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The use of various strains of *Rhizobium tropici* for inoculation of snap bean cultivars with a determinate growth pattern

Inoculação com estirpes de *Rhizobium tropici* em genótipos de feijão-vagem de crescimento determinado

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Abstract

The snap bean (*Phaseolus vulgaris* L.) is an economically important vegetable in Brazil. Bean plants can establish symbiotic associations with bacteria that fix atmospheric nitrogen. These associations show specificity of host plants to certain bacteria. Nitrogen fertilization constitutes a crop production cost, and the use of *Rhizobium* inoculation to supply nitrogen to crops may decrease the production costs. The aim of this study was to evaluate the effects of inoculation of three different snap bean cultivars with three *Rhizobium tropici* strains on agronomical performance of the plant. A completely randomized experimental design was used, with a 3 × 5 factorial scheme and four replicates per treatment. The factors tested were three snap bean cultivars (UEL 1, Alessa, and UEL 2), and five types of inoculation (uninoculated control, inoculation with SEMIA 4077, SEMIA 4080, SEMIA 4088, or with a mix of the three strains). The experiment was conducted in a greenhouse. Plant height, stem diameter, pod length, the number of pods per plant, and average weight and yield of commercial-grade pods, were determined. An analysis of variance was conducted using the F test, followed by Tukey's test ($p < 0.05$). Inoculation had a positive effect on all the parameters quantified. The beneficial effect was more pronounced in plants inoculated with the mix of *Rhizobium* strains.
Key words: Inoculant. Nitrogen. *Phaseolus vulgaris* L.

Resumo

O feijão-vagem (*Phaseolus vulgaris* L.) é uma hortaliça destaque no mercado nacional. O feijoeiro tem a habilidade de associar-se simbioticamente com bactérias responsáveis pela fixação biológica do nitrogênio, sendo observada neste processo a especificidade entre genótipo e bactéria. A adubação nitrogenada constitui parte dos custos de produção, os quais podem ser reduzidos pelo uso da inoculação para fornecimento de N à cultura. O objetivo desse trabalho foi avaliar o desempenho agronômico de três genótipos de feijão-vagem inoculados com três estirpes de *Rhizobium tropici.* O delineamento experimental foi inteiramente casualizado com quatro repetições, conduzido em casa de vegetação, resultando em fatorial 3x5: três genótipos (UEL 1, Alessa e UEL 2) e cinco condições de inoculação (testemunha sem inoculação, SEMIA 4077, SEMIA 4080, SEMIA 4088 e a mistura dos três isolados). Foram avaliados altura da planta, diâmetro do colmo, comprimento de vagens, número de vagens por

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planta, peso médio de vagens comerciais e produtividade. Os resultados foram submetidos à análise de variância pelo teste F ($p < 0,05$) e as médias comparadas pelo teste de Tukey ($p < 0,05$). Todas as variáveis responderam positivamente à inoculação, sendo que a mistura de estirpes mostrou-se mais promissora.

Palavras-chave: Inoculantes. Nitrogênio. *Phaseolus vulgaris* L.

Introduction

The snap bean, *Phaseolus vulgaris* L., is a leguminous plant originating from Central America (PINTO et al., 2007). It is one of the most consumed plants in Brazil, and its average production for the 2010 harvest was approximately 57 thousand tons (CEASA, 2010).

Because it is consumed without processing, its marketing standards include both morphological and physiological pod quality. In order to meet these standards, the plants must be supplied with adequate amounts of nutrients during cultivation because a deficient nutrient supply disrupts metabolic pathways, with detrimental effects on the plant (FAGERIA et al., 1996). Nitrogen (N) is the nutrient required in greater amounts by plants (GALLO; MIYASAKA, 1961) and should be supplied in adequate quantity, especially during flowering and pod formation and filling stages (ARF et al., 1999).

The appropriate management of N provision to plants is therefore a challenge for the bean industry because low N availability limits bean production, but excess N supply exceedingly increases production costs, in addition to posing environmental risks due to nitrate leaching and surface N runoff (SANTOS et al., 2003). Gaseous N losses due to denitrification and volatilization should also be considered (SIQUEIRA et al., 1994).

Bean plants form symbiotic associations with bacteria from the genus *Rhizobium*, which fix atmospheric N in root nodules. In some cases, this association may have low efficiency, insufficient to meet the host plant's N demand (GUARESCHI; PERIN, 2009). Nonetheless, depending on the growth conditions, the above approach can partially or completely replace N fertilization, thereby decreasing production costs (GRANGE et al., 2007).

Symbiotic N fixation in bean plants can be optimized through selection of more efficient *Rhizobium* strains, adapted to different regions and bean cultivars. Several factors affect the efficiency of the association, namely the ability of the inoculated *Rhizobium* strains to colonize the host plant; this phenomenon is due to symbiotic specificity and varies with the strain and the host plant (SMITH; GOODMAN, 1999).

Root colonization by a particular inoculated *Rhizobium* strain probably involves several phases of nodule formation, which can result in competition between different strains for nodule occupation and promote nodule development (SESSITSCH et al., 2002).

The aim of the present study was to evaluate the effects of inoculation of seeds of three snap bean cultivars (with a determinate growth pattern) with different strains of *Rhizobium tropici.* These effects were assessed by means of phytometric and plant yield parameters.

Materials and Methods

The experiment was performed in a greenhouse in the Department of Agronomy of the Agrarian Sciences Research Center of the *Universidade Estadual de Londrina* (UEL), located at 23°19′42″S, 51°12′11″W and altitude 594 m.

Soil samples were collected from the superficial soil layer (0-20 cm) of a Red Latosol, in the experimental area of the School Farm of UEL, and were placed in 4-L plastic pots. Before sowing, the soil samples were sifted (pore diameter 4.0 mm), and chemical analysis of the soil was performed, in order to determine soil fertility (PAVAN et al., 1992). The soil had the following characteristics: pH 5.6 (resulting from $CaCl_2$); 23.2 g kg^{-1} organic matter, 0.35 mg dm^{-3} P; 896 mg dm^{-3} K; 6.2 cmloc dm^{-3} Ca; 2.2 cmloc dm^{-3} Mg; 4.85 cmloc dm^{-3} H + Al; CTC: 15.5 cmloc dm^{-3}; V: 68.8%.

The soil was corrected on the basis of the chemical analysis, with application of urea at 15 kg ha^{-1} (corresponding to a half dose of N), 70 kg ha^{-1} P_2O_5, and 40 kg ha^{-1} K_2O (OLIVEIRA, 2003). A nutrient solution was applied in order to avoid nutrient limitation, according to Novais et al. (1991).

Throughout the experiment, the soil water content in the pots was maintained at 70% of field capacity by daily replacement of the water lost through evapotranspiration.

Seeds of snap bean cultivars UEL-1, UEL-2, and Alessa, with a determinate growth pattern, were used. Inocula of *Rhizobium* strains SEMIA 4077, SEMIA 4080, and SEMIA 4088, at 10^5 cells mL^{-1}, were produced in the laboratory. The strain mix was prepared by mixing 5 mL of each strain. Inoculation was performed at sowing, by applying 5 mL of the inoculum of a pure culture or strain mix. Three seeds were sown per pot, and thinned to one seedling per pot 15 days after seedling emergence.

The following parameters were determined: plant height (H), stem diameter (SD), shoot dry weight (SDW), the number of commercial-grade pods (NCP), length of commercial-grade pods (LCP), average pod weight (APW), and pod yield (Y). The H value was measured at the base of the plant up to the insertion of the last trifoliate leaf in the main stem. NCP was quantified by counting all pods longer than 10 cm. The pod yield was calculated using the equation Y(x) = Number of plants * NCP * APW[per 1000] ÷ 1000, and expressed in tons (FANCELLI; DOURADO NETO, 2007).

A completely randomized experimental design was used, with a 3 × 5 factorial scheme and four replicates per treatment. The tested factors were three snap bean cultivars (1: UEL 1, 2: Alessa, and 3: UEL 2) and five types of inoculation (1: uninoculated control, 2: strain SEMIA 4077, 3: strain SEMIA 4080, 4: strain SEMIA 4088, 5: strain mix).

An analysis of variance was conducted using the F test, followed by Tukey's test, at $p \leq 0.05$.

Results and Discussion

No significant interactions between the bean cultivar and inoculation were observed for SDW and LCP, but there was a significant of isolated effect on both parameters (Table 1). The significant effect of the bean cultivar was due to genetic differences among the cultivars. Nonetheless, the responses to the different types of inoculation were independent of the host plant genotype.

Table 1. Simplified analysis of variance for the parameters under study.

Variation sources	Mean Square						
	H[1]	SD[2]	SDW[3]	NCP[4]	LCP[5]	APW[6]	Y[7]
Cultivar (G)	158.04**	0.05**	2.93**	13.52**	1.13*	0.98**	4.85**
Inoculation (I)	123.04**	0.21**	6.39**	96.92**	24.43**	0.70**	61.10**
G × I	5.71**	0.01*	0.15^{ns}	5.33*	0.64^{ns}	0.45**	2.70**
C.V. (%)	4.24	6.73	9.20	12.64	4.47	5.28	11.36

*Significant at $p \leq 0.05$, **significant at $p \leq 0.01$, and ^{ns} non significant, according to the F test.
[1]H: plant height; [2]SD: stem diameter; [3]SDW: shoot dry weight; [4]NCP: the number of commercial-grade pods; [5]LCP: length of commercial-grade pods; [6]APW: average pod weight; and [7]Y: pod yield.

The inoculated plants yielded higher SDW than control plants did, regardless of whether the bacterial strains were inoculated alone or as a mix. This result was likely due to a higher N concentration in the plants, resulting in increased production of photoassimilates and in plant growth (LONG et al., 2006). Higher production of plant mass in inoculated plants has been reported previously (GUEDES et al., 2010; SOARES et al., 2006).

The inoculated plants yielded greater LCP than the control plants (Table 1), thus producing commercial-grade pods (longer than 10 cm). This finding is in agreement with the data of Nóbrega et al. (2010) and confirms a positive effect of *Rhizobium* inoculation on the host plants.

Inoculation had a positive effect on H, with inoculated plants showing higher H than uninoculated controls did (Table 2). This is because the symbiotic association can results in increased photosynthetic rates and root activity, increasing nodulation; the observed phenomenon is in line with the data from Gualter et al. (2008), who observed increased higher plant size after colonization.

Table 2. Plant height (H) and stem diameter (SD) in plants of different snap bean cultivars, inoculated with different strains of *Rhizobium tropici*.

Cultivar	Rhizobium strain				
	Control	SEMIA 4077	SEMIA 4080	SEMIA 4088	Mix
			Plant height (cm)		
UEL 1	26.60 Ad	32.40 Abc	30.43 Ac	32.95 Ab	37.18 Aa
Alessa	23.68 Bc	27.68 Bb	26.68 Bb	26.65 Bb	30.20 Ca
UEL 2	22.83 Bc	26.25 Bb	27.68 Bb	26.00 Bb	32.80 Ba
			Stem diameter (cm)		
UEL 1	0.85 Ac	1.03 Ab	0.99 Abc	1.09 Ab	1.35 Aa
Alessa	0.88 Ac	1.04 Ab	1.04 Ab	1.01 Abc	1.21 Ba
UEL 2	0.80 Ab	0.98 Aa	0.96 Aa	1.01 Aa	1.09 Ca

Averages followed by different lowercase letters within the same row, and uppercase letters within the same column, were significantly different according to Tukey's test, at $p \leq 0.05$.

Inoculation resulted in increased H and SD. These effects were more pronounced for cultivar UEL 1, and for inoculation with the strain mix, and to a lesser extent for strain SEMIA 4088 (Table 2). The possible reason is different colonization rates, which vary among the different *Rhizobium*-host plant combinations, indicating symbiotic specificity (PAFFETTI et al., 1998). The wider the host range, the lower the specificity of a given *Rhizobium* isolate (BORGES et al., 2007).

According to the above-mentioned parameters, plants inoculated with the three tested *Rhizobium* strains showed better agronomical properties than the uninoculated controls, independent of the plant cultivar (Table 3).

Optimization of N fixation depends on increased nodule activity and may explain the effects on the parameters measured. The host plant supplies carbohydrates originating from photosynthesis to the rhizobial bacteria, and in exchange receives N fixed by the bacteria (STRALIOTTO, 2002). This situation results in higher N uptake by the host plant.

Overall, cultivar Alessa yielded the highest NCP, regardless of treatment. Treatments 4 and 5 had similar effects on NCP for all plant cultivars. This result is consistent with the findings of Barros et al. (2013), who observed a greater number of pods per plant among inoculated plants.

Table 3. The number of commercial-grade pods (NCP), pod average weight (APW), and pod yield (Y) in plants of different snap bean cultivars, inoculated with different strains of *Rhizobium tropici*.

Cultivar	Rhizobium strain				
	Control	SEMIA 4077	SEMIA 4080	SEMIA 4088	Mix
	Number of commercial grade pods				
UEL 1	8.25 Ac	10.00 Bbc	11.50 Bb	11.50 Ab	15.50 Aa
Alessa	7.00 Ac	13.50 Ab	14.25 Aab	11.75 Ab	16.50 Aa
UEL 2	7.00 Ac	10.25 Bb	11.50 Bab	12.50 Aab	14.00 Aa
	Average pod weight (g)				
UEL 1	4.78 Aab	5.27 Aa	4.88 Bab	4.63 Bb	4.87 Bab
Alessa	4.53 Ab	5.15 Aa	4.78 Bab	5.21 Aa	4.98 Bab
UEL 2	4.59 Ac	5.15 Ab	5.84 Aa	5.10 Abc	5.74 Aa
	Pod yield (t ha^{-1})				
UEL 1	5.51 Ac	7.37 Bbc	7.84 Bb	7.44 Ab	10.52 Aa
Alessa	4.36 Ac	9.72 Aab	9.50 Ab	8.54 Ab	11.47 Aa
UEL 2	4.47 Ad	7.42 Bc	9.40 ABab	8.93 Abc	11.20 Aa

Averages followed by different lowercase letters within the same row, and uppercase letters within the same column, were significantly different according to Tukey's test, at $p \leq 0.05$.

The fact that no statistically significant differences in NCP were observed among the plants inoculated with some of the isolated strains and the strain mix or the control treatment (Table 3) indicates symbiotic specificity. This was the case for UEL 1 with treatments 1 and 2, for which no significant increase in NCP was observed when compared to control plants. Bassan et al. (2001) did not observe higher NCP in inoculated plants either.

APW was significantly higher for inoculated plats, except for UEL 1, for which no significant differences were observed among most of the treatments tested (Table 3). For the other cultivars tested, inoculation with the strain mix had a more pronounced positive effect on APW than the remaining treatments did, as was reported elsewhere about the parameters discussed above (XAVIER et al., 2006). This phenomenon was likely due to a higher N fixation activity because of the joint action of the three *Rhizobium* strains.

The remaining variations observed resulted from the already discussed symbiotic specificity. Almeida et al. (2010) also reported a positive effect of inoculation.

The highest pod yield was observed for treatment 5. Nonetheless, pod yield was higher for all inoculation treatments than for the control treatment (Table 3). Pod yield was higher for cultivar Alessa because, although some plants had lower pod weight than UEL 2, they produced a greater number of pods.

Yadegari et al. (2010) observed a significant effect of *Rhizobium* inoculation combined with plant growth-promoting bacteria in bean plants, resulting in higher yield parameters, namely NCP and yield. Higher plant yields resulting from *Rhizobium* inoculation have also been reported in other studies (PERES et al., 1994; ROMANINI JÚNIOR et al., 2007; GITTI et al., 2012). All the parameters measured manifested the same behavior in response to inoculation, confirming beneficial effects of the *Rhizobium* inoculation.

Conclusion

Inoculation had a beneficial effect on bean plants and can replace, partly, N fertilization with chemical fertilizers. All the bean cultivars tested showed positive responses to *Rhizobium* inoculation. The tested *Rhizobium* strains increased the yield parameters of the bean plant, especially when combined in a mix, which had more pronounced positive effects than did the bacterial strains applied alone.

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