SEED TREATMENT WITH *Bacillus subtilis* OR INDOL BUTYRIC ACID: GERMINATION AND EARLY DEVELOPMENT OF BEAN SEEDLINGS

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SUMMARY

The aim of this work was to study seedling development after inoculation of bean seeds with Bacillus subtilis *and to compare it with the use of different concentrations of a synthetic auxin, indol butyric acid (IBA). Two essays were conducted, one in the lab and another in greenhouse, designed as totally random and random blocks, respectively. The variables evaluated were germination, vigor classification and root length for lab seedlings and emergence and emergence speed index for greenhouse seedlings. In both assays root, shoot and total dry matter of seedlings were determined.* B. subtilis *inoculation improved seedling emergence in laboratory and in greenhouse, and increased total dry matter. In greenhouse, shoot dry matter obtained with the bacteria inoculation was similar to that obtained with the higher doses of IBA (14 and 28mg·kg^{-1} of seed), while root dry matter was similar to that obtained with auxin doses of 7 and 14mg·kg^{-1} of seed. Total dry matter was higher than the control in all treatments, either inoculated or treated with auxin. Used as a inoculant in bean seeds,* B. subtilis *promoted a better seedling initial growth.*

Introduction

Microrganisms are extremely important in the control of their own environment and in affecting the plant metabolism in a complex way (Bloemberg and Lugtenberg, 2001). The vast majority of the works about plant growth-promoting rhizobacteria (PGPR), consider these phenomena as due to a indirect effect associated with biological control of secondary pathogens (Coelho *et al*., 2007; Ashrafuzzaman *et al*., 2009). However, aspects as germination and seedling growth are affected by PGPR (Kloepper *et al*., 2004) even in stress conditions such as salinity (Mishra *et al*., 2010, Naz *et al*., 2009) or low temperature (Khan and Patel, 2007). PGPR also act in the plant growth by affecting nutrient absorption, which implies in a more rational use of fertilizers (Adesemoye *et al*. 2008; Vessey, 2003).

According to Yasdani *et al*. (2009) PGPR use and phosphorus solubilizer bacteria may be responsible for an annual decrease up to 50% in fertilizers use, without affect-

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TRATAMIENTO DE SEMILLAS CON *Bacillus subtilis* O ÁCIDO INDOL-BUTÍRICO: GERMINACIÓN Y CRECIMIENTO INICIAL DE PLÁNTULAS DE FRIJOL

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RESUMEN

El objetivo de este trabajo fue estudiar el efecto de la inoculación de semillas con Bacillus subtilis *y de dosis crecientes de la auxina sintética ácido indol-butírico (IBA) durante el crecimiento inicial de plántulas de frijol. Se realizaron dos ensayos, en condiciones de laboratorio e invernadero, los cuales siguieron un diseño completamente aleatorizado y de bloques aleatorios, respectivamente. En el laboratorio se determinó la germinación, la clasificación de vigor y ancho radicular, mientras que en el invernadero se evaluaron la emergencia y el índice de velocidad de emergencia. Al final de los dos ensayos se evaluó el peso seco de brotes, raíces y plántulas total. Los resultados indicaron que la inoculación con* B. subtillis *incrementó la emergencia, tanto en laboratorio como en el invernadero, y contribuyó a aumentar la masa total de las plántulas. En el invernadero, la masa seca de los brotes obtenidos con inoculación de bacterias fue mayor que el control y similar a las dosis más altas del regulador de crecimiento (14 y 28mg·kg^{-1}), mientras que la masa seca de la raíz fue similar a la dosis de 7 y 14 mg·kg^{-1}. La masa vegetal seca total fue superior en todos los tratamientos con* B. subtilis *o regulador de crecimiento, en comparación con el testigo. El uso de* B. subtilis *como inoculante de semillas de frijol es eficiente y promueve un mejor crecimiento de las plantas en sus primeras etapas.*

TRATAMENTO DE SEMENTES COM *Bacillus subtilis* OU ÁCIDO INDOL-BUTÍRICO: GERMINAÇÃO E CRESCIMENTO INICIAL DE PLÂNTULAS DE FEIJOEIRO

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e Nelson Barbosa Machado-Neto

RESUMO

O objetivo desse trabalho foi estudar o efeito da inoculação de sementes com Bacillus subtilis *e doses crescentes da auxina sintética ácido indol-butírico (IBA) durante o crescimento inicial de plântulas de feijoeiro. Foram conduzidos dois ensaios, em laboratório e casa de vegetação, delineados como inteiramente casualizado e em blocos casualizados, respectivamente. No laboratório foram determinados a germinação, a classificação de vigor e o comprimento de raiz, enquanto que em casa de vegetação, foram avaliados a emergência e o índice de velocidade de emergência. Ao final de ambos os ensaios, avaliaram-se a massa seca de parte aérea, raiz e total de plântulas. Os resultados indicaram que a inoculação com* B. subtillis *aumentou a emergência, tanto em laboratório quanto em casa de vegetação, e colaborou para incrementar a massa total das plântulas. Em casa de vegetação, a massa seca da parte aérea obtida com a inoculação com a bactéria foi superior ao controle e semelhante às doses mais elevadas do regulador de crescimento (14 e 28mg·kg^{-1}) e a massa seca de raiz foi semelhante às doses de 7 e 14mg·kg^{-1}. A massa seca total das plantas foi superior em todos os tratamentos que receberam* B. subtilis *ou o regulador de crescimento, em relação ao controle. O uso de* B. subtilis *como inoculante em sementes de feijoeiro é eficiente e promove-se um melhor crescimento da planta em seus estágios iniciais.*

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ing growth and corn production. For Ashrafuzzaman *et al.* (2009) the use of PGPR was efficient to improve the germination and rice growth, due to the auxin production increment and making the phosphorus more soluble, which in crops of high expressive importance for human feeding may be a new approach.

Plant growth regulators are substances that in low concentrations are able to affect the plant physiological processes. Their production as secondary microorganism metabolites, in the soil, is directly linked to substrates, including plant exudates and other residues, which may be affected by environmental conditions as salinity and oxygen concentration (Radwan *et al.*, 2005; Ashrafuzzaman *et al.*, 2009).

In some works (Khalid *et al.*, 2004; Radwan *et al.*, 2005) it was clear that plant growth increase by use of PGPR was related to the production of auxins, which are responsible for cellular growth, shoot and root elongation, fruit development, apical dominance and abscission delay (Taiz and Zeiger, 2004). Among the auxins, indol acetic acid (IAA) is the most studied one, and is also produced by PGPR (Khalid *et al.*, 2004; Khan and Patel, 2007; Ashrafuzzaman *et al.*, 2009; Naz *et al.*, 2009; Calvo *et al.* 2010; Mishra *et al.*, 2010). *Bacillus subtilis* produced IAA and IBA (indol butiric acid) as a response to soybean exudates (Araujo *et al.*, 2005) and increased growth in soybean, corn and cotton (Araujo, 2008); in this case, seed inoculation with PGPR exhibited the advantage of an interaction with the plant along its whole cycle.

Common beans (*Phaseolus vulgaris* L.), a highly consumed grain in Brazil, is normally grown by low yield farmers, with low input of fertilizers and pesticides, witch leads to low productivity. Brazil, though, produces around 3,3·10^{6} tons of common beans, with a mean yield of 882.6kg·ha^{-1}, being the second largest producer of beans (CONAB, 2010); however, in irrigated areas, it can reach up to 3000kg·ha^{-1}. This high production is directly linked to the fact that beans are a basic food of the population and one of the main sources of protein in the diet (EMBRAPA, 2004).

However, the bean plant is sensitive to water deficit after sowing. Values of -0.15MPa in the soil induce the first symptoms of deficiency in the primary leaves, and at -0.35MPa germination and cell elongation may be drastically reduced. On the other

TABLE I
VARIANCE ANALYSIS OF GERMINATION (G), VIGOR CLASSIFICATION (CV), SHOOT (SDM), ROOT (RDM) AND TOTAL (TDM) DRY MASS AND ROOT/SHOOT RATE (R/S) OF COMMON BEAN SEEDS TREATED WITH DIFFERENT IBA CONCENTRATIONS AND *B. subtilis* AFTER SEVEN DAYS OF SOWING IN THE LAB

	Mean squareF test for all treatments						
Variation source	G	CV	CR	SDM (g)	RDM (g)	TDM (g)	R/S rate
Treatament	389.42**	369.55**	4.631**	0.5204**	0.5733**	1.1856**	0.3927**
Residue	63.66	60.21	0.7734	0.0174	0.0101	0.0327	0.0164
VC (%)	9.58	10.94	10.06	11.23	13.52	9.43	19.22
	Mean squareF test for all treatments						
Treatment	426.27**	365.07**	3.419*	0.2083**	0.6686**	1.436**	0.3367**
Residue	65.55	49.67	0.837	0.017	0.0033	0.0213	0.0193
VC (%)	9.98	9.95	10.95	12.32	7.02	7.70	18.26

*, ** significant at 5% and 1% by the F test, respectively. VC: variance coefficient.

hand, in lab conditions, germination was still observed at a simulated water deficiency of up to -0,9MPa (Machado Neto *et al*., 2006, Custódio *et al*., 2009, Coelho *et al*., 2010). Common bean is cultivated in marginal soils, with fertility problems related to nitrogen and phosphorus deficit (Barbosa Filho *et al*., 2003) and yield reduction due to sanitary problems (Sartorato *et al*., 2003), from sowing to harvest. This means increasing agrochemicals use. PGPR should be an alternative for the rational production of this crop, minimizing several impacts of modern technologies on the environment.

TABLE II
GERMINATION (G) AND SEED VIGOR CLASSIFICATION (VC) OF BEAN SEEDS TREATED WITH DIFFERENT CONCENTRATION OF IBA (mg·kg^{-1} seed) AND *B. subtilis* (10g·kg^{-1} seed) AFTER SEVEN DAYS OF SOWING IN THE LAB

Treatament		G (%)	CV (%)
B. subtilis		88 a[1]	81 a
Indol-butyric acid	0	76 b	64,5 b
	1.75	69 b	61 b
	3.5	76 b	70,5 b
	7	77 b	64 b
	14	93 a	82 a
	28	94 a	83 a
Control		71 b	62 b

[1] Means followed by the same letter are not statistically different by Scott-Knott (p >0.05).

The objective of this work was the study bean seed inoculation with *Bacillus subtilis* and to compare this treatment with the use of increasing doses of indolbutyric acid, during the germination and initial growth of bean seedlings.

Material and Methods

The work consisted of two experiments. The first one was carried out in the laboratory and the second in a greenhouse. The bean seeds used in both experiments were *Phaseolus vulgaris* cv Perόla, a carioca type, with normal cycle, semi erect, indeterminate growth, with a 100 seeds mass of 23-25g (EMBRAPA, 2004). Used seeds were the ones retained in a 6.3mm circular holes sieve.

Immediately before sowing, the seeds were treated with 5ml of indol-butyric acid (IBA) solution (0, 0.35, 0.7, 1.4, 2.8 and 5.6mg·ml^{-1}) per kg of seed for setting up the treatment (0, 1.75, 3.5, 7.0, 14 and 28mg·kg^{-1} seed). IBA was diluted in 3ml ethanol for the highest concentration and the volume completed to 10ml with water. The low concentration solutions were obtained by dilutions with a 30% ethanol solution (Remans *et al*., 2008). One treatment with *B. subtilis,* PRBS-1 (accession number AY504952, NCBI; Araújo *et al*., 2005), in the concentration of 10^9 cells/g as recommended by Araujo (2008), was used in the proportion of 10g inoculant per kg of seed. For a higher adhesion seeds were previously wetted with a 10% sucrose solution (Khalid *et al*., 2004) using a dose of 5ml. Two controls, one treated with 30% ethanol and a second with non treated seeds were used.

In the lab experiment, all treatments were used, with four replications per treatment, containing 50 seeds each, enveloped by three paper sheets, two as base and one as cover. They were rolled and placed in polyethylene bags and wetted with water 2.5 times their weight. Germination was carried out at 25ºC, and evaluated at seven days after sowing, counting normal, strong, weak and abnormal seedlings as well as dead seeds (BRASIL, 2009; Nakagawa, 1999). The results were expressed as percentages. Seedling performance was evaluated by a germination test; shoot and root were separated, placed in paper bags and dried at 60°C for 48h. Dried materials, were let to cool down in desiccators, and weighted in an analytical balance with a precision of 0.001g (Nakagawa, 1999). The relation between shoot and root dry weights was determined.

Based on germination data obtained in the laboratory, an experiment was conducted in the greenhouse, in which soil was conditioned in pots and seeds from the control, AIB treated (7, 14 and 28mg·kg^{-1}) and inoculated with *B. subtilis* treatments were chosen, based on their lab performance. As there were no differences between seeds treated or not with ethanol (Tables I and II) the latter was omitted. The pots, of 8 liters capacity, were filled with agricultural soil collected in the 0-20cm layer of a Distroferric Red Argisoil (a sandy loam soil). The soil was air dried and passed through a sieve with 2mm mesh. Soil samples were taken for characterizing chemical attributes and granulometry, with the following results: pH ($CaCl_2$ 1mol·l^{-1}) 5.1; organic matter 11g·dm^{-3} or 0,92%; P_{resin} 10mg·dm^{-3} or 10ppm; H+Al 17mmol$_c$·dm^{-3}; K 1,9mmol$_c$·dm^{-3}; Ca 18mmol$_c$·dm^{-3}; Mg 7mmol$_c$·dm^{-3}; SB (sum of bases) 27mmol$_c$·dm^{-3}; CEC (cation exchange capacity) 44 mmol$_c$·dm^{-3}; base saturation 62%; sand 740g·kg^{-1}; silt 80g·kg^{-1}; and clay 180g·kg^{-1}. Field capacity on non structured (sieved) soil was determined at -0.03MPa in the Richards extractor, and the value obtained was 165g·kg^{-1} of water. Dolomitic limestone was added to the sieved soil to elevate its base saturation to 70%. After liming, the soil was maintained in plastic bags for 20 days with moisture content close to field capacity. Four pots with 50 seeds per treatment were used.

Daily counts were made to calculate the maximum percentage of emergence and the emergence speed index (ESI; Nakagawa, 1999) in each treatment. At 18 days after sowing, plants were harvested, washed

TABLE III
ROOT LENGTH (RL), SHOOT (SDM), ROOT (RDM) AND TOTAL (TDM) DRY MASS AND ROOT/SHOOT RATE (R/S) OF BEAN SEEDS TREATED WITH DIFFERENT CONCENTRATIONS OF IBA (mg·kg^{-1}·seed) AND *B. subtilis* (10g·kg^{-1}·seed) AT SEVEN DAYS AFTER SOWING IN THE LAB

Treatment		RL (cm)	SDM (g)	RDM (g)	TDM (g)	R/S rate
B. subtilis		10.18 a	1.92 a	0.42 c	2.35 a	0.21 c
Indol-butyric acid	0	9.22 a	0.91 b	0.57 c	1.48 b	0.63 b
	1.75	7.99 b	0.91 b	0.52 c	1.42 b	0.58 b
	3.5	8.60 b	1.04 a	0.61 b	1.65 b	0.59 b
	7	7.62 b	0.91 b	0.56 c	1.47 b	0.62 b
	14	9.51 a	1.48 a	1.20 b	2.69 a	0.81 b
	28	7.14 b	1.15 a	1.47 a	2.62 a	1.32 a
Control		9.65 a	1.05 a	0.58 c	1.62 b	0.54 b

Means followed by the same letters are not significant by the Scott-Knott test.

in a sieve and split into canopy and root. They were dried at 60°C for 48h to obtain the dry matter of root, shoot and their ratio.

In the laboratory, the experimental design used was completely randomized with eight treatments and four replications. The greenhouse experiment was conducted in a randomized block design with four blocks and five treatments per block. The percentage data were transformed to arcsine $(X/100)^{1/2}$. The F test was applied for variance analysis; when this was significant, polynomial regression for levels of IBA (quantitative treatments) was used to analyze and determine significant equations with lower polynomial degree and higher determination coefficient (R^2). Means of all treatments (IBA concentrations and *Bacillus* use) were compared by Scott-Knott test ($p<0.05$), which is a method for grouping means, distinguishing results without ambiguity (Bhering *et al.*, 2008) as for example Tukey's test. The SISVAR software was used (Ferreira, 2008).

Results and Discussion

Analysis of variance of the first experiment (Table I) showed that germination, vigor classification, root length, shoot dry mass, root dry mass, total dry mass and root/shoot ratio were significant by the F test for both treatments, either for the qualitative and quantitative data (IBA), indicating the need to compare means in the first case and a regression analysis in the second.

Germination and vigor classification were higher in the seeds inoculated with *B. subtilis* and treated with the higher auxin concentrations (IBA) in the laboratory test (Table II). This was also observed in rice (Ashrafuzzaman *et al.*, 2009), chickpea (Khan and Patel, 2007, Mishra *et al.*, 2010), soybean (Naz *et al.*, 2009) and a perennial crop (*Pinus*; Kloepper *et al.*, 2004)

Treatments with *B. subtilis* and auxin did not increase root and shoot dry mass compared to control (Table III). Root dry mass was higher with the highest dose of IBA, and was not enhanced by inoculation with *B. subtilis*. However, seed inoculation produced higher seedling total mass, which was more influenced by the shoot than by the root mass (Table III). The root/shoot values were <1 for most of the treatments. The highest and unique value >1 (1.32) resulted from the treatment with auxin at 28mg·kg^{-1} seed. The lowest value (0.21) was observed in the treatment with *B. subtilis* (Table III). The root/shoot ratio indicates reserve allocation from the cotyledons to the different organs. In this case, auxin (IBA) induced the highest mass transfer to root development at shoot expense at the highest dose, while the bacteria induced greater mass allocation to shoot. According to Weber *et al.* (2000) plant growth provided by diazotrophic bacteria can be attributed mainly to the plant production of growth regulator substances; however, PGPR can change dry matter allocation, root morphology and biomass increase, enabling plants to better exploit soil volume and nutrient absorption (Malik *et al.*, 1997). Although the inoculation with *B. subtilis* in the lab, on paper substrate, promoted the bean seedling overall development, the ratio root/shoot did not indicate preferential allocation of biomass to root growth.

An analysis of auxin doses (IBA) indicated that seed germination and vigor classification responded to the increase in the concentration of hormone. Each 1mg·kg^{-1} increase in IBA concentration led to an increase of 0.87% and 0.78% in the germination and vigour classification (graphs not shown), according to the equations $Y=73.3+0.87x$ with a determination coefficient $R^2=0.7868$ for germination and $Y=63.8+0.78x$, R^2 of 0.7343, for vigour classification, both significant at 1% by the F Test, with the equation coefficients also significant at 1% by the same test. Root length did not respond to increase in the IBA doses tested (Figure 1a).

Shoot and total dry mass showed the maximum level (shoot maximum growth) in a concentration of 17.81mg·kg^{-1} (Figure 1b) and seedling total dry mass in 27.12mg kg^{-1} (Figure 1d). Root dry weight indicated that the increase of each 1mg in the growth regulator

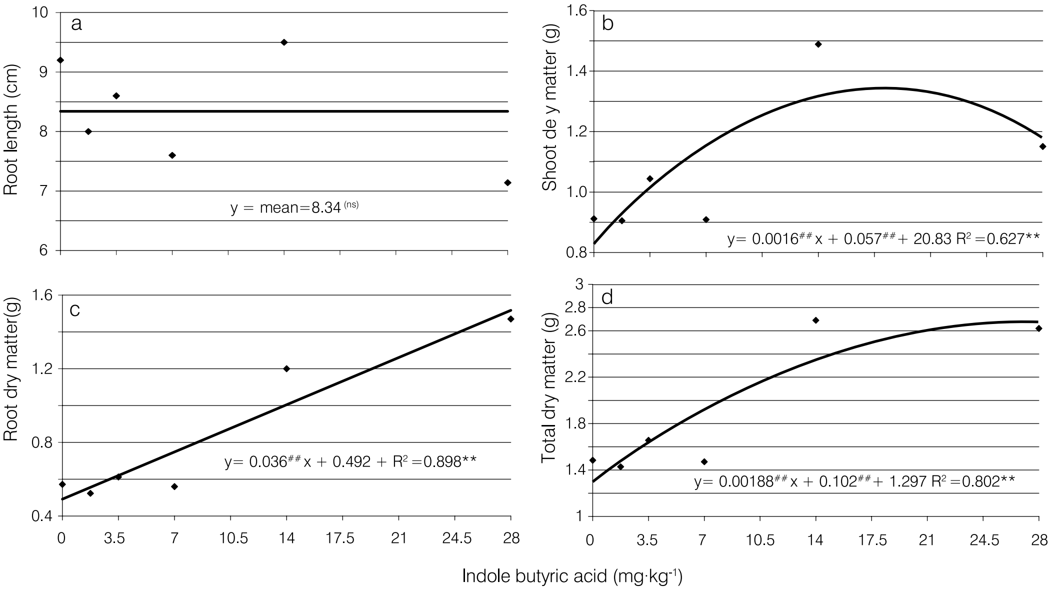

Figure 1. Root length (a), shoot dry mass (b), root dry mass (c) and total dry mass (d) of bean seeds treated with different concentrations of IBA at 7 days after sowing in the laboratory.
R^2: correlation coefficients of the equations, **: significant by the F test at 1%, $^{\#\#}$: significance of the coefficients of the regression equations for the F test at 1%, ns: not significant.

TABLE IV
SUMMARY OF ANALYSIS OF VARIANCE OF THE EMERGENCE (E) EMERGENCE SPEED INDEX (ESI), SHOOT DRY MASS (SDM), ROOT DRY MASS (RDM), TOTAL DRY MASS (TDM) AND ROOT / SHOOT (R/S) RATE OF BEAN SEEDS TREATED WITH DIFFERENT CONCENTRATIONS OF IBA AND *B. subtilis* AT SEVEN DAYS AFTER SOWING IN THE GREENHOUSE

	Mean square F Test for all treatments					
Variation source	E	ESI	SDM	RDM	TDM	R/S rate
Treatment	2352.70**	1087.77**	60.28**	94.864*	245.24*	0.600*
Block	2259.40*	1152.47**	66.28**	100.39*	299.23**	0.3402^{ns}
Residue	382.56	103.24	9.54	20.26	49.48	0.1505
VC(%)	26.29	22.10	29.30	20.35	22.42	28.42
	Mean square F Test for all treatments					
Treatment	1310.91*	749.43**	53.06**	113.80*	249.78*	0.7945*
Block	3019.58**	1301.34**	92.92**	97.30*	357.01**	0.2795^{ns}
Residue	249.13	70.18	3.14	21.36	36.76	0.1991
VC(%)	24.60	21.58	18.59	24.15	20.32	23.49

*, ** significant at 5% and 1% by the F test, respectively. VC: variance coefficient.

TABLE V
SEEDLING EMERGENCE (E) AND EMERGENCE SPEED INDEX (ESI) OF BEANS TREATED WITH DIFFERENT CONCENTRATIONS OF INDOL-BUTYRIC ACID (mg·kg^{-1} seed) AND *Bacillus subtilis* (10g·kg^{-1} seed) AFTER 18 DAYS OF SOWING

Treatment		E (%)	ESI
B. subtilis		87 a	52.15 a
Indol-butyric acid	7	49 b	24.18 b
	14	43 b	28.27 b
	28	68 a	43.45 a
Control		24 b	10.19 c

Means followed by the same letters are not significant by Scott-Knott Test at 5%.

concentration led to an increase of 0.036g root dry magnitude (Figure 1c). In the root/shoot analysis (Figure 2), the linear regression showed that each 1mg increase in IBA, under laboratory conditions, led to an increase of 0.026 in the R/S, indicating that the IBA is an auxin acting in the rooting (Castro and Alvarenga, 2001).

Analysis of variance of the greenhouse experiment (Table IV) indicated that the variables emergence, emergence speed index, shoot dry mass, root dry mass, total dry matter and root/shoot ratio were significant accordin to the F test for both treatments for the quantitative and qualitative data (IBA), indicating the need for comparison of means in the first case and in the second a regression analysis.

In greenhouse conditions, treatment with *B. subtilis* showed similar results to the highest concentration of IBA on the emergence speed index and emergence, superior to other treatments (Table V). The emergence of small plants in the control treatment may have been due to limiting factors in the soil, such as the presence of pathogens, since the seeds had not been previously treated with fungicides. Treatments with some organisms may protect the plants by synthesizing compounds that are beneficial to the seedling either by turning the seedling stronger or by secreting molecules that may have adverse effects on the pathogens.

In relation to the shoot dry mass, treatment with *B. subtilis* and IBA at concentrations of 14 and 28mg·kg^{-1} had similar results, superior to the control. In the evaluation of root dry mass, treatment with the bacterium and the IBA, at 7 and 14mg·kg^{-1} proved to be superior to other treatments. In the plant total dry mass, the control showed lower performance (Table VI). As for the root/shoot ratio, treatment with *B. subtilis* promoted a more balanced relationship with the nearest one, indicating proportionality in root growth and shoot. This result differs from that obtained in the laboratory test, in which the biological treatment produced the lowest root/shoot ratio among the treatments (0.21). This could be because the *B. subtillis* in the soil may have to counterattack other microorganisms, producing less auxin or having it diluted in the soil, which also decreases the amount of auxin instead of promoting root growth. *B. subtillis* also produces antibiotics what could improve the resistance of the seedlings to soil born pathogens (Araujo *et al.*, 2005).

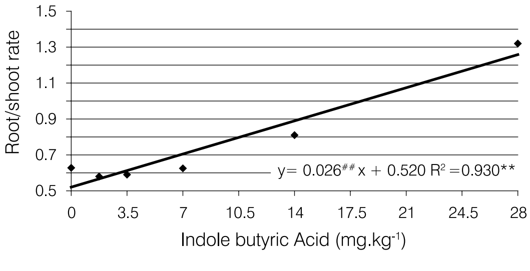

Figure 2. Root/shoot ratio of bean seeds with different concentration of IBA (mg·kg^{-1} seed) at 8 days after sowing in pot. R^2: correlation coefficient, **: significant by the F test at 1%, $^{\#\#}$: significance of the coefficients of the regression equations for the F test at 1%, ns: not significant.

TABLE VI
SHOOT DRY MASS (SDM), ROOT DRY MASS (RDM), TOTAL DRY MASS (TDM) AND ROOT/SHOOT RATIO (R/S) OF BEAN SEEDLINGS TREATED WITH DIFFERENT CONCENTRATIONS OF IBA (mg·kg^{-1} seed) AND *B. subtilis* (10·g kg^{-1} seed) AT 18 DAYS AFTER SOWING IN POTS

Treatment		SDM (g)	RDM (g)	TDM (g)	R/S
B. subtilis		14.59 a	13.91 a	28.51 a	0.95 b
	7	8.99 b	14.29 a	23.29 a	1.44 a
Indol-butyric acid	14	11.59 a	15.41 a	27.00 a	1.38 a
	28	12.90 a	8.06 b	20.96 a	0.68 b
Control		4.64 b	4.10 b	8.75 b	0.68 b

Means followed by same letters are not statistically different by the Scott-Knott test at 5%.

The regression studies for the concentration of IBA applied to the seeds in the greenhouse test showed that IBA increased the emergence and the emergence speed index of the beans linearly (graphs not shown). For each 1mg·kg^{-1} increase in the concentration of the growth regulator there was an increase of 1.39% in the emergence of beans, according to the equation $Y= 28.6 +1.39x$, with coefficient of determination $R^2= 0.8423$, and 1.12 at emergence speed index, according to the equation $Y= 12.75 +1.24x$, $R^2= 0.9649$, being the equations and coefficients significant at 1% by F test.

The IBA treatment induced greater growth of shoots up to a concentration of 24.12mg·kg^{-1} seed, calculated as the maximum (Figure 3a). Root growth occurred up to a maximum at the concentration of 15.37mg·kg^{-1} seed (Figure 3b). The concentration of 17.19mg·kg^{-1} seed of IBA solution was calculated as the maximum for the production

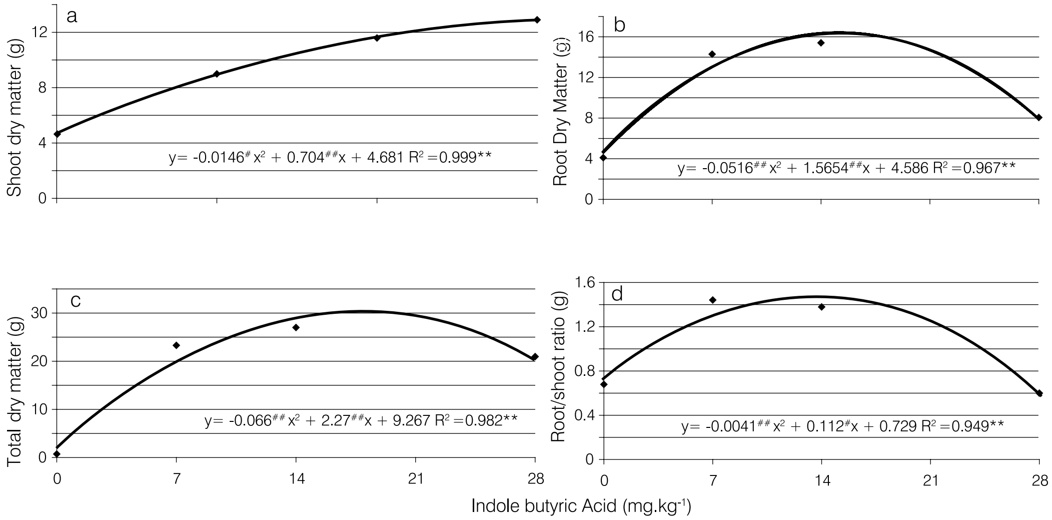

Figure 3. Shoot dry matter (a), root dry matter (b), total dry matter (c) and root/shoot ratio (d) of bean seeds with different concentration of IBA (mg kg^{-1} seed) at 8 days after sowing in pot. R^2: equation determination coefficient; *, **: significant by F test (p0.05 and 0.01, respectively); $^{\#}$, $^{\#\#}$ equation coefficient significance regression by F test ($p<0.05$ and 0.01, respectively).

of total dry mass (Figure 3c). However, the ratio root/shoot reached a maximum with 13.67mg of auxin per kg^{-1} of seed (Figure 3d).

The effects of plant growth regulators on beans have been demonstrated to be positive for plant development (Vieira and Castro, 2001). In this paper it was noted that seed treatment with synthetic auxin in larger doses provided an increase of germination and plant development in the laboratory and greenhouse. It was also observed that different doses of auxin employed in the treatment modified the root/shoot ratio. This effect is clearer in the quadratic fitting found with the increasing doses of the growth regulator (Figure 3d). It is well known that the effects of auxin in root development can change from negative to positive with increasing doses of growth regulator (Taiz and Zeiger, 2004).

The seed treatment with *B. subtilis* led to an increase in germination and plant development. This result may be associated with the indirect beneficial effect of rhizobacteria that, besides the direct promotion of growth, also have control effects on plant pathogens (Araújo *et al.*, 2005). The rhizobacteria performance on beans development confirmed what was found by Araújo (2008), who observed gains in developing soybean, corn and cotton when the same bacterial strain was seed innoculated. Lazzaretti and Melo (2005) working with inoculation of *B. subtilis* in beans, also concluded that the use of rhizobacteria is a promising technique to increase root nodulation and to promote growth of bean plants.

The production of auxin by the same strain of *B. subtilis* used in this work was proved in the lab, in a study with soybean seeds (Araujo *et al.*, 2005). On the other hand, a survey of PGPR isolated from wheat rhizosphere showed that the amount of indole compounds released by the rhizobacteria in the culture medium under aseptic conditions ranged from 1.8 to 24.8mg·l^{-1} (Khalid *et al.*, 2004). Performance of *B. subtilis* in this paper was similar to that found by seed treatment with higher doses of plant growth regulator in the shoot dry mass production, emergence and emergence speed index, which could likely prove the production of regulatory growth substances in the interaction with the beans, and in pot conditions the results were more promising than those observed in the laboratory. Probably, in contact with the soil, the bacteria found better conditions for growth and their interaction with bean roots was stronger than in the laboratory, where paper was used as substrate for germination.

Summarizing, *B. subtilis* was beneficial for crop establishment (emergence and seedling vigor) and also provided increases in plant growth comparable to the higher levels of IBA in this phase, which also influenced the bean germination and proved to be efficient both for shoot and root growth. At higher doses auxin increased, in the lab, shoot growth at the expense of the root system, and lower doses in the field promoted root growth in shoot detriment. The benefits derived from the interaction between bean and *B. subtillis* can be extended for the whole cycle of the plant. On the other hand, IBA effects were ephemeral and could be observed only when the substance was still present in the seed or seedling.

Conclusion

Bacillus subtilis should be used as bean inoculant as it promotes a better growth in the early plant stages.

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