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Original Research Article

Evaluation of potential vegetal growth by using endophytic bacteria

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 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 (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

The corn (*Zea mays* L.) it's one of the oldest crops of the world and also have a big relevance on the world economy. Besides being a commodity, it also have importance on the agriculture for small families.

One of the most required elements of the corn crop is nitrogen (N), because from this element is that basic processes of plant metabolism are performed, since it is part of proteins, nucleic acids, cellular constituents and hormones (Taiz Et al., 2017). However, this element is considered the most expensive for the crop because besides the high costs to make it in an inorganic form because it takes to much energy on the making process the N is required on high quantities for plants of the Poaceae family such as the corn. This high provision of N on its inorganic form as a industrialized chemical is a very common practice on the actual growing methods once there is an incessant search for increased productivity. And, because of that, this nutrient is often used on higher amounts than the plants need (Epstein & Bloom, 2006).

When we give N on excess negative environmental impacts can happen such as the contamination and eutrophication of water bodies, reduction of microflora and micro fauna native to the soil, besides soil acidification. Seeking for a more sustainable production system that preserves the environment and allow the farmer to have more profitability by minimizing the production cost the use of biotechnology has been used. One example of this can be the use of bacteria that can promote the vegetable grow isolated from the plants itself or from the soil. Those bacteria can help the plant on its development by different

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mechanisms like: biological fixation of nitrogen, Phosphate solubilization, induction of resistance to diseases, production of organic acids and indole-acetic acid, among others (Glick, 2013).

On the other hand, those interactions between growing promotion bacteria and plants from the Poaceae family are not completely elucidated, once the answers for its use are not always predictable because at an associative interaction both organisms gain advantages but is not known if there is a prevalence from one on the other. The use of different bacteria species not focusing only on the gender *Azospirillum* as well as the use of different corn genotypes can help to comprehend those microorganisms (Moreira *et al.,* 2014).

The present research had as its objective verify the vegetable growing promotion potential of five bacteria strains in order to determine the best period of interaction between plant and bacteria.

2. MATERIAL AND METHODS

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 and which strain was considered a treatment. Besides these was performed a control treatment which had no inoculant. Strains were gentle provided by Parana Federal University, Campus Palotina, and also they were kept by successive reproduction on solid growth medium and kept at incubators set to 28ºC.

The strains were isolated from different crops from previous experiments being the *B. ambifaria* and *Burkholderia* sp., were isolated from corn roots and *P. ananatis* is from the 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*.

In addition to the variable inoculation, the analyzes were done in two distinct periods, at 7 and 14 days after inoculation.

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 (2001).

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 an incubator shaker kind at 120 rotations per minute (rpm) and 28ºC overnight. After this time an 1ml aliquot was collected from the Falcon tube and transferred to 250 ml Erlenmeyer filled with 20 ml of liquid DYGS medium going back 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 0.9% saline solution.

To the essay performed *in vitro* seeds of the corn genotype (*Zea mays* L.) simple hybrid PIONEER® 30F53 YH were washed on acidified hypochlorite solution for 20 minutes at constant agitation followed by 3 washes on distilled and autoclaved water in a laminar flow chamber. Posteriorly at laminar flow chamber the seeds were deposited inside 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.

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

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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 (Murashige & Skoog, 1962) suppressed of nitrogen (NH_4NO_3 and KNO_3) and pH adjusted to 5,8. Were added polypropylene balls to the tube making a layer of 5 cm that worked as a support for the seeds on the medium so those did not submerge on the culture medium. Each tube received one pre-germinated seed with 25 repetitions per treatment. 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.

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 were the evaluated parameters.

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 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 weight. The same samples were left in hot air oven with forced circulation at 54ºC for 72 hours to obtain the aerial and root dry weight.

To determine nitrogen content on the aerial part were weight 100 mg from the dry sample in triplicate grinded with a mortar and kept on test tubes adapted to protein distiller Kjeldahl. Wass 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 increased another 50ºC on the temperature until it reach 400ºC.

To the distillation were used erlenmeyer of 125 ml filled with 10 ml of H_3BO_3 previously made. 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 (Silva, 2002).

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.

To evaluate the epiphytical bacterial cell count was taken 1 plant to laminar flow chamber were the roots were separated from its aerial part. On the roots was performed a tripled wash with autoclaved and distilled water and posteriorly it was added to microtube with 900µl 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 900µl os saline solution at 0,9% and this was the dilution of 10^{-1}, from that dilution were taken another 100µl 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 100µl 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 autoclaved water. Next the same root was macerated in mortar filled with 900µl 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

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where each micro drop was considered a repetition (Romeiro, 2001). 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 on microscope with a 10-fold increase =, each treatment was observed in triplicate.

The experimental design was completely randomized (CRD) and the results were submitted to variance analysis at 5% of probability and when they presented significant difference was performed an average comparison test from the Tukey at 5% of probability using the CoStat software (Cohort Software, 2003).

3. RESULTS AND DISCUSSION

On the Table 1 are presented the referent data to the microbial population on the corn plant roots at 7 and 14 days after inoculation with the studied bacteria.

Observing the data present on Table 1 its verified that on the treatment control had 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 even though for long periods of storage as reported for Moreira& Siqueira (2006). What reinforces this is the fact that 14 days after inoculation there was no microbial load epiphytically or endophytically.

Neiverth (2011) working with the inoculation of *H. seropedicae* on wheat cultivars also obtained epiphytic and endophytic growth on the other hand 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.

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** (UFC 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.	$2 . 10^5$	$1,4 . 10^6$	$6,6 . 10^4$	$6,6 . 10^5$

However, Dall'Óglio-Chaves (2013) 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 notice that the bacteria *P. ananatis* was more aggressive on the colonization once its population presented in greater number on both epiphytic and endophytic way

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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 with the passage of time the population tend 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.,* (2012) 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 evaluated 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 (2011) when evaluating dry aerial weight on 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.

To the parameter aerial dry weight, the biggest averages were obtained on the treatments control and *A. brasiliense* and the smallest on the treatment inoculated with *H. seropedicae.*

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.

To the parameter root fresh weight, the treatment that had shown the biggest average was inoculated with *H, seropedicae*, being that this did not differ statistically from the treatment control, *A, brasilense* and *P. ananatis.* The smallest average was found in the treatment inoculated with *Burkholderia* sp. Neiverth (2011) 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.

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To the parameter plant height did not occur statistic differences among treatments. Dall'Óglio-Chaves (2013) performing study with the inoculation of different bacteria on wheat cultivars *in vitro* for 5 days verified for the parameter plant height 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.*

On the parameter aerial N percentage no significant statistic differences occurred on all evaluated treatments. Neiverth (2011) 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 (2013) 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 being that the inoculation was benefic for the wheat plants who received the species *Agrobacterium tumenfaciens, A. brasilense, H. seropedicae* and *Burkholderia* so., 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 (2014), which is 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 (2006) 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 the treatments both in control and inoculated, once the plants were in the beginning of vegetative stage.

Araujo *et al.,* (2013) by 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 bacteria capacity of phytohormones production. Also observed that the genetic breadth of the used material had influence 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.

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

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

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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.

To the parameter root length, the highest average was found in the treatment *A. brasilense,* which did not differ statistically from the treatment control. Sala *et al.,* (2005) by inoculating endophytic bacteria isolated from the same cultivar on genobiotic conditions with N restriction observed that the length of the main root was bigger on the treatments with inoculation in relation to the control for one of the tested cultivars, 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 among bacteria and plant.

To the parameter aerial N % the highest averages were obtained on the control and *B. ambifaria,* which differed statistically just from the treatment *P. ananatis.* Sala *et al.,* (2007) 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 similar 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 content on Table 3 it is possible to identify that the bacteria did no promote the corn growth without the supplement N and, in some situations, they also caused damage to the development. In the case of *Burkholderia* sp., it is possible to say that 14 days after the inoculation its visible its deleterious effect on the plant when observing the parameters root length and root dry weight once the radicular system has the function the fixation of the plant and abortion of water and nutrients being those essential to the plant growth and development (Zona *et al*., 2006).

Moreira & Siqueira (2006) 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.,* (2013) 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 present study when testing the same strain did not occur gains for the evaluated parameters although the hybrid was simple.

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Pimentel *et al.,* (2008) by inoculating *A. brasilense* in coffee detected that only one of four tested cultivars presented an increase in the dry leaves biomass and expansion of the foliar area contrary the other treatments did no differ from the control (without inoculation) to the vegetative parameters evaluated. Barbosa *et al.,* (2012) 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 (2011) performing promotion of vegetable growth essay 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.

At 7 days after inoculation was possible to observe differences between the morphological aspects of the roots as shown on the Figure 1. Is noticeable that the bacteria *H. seropedicae* promoted a greater appearance of root hairs being possible to compare to the control test what reinforces that is the bigger root fresh weight (Table 2) found on this treatment. Neiverth (2011) 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.

Zonta *et al.,* (2006) highlight the importance of root hair on the gathering of nutrients process because they increase the surface of radicular absorption. It is possible to also verify that both bacteria from the gender *Burkholderia* promoted 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.

At 14 days after inoculation it is hard to notice the differences among the treatments (Figure 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 the treatment control showed, probably, a larger region of root elongation (Zonta *et al.,* 2006).

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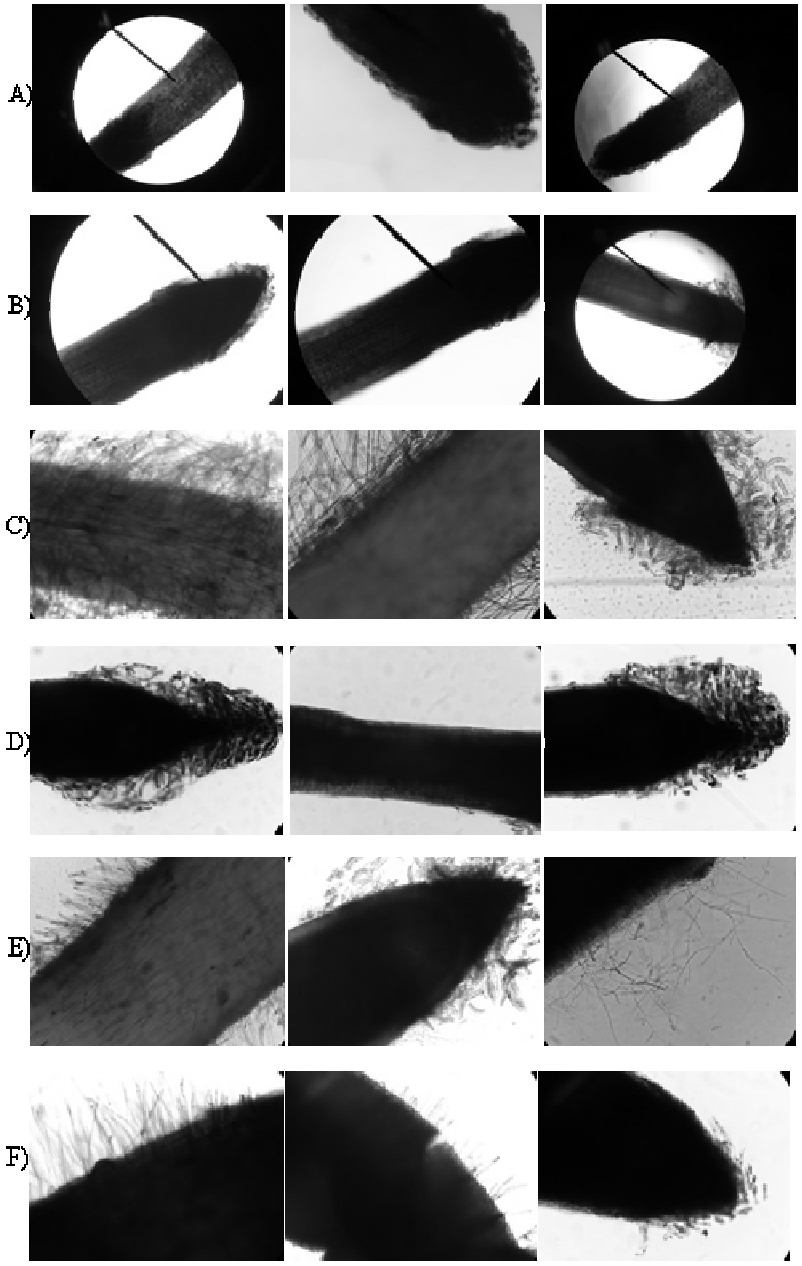

Figure 1: Images obtained through the observation in optical microscope with increase of 10 times of the roots from the plants at 7 days after the inoculation of the treatments: Control (A); *A. brasilense* (B); *H. seropedicae* (C); *P. ananatis* (D); *B. ambifaria* (E); *Burkholderia* sp. (F).

By observing the Figure 2 to the bacteria *Burkholderia* sp can be seen 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 (2006) 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.

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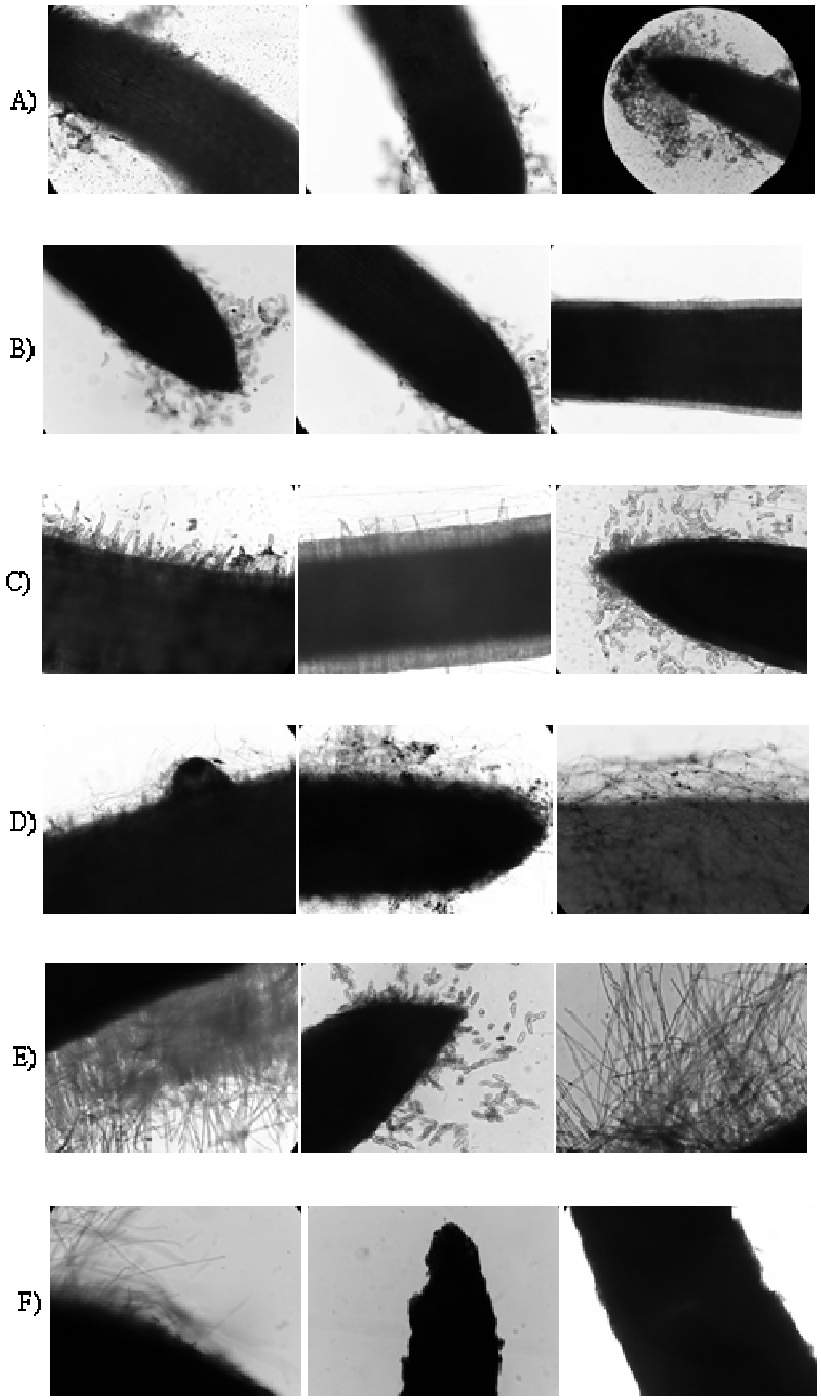

Figure 2: Images obtained through the observation in optical microscope with increase of 10 times of the roots from the plants at 14 days after the inoculation of the treatments: Control (A); *A. brasilense* (B); *H. seropedicae* (C); *P. ananatis* (D); *B. ambifaria* (E); *Burkholderia* sp. (F).

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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.

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