	738,23 ab	184,57 a	11,96 bc	7,85 c	3,51 b
Burkholderia sp.	306,27 a	42,34 a	647,28 b	171,07 a	9,46 c	4,58 d	3,93 ab

** Distinct letters in the column represent statistic difference by the Tukey test p<0,05*

isolated from the same cultivar on genobiotic conditions, with N restriction, observed that the length of the main root was bigger on the inoculated treatments in relation to the control, for one of the tested cultivars whereas for another, only one bacteria was able to promote significant increase on the root length in relation to the control, this shows the complexity of the interaction between bacteria and plant.

The highest aerial N % averages were obtained on the treatments control and *B. ambifaria,* which differed statistically just from the treatment *P. ananatis.* Sala et al. [21] on study performed with 3 strains (*A. brasilense, Achromobacter insolitus, Zoogloea ramigera*), 3 levels of Nitrogen fertilizer (0, 60 and 120 kg ha^{-1}) and two wheat cultivars (ITD-19 and IAC-370) on field conditions, obtained comparable results to the present research once were performed analysis on the vegetative stage of the crop and when there was no use of the N supplement, the inoculation generally reduced the aerial dry weight and accumulation of N on the leaf.

Observing the data on Table 3 it is possible to identify that the bacteria did not promote the corn growth without the N supplement and, in some situations, they also caused damage to the development. In the case of *Burkholderia* sp., it is possible to say that at 14 days after the inoculation its visible a deleterious effect on the plant when observing, mainly, the parameters root length and root dry weight, once the radicular system has the function of fixation of the plant in the soil and absorption of water and nutrients being those essential to the plant growth and development [22].

Moreira & Siqueira [14] emphasize that, under low fertility conditions, the microorganism can compete against the plant for nutrients, causing a decrease on its content on the vegetable tissues and, consequently, a deficiency of elements on the plant. This happen because the time for the microorganisms to develop is way faster than the cells on the roots, resulting in a faster incorporation of nutrients into the microbial biomass.

Araujo et al. [19] while working with *H. seropedicae* strain, observed that this bacteria provided gains related to dry matter accumulation and N on the foliar tissue in hybrids with lower level of genetic amplitude, in other words, triple and simple hybrids. In the present study, when testing the same strain, e not occurred gains for the evaluated parameters although the hybrid was simple.

Barbosa et al. [17] confirmed that is necessary to find cultivars that fit into the use of this biotechnology artifice because there are many complex and less evident factors on this interaction.

Neiverth [15] performing promotion an essay of vegetable growth, with different wheat cultivars, considered that the period of interaction among plant and bacteria to the evaluated parameters were better on the period of 7 days after inoculation. On the present research was possible to notice significant difference to the parameters evaluated at 7 and 14 days after inoculation.

Differences between the morphological aspects of the roots were possible to be seen after inoculation as shown on the Fig. 1. Is noticeable that the bacteria *H. seropedicae* promoted a greater appearance of root hairs, being possible to compare with the control test what reinforces that is the bigger root fresh weight (Table 2) found on this treatment. Neiverth [15] observing the root morphology of wheat at 7 days after inoculated with *H. seropedicae in vitro* and without N restriction, obtained a perceptible increase on the root hair.

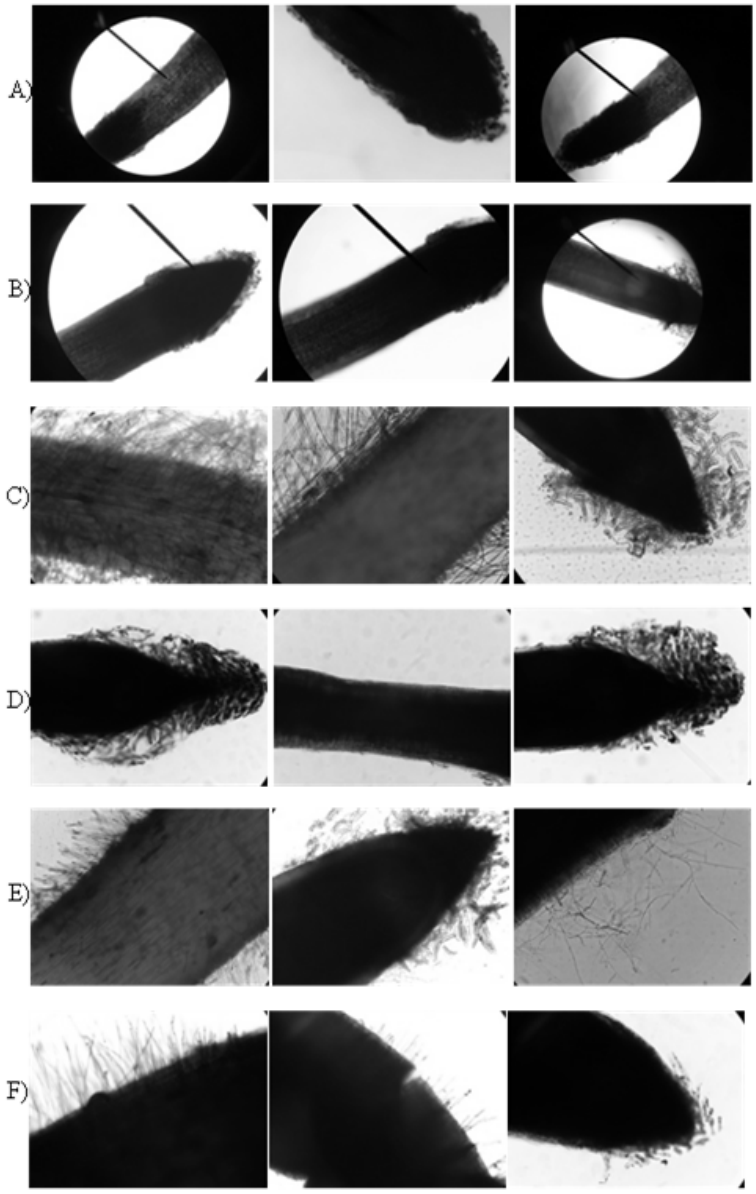

Fig. 1. Images obtained through the observation in optical microscope with increase of 10 times of the roots from the plants at 7 days after the inoculation of the treatments: Control (A); *A. brasilense* (B); *H. seropedicae* (C); *P. ananatis* (D); *B. ambifaria* (E); *Burkholderia* sp. (F).

Zonta et al. [22] highlight the importance of root hair on the gathering of nutrients process because they increase the surface of radicular absorption. It is also possible to verify that both of the bacteria from the gender *Burkholderia* promoted an appearance of root hair, however, in a discretely way and, observing the data referent to the radicular portion on those treatments on Table 2, it is noted that it did not have a good development when compared with the control.

It is hard to notice the differences among the treatments fourteen days after inoculation (Fig. 2). The bacteria *B. ambifaria* had shown a formation of more root hair when compared to 7 days after inoculation standing out the treatment with *H. seropedicae*. Both 7 and 14 days after inoculation, the treatment control did not show root hair growth, however, its results to the radicular parameters on Table 2 and 3 are always found with bigger averages, mainly on the question root length. Due to this we can infer that

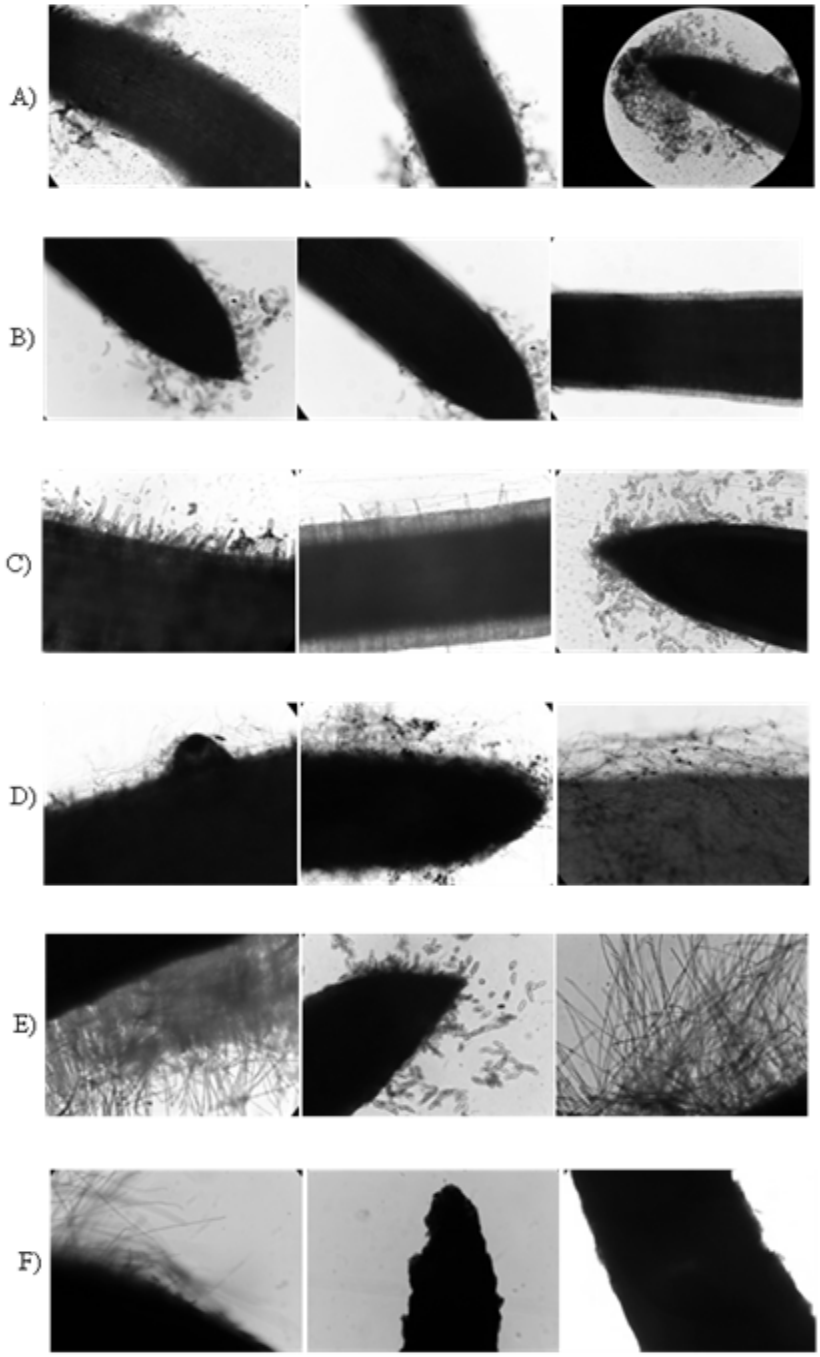

Fig. 2. Images obtained through the observation in optical microscope with increase of 10 times of the roots from the plants at 14 days after the inoculation of the treatments: Control (A); *A. brasilense* (B); *H. seropedicae* (C); *P. ananatis* (D); *B. ambifaria* (E); *Burkholderia* sp. (F).

the treatment control showed, probably, a larger region of root elongation [22].

By observing the Fig. 2, to the bacteria *Burkholderia* sp, it is visible a damage to the root apex, this fact agrees with the obtained on Table 3 where to the root length and plant height parameters the treatment that received this inoculation had shown lower averages.

Moreira & Siqueira [14] highlight that, among the morphological and physiological effects that the

endophytic microorganisms are capable of, are the radicular tissue damage, alterations on the metabolism, utilization of some components and exudates, enzyme excretion, toxins, alteration on the availability, accessibility and assimilation of mineral nutrients.

4. CONCLUSION

Both at 7 and 14 days it is possible to verify the interaction between plant and bacteria by means of the evaluated parameters.

The bacteria have not shown promise to growth promotion of the hybrid 30F53 YH *in vitro* on nitrogen restriction conditions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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