ABSTRACT

The increase in productivity in soybean cultivation has been achieved by many Brazilian producers, due to the technological advances related to soil management, mineral nutrition, pest and disease control, genetic improvement, and the use of beneficial microorganisms via inoculation and co-inoculation. However, the application of inoculants based on Bradyrhizobium japonicum and Azospirillum brasilense still needs to be better studied among the many soybean cultivars available on the market. Therefore, the objective of this study is to evaluate the production increase in three soybean cultivars, through the application of synthetic hormones, inoculant and co-inoculant. The experimental design was a randomized complete block design with four repetitions, in a 3x6 factorial.
factorial scheme, with three soybean cultivars (Desafio 8473, TMG 1180 and CD 2737) and six seed treatments (Control, Inoculation, Hormonal, Co-inoculation, Co-inoculation + Hormonal and Inoculation + Hormonal). The treatments with the diazotrophic bacteria (Inoculation + Co-inoculation) and Hormonal did not influence the yields of cultivars 'CD 2737' and 'Desafio 8473', which can only be cultivated with the standard seed treatment of the region. The 'TMG 1180' cultivar has it's yield increased when it's seeds are inoculated with Hormonal seed treatments and with diazotrophic bacteria in Co-inoculation.

**Keywords:** Biological fixation; bacteria fixing; seed treatment.

## 1. INTRODUCTION

In the last decades, the area planted with soy has increased significantly in Brazil. Focusing on efficient management and use of technologies, aiming to reduce costs and increase productivity, enabling producers to participate in increasingly globalized and competitive markets [1].

Considering this scenario, the understanding of the productive process by which the plants are being submitted, such as: soil conditions and mulch; leaf area; grain filling; water demand; mineral nutrition; characteristics of cultivars; population density; seeding quality; allied with the appropriate use of technology and modern management tools [2], are of high importance to the success of the crop.

Several problems are often associated with early-stage soybean cultivation, so that the use of efficient seed treatment is often necessary to minimize economic losses caused by abiotic or biotic agents, consequently the correlation of products with a distinct mechanism of action may constitute a safer alternative for the stability of the vegetable in the field [3].

Among the biotic factors that can cause damage to the farmers in the initial phase of the crop are fungi, bacteria, nematodes and insects. And one of the strategies that can be adopted by the producers is the handling with efficient products in the treatment of seeds, associated with the cultivar choice. In this context and in particular for the improvement of rhizosphere activity, some types of hormonal products have been used in association with beneficial microorganisms such as diazotrophic bacteria *Bradyrhizobium japonicum* and *Azospirillum brasilense*.

The use of stimulating hormones in soybean cultivation is recent, but may be defined as the group of hormones in the auxin group, gibberellins, cytokinins, amino acids, vitamins and minerals [4]. Currently, the use of plant regulators in rice, corn, beans, cotton and soybean crops has increased productivity, although their use is still not a routine practice among high-level technological crop producers [1].

The use of these additives can modify the morphological and physiological responses of soybean plants, thereby, it is necessary to simultaneously identify several characters [4] through the selection of different seed treatments, with the objective of selecting strategies agronomically superior and desirable to the producer [5].

However, the conventional treatment of seeds together with the combination of inoculation and co-inoculation in plants may present contradictory results, which may stimulate or inhibit nodule formation and root system development. In this context, the objective of this study is to evaluate the production increase in three soybean cultivars in the Southwest region of Goias, using a combination of synthetic hormone, inoculation and co-inoculation.

## 2. MATERIALS AND METHODS

The study was conducted between November 27, 2017 and March 28, 2018, at the Experimental Farm Luís Eduardo de Oliveira Salles, belonging to UNIFIMES, a rural area of the municipality of Mineiros, GO, Brazil. Geographically it is at latitude 17º58' S and longitude 45º22' W and with approximately 800 m of altitude. Average temperature of 22.7°C and average annual precipitation of 1695 mm, occurring mainly in spring and summer. The experimental area is classified as Aw climate (hot to dry) [6].

The soil in the 0-20 cm layer had the following characteristics: hydrogen potential 4.1; phosphorus 3 in mg dm⁻³; potassium 0.6, calcium
5, magnesium 3, aluminum 4, potential acidity 29 in mmol c dm\(^{-1}\); clay 80, silt 30 and sand 890 in g dm\(^{-3}\). The analysis was carried out at the UNIFIMES Soil Chemistry and Fertility Laboratory, according to the EMBRAPA methodology [7]. The soil was classified as Quarantine Neurosol of sandy texture [7].

The experimental design was a randomized complete block design with four replications, in a 3x6 factorial scheme, corresponding to three soybean cultivars (Desafio 8473, TMG 1180 and CD 2737) and six seed treatments (Control, Inoculation, Hormonal, Co-inoculation, Co-inoculation + Hormonal and Inoculation + Hormonal).

The control treatment was established with the application of the Maxim product (Fludioxonil 2.5%, Metalaxyl-M 1%) and Cruiser (Metalaxyl-M 2%, Thiabendazole 15%, Fludioxonil 2.5%, Thiamethoxam 35%) using 50 ml of each for 50 kg of seed. For the diazotrophic inoculation, the Bioma Nod Soja (Bradyrhizobium japonicum) inoculant formulated from the strains SEMIA 5079 and SEMIA 5080 at the concentration of (7.2x10^5 cells mL\(^{-1}\)), used dose of 100 ml for 50 kg of seed was used. The hormone treatment was composed of 40 ppm of indole-3-acetic acid + 40 ppm of indole-bicyclic acid and 20 ppm of Gibberellin, with a dose of 75 ml for 50 kg of seeds. Diazotrophic co-inoculation was determined by the application of Bioma Maiz (Azospirillum brasilense) formulated from Ab-V5 and Ab-V6 Strains, in the concentration of (5x10^8 ml\(^{-1}\) cells) at a dose of 100 ml for 50 kg of seed. The seeds were treated in polyethylene bags.

Soil preparation was carried out in the conventional system with plowing and harvesting. Twenty seeds per linear meter of the cultivars Desafio 8473\(^{\circ}\) and CD 2737\(^{\circ}\) were distributed in the sowing, in addition of 15 seeds per meter of the cultivar TMG 1180\(^{\circ}\). Fertilization was carried out simultaneously with sowing with 200 kg ha\(^{-1}\) of monoammonium phosphate and 30 days after 120 kg ha\(^{-1}\) of potassium chloride was implemented. The management of weeds, pests and diseases were carried out whenever necessary, respecting the good practices of integrated pest management.

At the end of the experiment it was evaluated: height of the plant and the first reproductive node in cm; number of vegetables with 1, 2, 3 and 4 grains in one; number of vegetables and grains per plant in units; weight of a thousand grains in g; and yield in sc ha\(^{-1}\) [8]. Soon after, the data obtained were submitted to the assumptions of the statistical model, verifying normality [9] and homogeneity of variances [10]. Afterwards, the analysis of variance was carried out in order to identify the interaction between the soybean genotypes x seed treatment, when verifying significant interaction they were broken down to the simple effects and in the absence with main effects through the grouping test of averages of Scott-Knott, at 5% probability [11]. Subsequently, the variables of each spacing were subjected to Pearson's correlations in order to understand the trend of association, with its significance based on a 5% probability by the t test. The canonical variable biplot method was used, where it was possible to visualize the general variability of the experiment and the multivariate trends. The analyzes were performed on the Rbio do R interface [12], in addition to the Software Genes [13].

3. RESULTS AND DISCUSSION

Significant effects were observed involving soybean cultivars and seed treatment except for the variable plant height, number of pods with 1 and 4 grains and weight of 1000 grains. Among the cultivars evaluated 'Desafio 8473' showed lower plant height than the other cultivars. However, the development of seed treatments within the cultivars did not influence plant height (Fig. 1a), corroborating with Prieto et al. [14] which did not find variations in plant height inoculated with B. japonicum. However, Bulegon et al. [15] found a positive response with Co-inoculation in comparison to the other seed treatments. The height of soybean plants is a feature strongly influenced by environmental conditions, where higher relative maturity groups provide later cycle plants and larger plants.

The treatments of hormonal and co-inoculation seeds reduced the insertion height of the first reproductive branch of the cultivar 'Desafio 8473' (Fig. 1b), a fact that may be detrimental to mechanized harvesting [5]. What is desirable is that the height of the 1st reproductive node is superior to 10 cm and inferior to 15 cm favoring the mechanized harvest, indicating a productive vegetative biotype in the soybean crop. Paula Barbosa et al. [16] states that only the inoculation of seeds with nitrogen-fixing bacteria is capable of supplying nitrogen necessary to ensure the vegetative development of plants. Plants with inefficient inoculation may suffer from stress due to lack of nitrogen in their metabolism, causing
precocity in flowering and reduction in the insertion height of the 1st reproductive branch.

The 'Desafio 8473' cultivar presented the highest number of pods with 1 (Fig. 2α) and lowest number with 4 grains (Fig. 2γ) in all seed treatments. The frequency of pods with 2 grains (Fig. 2β) and 3 grains (Fig. 2μ) was more present in 'TMG 1180' for all seed treatments. In the Southwest region of Goias, it is common for the pods of soybeans to have an average of 2.5 grains, a number that in many cases can be used as a component of fixed yield. The number of grains per plant is one of the main components of yield of the crop, allied to the population and the weight of a thousand grains. Bulegon et al. [15], obtained different results among soybean varieties, related to treatments with diazotrophic bacteria, where such variations are due to external factors, such as competition of resources by other bacteria, resulting in changes in results from one work to the other. As verified by Paula Barbosa et al. [16] when testing the relationship between mineral fertilizer and inoculation, it was verified that all parameters studied did not present significant variation in the soybean crop.

The cultivar 'TMG 1180' presented the highest number of pods (Fig. 3α) and grains per plant (Fig. 3β) among all seed treatments. The treatment of hormonal seed was the most efficient for all the cultivars providing elevation in these variables. Hormonal seed treatment may be an alternative for producers to raise seedling establishment potential in the field. As well, Chaves and Guiscem [3] demonstrated that the treatments of seeds with vitamins (C, B9 and B1) had a significant effect on the germination of the soybean plant.

There was a decrease in the weight of one thousand grains for the cultivars 'Desafio 8473' and 'CD 2737' when they received the treatment with Co-inoculation as evidenced in Fig. 3μ. For Prieto et al. [14], the weight of a thousand soybeans, was not influenced by the application of biostimulants, biofertilizers and inoculant, as observed in this work, as well as Hipólito and Borges [2] when treating seeds with bioregulators and diazotrophic bacteria in different nutritional and physiological handlings.

The yield of grains was higher than the others when under treatments Hormonal and Co-inoculation for cultivar 'TMG 1180'. While the cultivars 'Desafio 8473' and 'CD 2737' showed the highest values of grain yield when submitted to hormonal treatment (Fig. 3). Differently from Kavalco et al. [4] when stating that soybean yield is not influenced by the hormone concentrations applied via seed treatment. The same happened with Siqueira et al. [17] when testing in soybean seeds products with micronutrients, bioregulators and conventional management.

Inoculation did not present a satisfactory result for 'TMG 1180' (Fig. 3γ) probably influenced by conventional soil management, where soil moisture and temperature factors are more limiting to soil biota, reducing the efficiency and action of B. japonicum and even of A. brasilense. Hungary et al. [18] justifies that the Cerrado biome has high temperatures and soils with high sand content and low water retention capacity, which makes the environmental conditions hostile to the survival of the biological inoculations. Such conditions in a conventional system reduce the symbiotic efficiency and exploitation of natural resources by soybean plants, mitigating the technique of seed treatment.

Table 1 presents the descriptive analysis of costs with seed treatment. Among the treatments Co-inoculation and Co-inoculation + Hormonal had the highest acquisition values, R$ 117.60 and R$ 129.15, respectively. The other treatments represent an average of values ranging from R$ 70.00 to R$ 88.50.

According to Hennig [19], the treatment of soybean seeds has been widely adopted because, besides allowing the germination of infected seeds, it controls pathogens transmitted by the seeds and protects the seeds of the fungi in the soil, allowing greater potential for the initial development of the culture and establishment of the initial booth, at reduced costs. The values found by Mastela [20], are in line with those presented in the present work, since the increases in seed treatment would have similar values.

For Oliveira et al. [21], the analysis of the costs and the profitability of the activities, are management tools that aid in decision making and are fundamental for the rural producer. In this way the test of new technologies assists the producer in the moment of decision making, relating important points such as production costs and productivity increase, being these factors preponderant for the insertion and acquisition of new products and technologies.
Fig. 1. Average plant heights (α) and insertion of the 1st reproductive branch (β) of soybean cultivars (Desafio 8473, TMG 1180 and CD 2737) when submitted to different seed treatments (Witness, Inoculation, Hormonal, Co-inoculation, Co-inoculation + Hormonal, Inoculation + Hormonal). UNIFIMES, Mineiros, GO, 2019

Averages followed by the same lowercase letter between the cultivars within each seed treatment and upper case between seed treatments within each cultivar do not differ statistically from each other by the Scott-Knott test at a 5% probability level.
Table 1. Descriptive analysis of cost R$ per hectare in different treatments of seeds in soybean cultivars. UNIFIMES, Mineiros, GO, 2019

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Description</th>
<th>Hectare dose ml p.c</th>
<th>Hectare price R$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Desafio 8473</td>
<td>TMG 1180</td>
<td>CD 2737</td>
</tr>
<tr>
<td>Witness</td>
<td>Maxim-Cruiser</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>140</td>
<td>120</td>
<td>140</td>
</tr>
<tr>
<td>Inoculation</td>
<td>Maxim-Cruiser+Bioma Nod</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>140+140</td>
<td>120+120</td>
<td>140+140</td>
</tr>
<tr>
<td>Hormonal</td>
<td>Maxim-Cruiser+Prime Vital</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>140+105</td>
<td>120+90</td>
<td>140+105</td>
</tr>
<tr>
<td>Co-inoculation</td>
<td>Maxim-Cruiser+Bioma Nod-Bioma Maíz</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>140+280</td>
<td>120+240</td>
<td>140+280</td>
</tr>
<tr>
<td>Co-inoculation+Hormonal</td>
<td>Maxim-Cruiser+Bioma Nod-Bioma Maíz+Prime Vital</td>
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<td>140+280+105</td>
<td>120+240+90</td>
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<td>Inoculation+Hormonal</td>
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<td></td>
<td>140+140+105</td>
<td>120+120+90</td>
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</tr>
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</table>

Seeds in kg ha⁻¹ of cultivars = Desafio 8473: 70; TMG 1180: 60; CD 2737: 70.
Price in R$ L⁻¹ of the products = Maxim XL: 120.00; Cruiser: 380.00; Bioma Nod: 50.00; Prime Vital: 110.00; Bioma Maíz: 120.00.
Source: Averages of the local resales obtained in the conduction of the work.
Fig. 2. Averages for number of pods with 1 grain (α), 2 grains (β), 3 grains (μ) and 4 grains (γ) of soybean cultivars (Desafio 8473, TMG 1180 and CD 2737) when submitted to different seed treatments (Witness, Inoculation, Hormonal, Co-inoculation, Co-inoculation + Hormonal, Inoculation + Hormonal). UNIFIMES, Mineiros, GO, 2019

Averages followed by the same lowercase letter between the cultivars within each seed treatment and uppercase between seed treatments within each cultivar do not differ statistically from each other by the Scott-Knott test at a 5% probability level.

The multivariate analysis with the correlation of the variables allowed to characterize the variables that discriminated the most in the formation and differentiation of the variables of soybean cultivars (Fig. 5). For the graphic presentation of the canonical variables, the first two main components were used to represent the discriminatory power of the soybean attributes, in the different treatments analyzed (Fig. 4).

Along dimension 1 (main axis), the treatments with 'CD 2737' were arranged in the lower median portion, in the upper right portion are arranged the treatments belonging to the cultivar ‘Desafio 8473’, and on the left the treatments
belonging to 'TMG1180' which shows clear separation between cultivars, their environmental adaptability and response to yield components (Fig. 4). The variables of TVP, TGP and PMG were more associated with cultivar 'TMG 1180'.

REND was more influenced by 'CD 2737', while 'Desafio 8473' was far apart for all variables. These results demonstrate the importance of choosing and positioning the genetic material in the field.

Fig. 3. Averages for number of pods (α) and grains per plant (β), weight of one thousand grains (μ) and yield (γ) of soybean cultivars (Desafio 8473, TMG 1180 and CD 2737) when submitted to different seed treatments Witness, Inoculation, Hormonal, Co-Inoculation, Co-inoculation + Hormonal, Inoculation + Hormonal. UNIFIMES, Mineiros, GO, 2019

Averages followed by the same lowercase letter between the cultivars within each seed treatment and upper case between seed treatments within each cultivar do not differ statistically from each other by the Scott-Knott test at a 5% probability level.
Fig. 4. Analysis of canonical variables of averages of yield components of soybean cultivars, DVT: total pods per plant; TGP: total grains per plant; and REND: yield. Mineiros-GO, UNIFIMES, Brazil, 2019

Fig. 5. Network of phenotypic correlations of soybean characteristics. The red lines represent negative correlations and the green ones represent positive corrections. The line thickness is proportional to the magnitude of the correlation. The highlighted lines present correlation in a module greater than 0.6. Variables of adult plants: V1: plant height; V2: height of the first reproductive node; V3: pods with 1 grain; V4: pods with 2 grains; V5: pods with 3 grains; V6: pods with 4 grains; V7: pod per plant; V8: grains per plant; V9: weight of a thousand grains; and V10: yield. Mineiros-GO, UNIFIMES, Brazil, 2019

The analysis of canonical variables explains 81.8% in the first factor and 16.2% in the second, totaling in the first two canonical variables the explanation of 98% of the total variance found,
with a loss of explanation of only 2%. This allows a satisfactory explanation of the variability manifested among the treatments (Fig. 4). For Ferreira et al. [22] this tool can be used efficiently in works involving other phytotechnical themes such as the potential for plantability and final performance of plants in the field. However, for works with different soybean genotypes, this tool demonstrates the degree of similarity among the desired characteristic of the genotype implanted in the field.

In the interpretation of correlations, three aspects must be considered: Magnitude, direction and significance. Positive correlation coefficient estimates indicate the tendency of one variable to increase when the other increases, negative correlations indicate tendency of one variable to increase while the other one decreases [23].

In the analysis of correlation network, the formation of a positive cohesive group was observed between the variables of pods with 2 grains, pods with 3 grains, pods with 4 grains, pods per plant and grains per plant. Positive correlation was also observed between the weight of a thousand grains and yield. Carpentieri-Pipolo et al. [24] verified that the number of pods per plant is the main contributor to grain yield in legumes, since it has the highest correlations with grain production and pods with 1, 2 and 3 grains may influence the size of seeds that will be produced and consequently in productivity. Another observation was the number of pods with 1 grain that presented negative phenotypic correlation with plant height, pods with 2 grains, pods with 3 grains, pods per plant and grains per plant (Fig. 5). Zuffo et al. [25] and Smiderle et al. [26], also found strong correlations between soybean characters. This study contributes to the choice of specific products for soybean culture, where specific sites can contribute to other metabolic routes, enhancing other characters.

4. CONCLUSION

The treatments with the diazotrophic bacteria (Inoculation + Co-inoculation) and Hormonal did not influence the yields of the cultivars "CD 2737" and "Desafio 8473", which could only be cultivated with the standard seed treatment of the region, with the former having a higher productive ceiling.

The 'TMG 1180' cultivar has high yield when its seeds are inoculated with Hormonal seed treatments and with diazotrophic bacteria in Co-inoculation.

The choice of the genetic material and its positioning are of high importance for the elevation of soybean yield components, and may have as an aid in decision making the application of multivariate tools.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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