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Co-inoculation in common beans can substitute mineral fertilization

INTRODUCTION

Due to its high protein content, common beans (*Phaseolus vulgaris*) is a staple food in Brazil and in many other developing countries. One of the constraints for high bean yields is Nitrogen (N) mineral nutrition, which is expensive due to the huge amounts of fossil energy needed for its production. The use of improved microorganisms capable of doing the Biological Nitrogen Fixation (BNF) and freely fixing N from the air is an alternative for poor and developing countries to produce beans at a much lower financial and environmental costs.

The N fixing process is performed by the symbiosis between the bacterium *Rhizobium* and the bean plant, in root nodules where the bacteria find shelter and energy in exchange to the N they fix from the atmospheric N_2, through the enzyme nitrogenase, which reduces N_2 to Ammonium, which is used by the plant to build proteins and other compounds.

For decades elite *Rhizobium* strains have being selected to supply the entire N needed by plants to produce high yields (Hungria et al., 2006). On other way, the bacteria of the genus *Azospirillum* act synthesizing growth promoters (fitohormones), which favors root nodulation and BNF by R*hizobium*. The co-inoculation of both bacteria in soybean can produce early and abundant nodulation resulting in higher yields (Hungria et al., 2015).

The legume plant photosynthetic ability is important to supply the needed energy to keep the effectiveness of the relationship between the plant and the symbiotic microorganisms in the BNF process. Root nodules are decayed by the reduction of photosynthetic efficiency (Bano and Iqbal, 2016).

The aim of this research work was to test co-inoculation of *Bradyrhizobium* and *Azospirillum* in common beans (*P. vulgaris*) in an acid soil (pH 4.2), to confirm their ability to substitute mineral N fertilization in this staple food cropping system.

MATERIAL AND METHODS

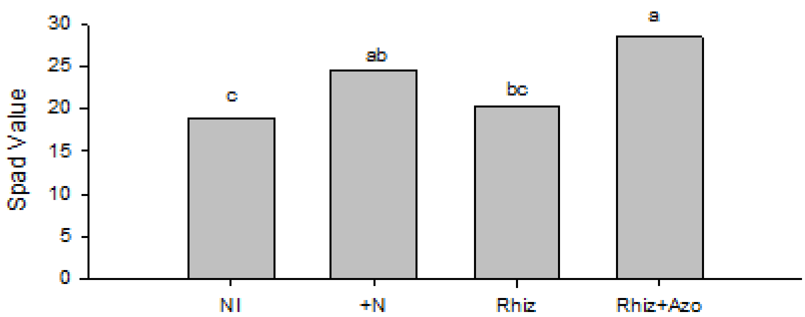
An experiment was set in 20 trays containing 45 liters of soil (pH 4.2), in a greenhouse. Treatments were 1) NI (non-inoculated or control); 2) +N (2 g per tray of mineral N as urea); 3) *Rhiz* (inoculated with *Rhizobium tropici*); *Rhiz+Azo* (inoculated with *Rhizobium tropici* and *Azospirillum brasilense*). The treatments were displayed in a Randomized Complete Block Design, with 5 blocks (reps). Each tray was sowed with 20 seeds on May 30, and one week after germination the bean plantlets were thinned to 12 plants distributed in two lines, which were left growing. In mid cycle (Jul. 4th) 6 bean plants (one line) were collected to assess shoot and root dry matter, nodulation (mass and number), and 6 plants were kept developing until maturity for harvesting the grains.

The mid cycle parameters measured were Shoot Dry Weight (g/6 plants); Root Dry Weight (g/6 plants); Number of Nodules (modules/6 plants); and Mass of Nodules (g/6 plants). At maturity the plants were harvested and Yield (g/6 plants) was assessed.

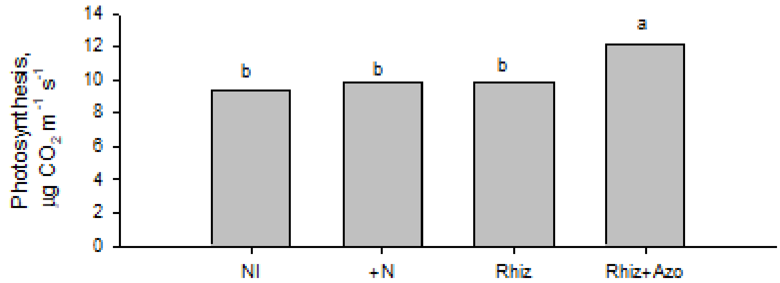
In the experiment during the plants cycle we also measured two parameters, which indicate the quality of the photosynthetic effectiveness of the bean plants. Since the greenness of the leaves are due to the chlorophyll content in them, by measuring the degree of greenness it is possible to have an idea how well the plants are being photosynthesizing (Al-Barzinji et al., 2016). The second parameter we measure was a more direct assessment of the uptake of CO_2 by the leaves (in μg CO_2.m^{-1}.s^{-1}) with a portable photosynthesis apparatus (LI-COR 6400). Both measurements were made at four dates (03 Jul.; 09 Jul.; 17 Jul.; 31 Jul. 2018), during the development of the bean plants, and the data presented are the average of these for sampling dates.

RESULTS AND DISCUSSION

The leaf greenness (SPAD values) in the treatment *Rhiz+Azo* was higher than in the *Rhiz* and NI treatments, but equivalent to the +N treatment (Figure 1A). This means that the N fixed in the co-inoculation treatment was equivalent to the provided by the mineral N fertilizer for plants to build chlorophyll. Regarding photosynthesis, the *Rhiz+Azo* treatment presented significantly higher values of CO_2 uptake than all the other treatments (Figure 1B). These results show that inoculation with *Rhiz+Azo* was the best treatment promoting higher photosynthetic rates than the other treatments.



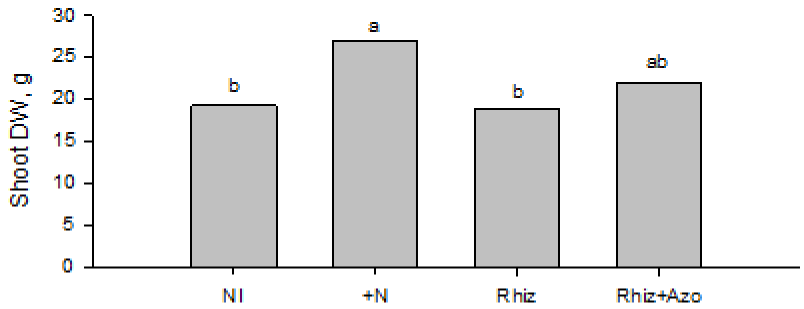
(A)



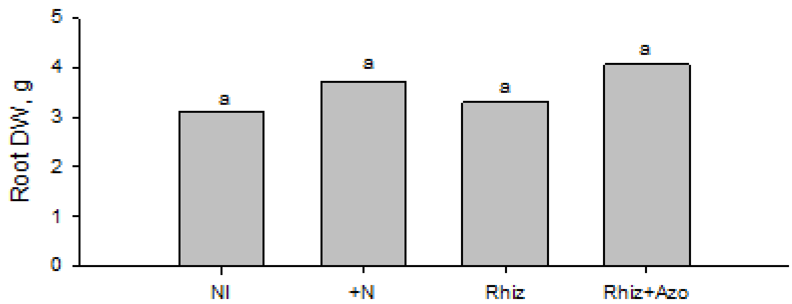
(B)

Figure 1. SPAD values (A) and photosynthesis (μg CO^2. m^{-1}.s^{-1}) (B) measured in leaves of bean (*Phaseolus vulgaris*) submitted to four treatments (NI, non-inoculated; +N, mineral nitrogen fertilization; *Rhiz*, inoculated with *Rhizobium*; and *Rhiz+Azo*, inoculated with *Rhizobium* and *Azospirillum*). Means represented by columns with different letters are significantly different from each other (Duncan, $p \leq 0.05$). Londrina, PR, Brazil, 2018.

Resembling the SPAD data, plant growth was equally responsive to mineral fertilizer and co-inoculation with *Rhiz+Azo*, especially for shoots (Figure 2A). These results, emphasizes the early response to co-inoculation compared with the single inoculation with *Rhizobium*. Stimulation to nodulation promoted by co-inoculation of rhizobia and *Azospirillum* have been demonstrated (Hungria et al., 2013, 2015). Evaluations of mid season may represent the yield potential of the crop and allow checking the effects of treatments that were applied at the beginning of the crop cycle, like fertilizers and inoculation. Root dry weight, on the other hand, did not differ significantly among the treatments (Figure 2B).



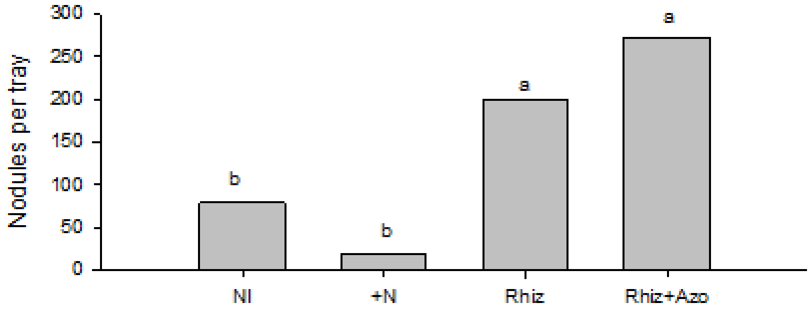
(A)



(B)

Figure 2. Shoot (A) and root dry weights (g/6 plants) (B measured in bean (*Phaseolus vulgaris*) submitted to four treatments (NI, non-inoculated; +N, mineral nitrogen fertilization; *Rhiz*, inoculated with *Rhizobium*; and *Rhiz+Azo*, inoculated with *Rhizobium* and *Azospirillum*). Means represented by columns with different letters are significantly different from each other (Duncan, $p \leq 0.05$). Londrina, PR, Brazil, 2018.

Nodulation, i.e., number of nodules (Figure 3A) and mass of nodules (Figure 3B) showed the most stunning effect of treatments. As observed, nodulation in the non-inoculated treatment was notoriously low and was further decreased in the treatment that received mineral N fertilizer. The low number of nodules in the non-inoculated treatment is a consequence of the low population of Rhizobia in the soil and strengthens the needs for using inoculants at the sowing. Despite not significantly different from the non-inoculated treatment, the addition of mineral N decreased the nodulation still further. This is a typical plant reaction, which suppresses the nodulation when there is enough mineral N in the surrounding soil. On the other hand, the single inoculation with *Rhizobium* increased the number of nodules, which an even bigger increase in the plants that received *Rhizobium* and *Azospirillum* in co-inoculation. The further increase in nodulation due to co-inoculation have been reported (Hungria et al., 2013, 2015) and is a consequence of a plant physiologically more active. As the BNF process is a high energy demanding process, plants with higher photosynthetic capacity (Figure 1B), can support the biological process more effectively.



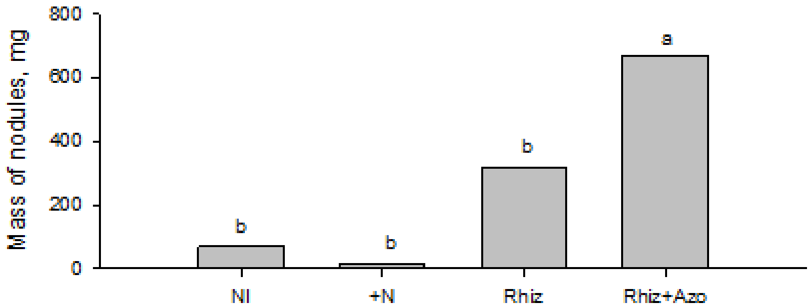


Figure 3. Number (A) and Mass of nodules (B) per tray (g/6 plants) measured in bean (*Phaseolus vulgaris*) submitted to four treatments (NI, non-inoculated; +N, mineral nitrogen fertilization; *Rhiz*, inoculated with *Rhizobium*; and *Rhiz+Azo*, inoculated with *Rhizobium* and *Azospirillum*). Means represented by columns with different letters are significantly different from each other (Duncan, $p \leq 0.05$). Londrina, PR, Brazil, 2018.

The grain yield of the co-inoculated plants was similar to the yield of the mineral N-supplied plants. This means that the use of co-inoculation, even under harsh soil conditions (e.g., low fertility, low pH) can increase the plant performance and result in yield levels similar to the ones obtained by supplying mineral N to the crop (Figure 4). This is extremely important in underdeveloped areas where the access to the chemical fertilizers is limited. Besides being cheaper, the biological N supply stimulated by the co-inoculation is environmentally friendly. In contrast, mineral N in the soil is usually exposed to losses from the cropping system, either by leaching to the groundwater or as greenhouse gasses, resulting in environmental problems.

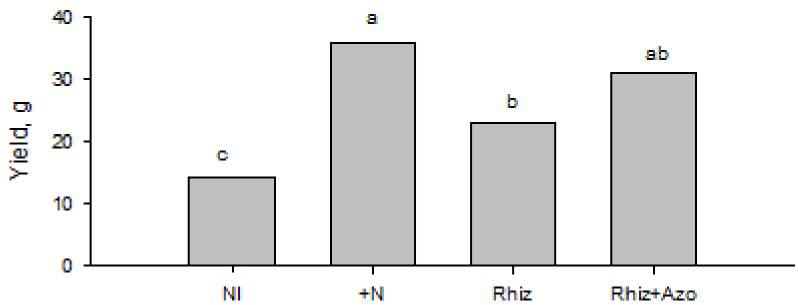


Figure 4. Yield per tray (g/6 plants) assessed at maturity (harvesting) in bean (*Phaseolus vulgaris*) submitted to four treatments (NI, non-inoculated; +N, mineral nitrogen fertilization; *Rhiz*, inoculated

with *Rhizobium*; and *Rhiz+Azo*, inoculated with *Rhizobium* and *Azospirillum*). Means represented by columns with different letters are significantly different from each other (Duncan, $p \leq 0.05$). Londrina, PR, Brazil, 2018.

CONCLUSION

For being a much less expensive and more environmentally friendly alternative, the biological N fixation can help farmers to increase common bean production spending much less money and preserving the environment by substituting the mineral N fertilization by inoculating the bean seeds with Rhizobium + Azospirillum, contributing to the world food security efforts.

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