

METHODOLOGY OF ADDITION DIFFERENT DOSES OF BORON AND ZINC IN THE COATING OF PERENNIAL SOYBEAN SEEDS

Abstract - Most Brazilian soils present deficiencies of B and Zn, demanding the supply of these nutrients so that production is not limited. Thus, the objective of this work was to test application methodology and doses of B and Zn in perennial soybean seeds. The physical, physiological and nutritional characteristics of the covered seeds and the initial growth of the plants were evaluated in laboratory and greenhouse. For laboratory tests, a completely randomized design was used, and randomized blocks were used in greenhouse tests. Methods 1 and 2 increase the B + Zn contents in the seeds by approximately 97 times and 22 times, respectively. The micronutrient application methodology in the 6th coat layer promoted greater increases in seed mass and higher levels of B and Zn. Consequently, the plants originated from these seeds had their vigor reduced. Both methods did not significantly influence the initial plant growth.

Keywords: layers; materials; nutrition; vigor

1. INTRODUCTION

The *Neonotonia wightii* is a herbaceous Fabaceae, which has a high nutritional value, high palatability, good ability to intercropping and good natural reseeding, which is approved as one of the most important tropical Fabaceae, being suitable for haymaking, formation of pasture and green manure [1, 2].

Due to the importance of nutritional elements for the development and growth of

plants, some practices with the goal of increasing crop productivity, have been causing a nutritional imbalance, leading to phytotoxicity of some nutrients and to the deficiency of others, even when they are at appropriate concentrations in the medium. However, the majority of soils have a deficiency of B and Zn, micronutrients which, although required in small quantities, are harmful to the development and yield of plants when restricted. The delivery of these nutrients through fertilizers, to adequately meet the nutritional requirement of the crops, so that production is not limited, is the most used option in Brazilian agriculture [3, 4].

As an option to the traditional methods of providing nutrients to plants, comes coating the seeds, which in addition to the initial advantages of increasing the size and changing the shape and texture of the seeds, so that there is less loss at the time of mechanized planting, also allows the application of nutrients along with the materials used in its manufacture, always aiming to improve seed performance, both physiologically and economically [5, 6].

Given the above, the objective of this study was to evaluate the application methods and doses of B and Zn in the quality of coverage, in the vigor and initial growth of perennial soybean seedlings cv. Comum.

2. MATERIALS AND METHODS

2.1 Experimental Location

This study was divided in two experiments, denominated Method 1 and Method 2. Both were developed in the laboratory and in a greenhouse at the State University of Norte Fluminense, in the period of October / November 2015. Commercial seeds of *Neonotonia wightii* were acquired from the BRSEEDS[®] Company, which were previously submitted to

47 scarification between two sheets of sandpaper iron number 36.

48 **2.2 Seed Coating**

49 For both experiments, the coating process of the seeds used one coater, model N10
50 Newpack and the methodology was adapted from [7]. The coater was regulated so that the
51 vessel would rotate at 90 rpm; the compressed air that drives the adhesive solution would be
52 at a pressure of 4 bar and the spray of the solution, when triggered, lasted for 1 second; and
53 the hot air blower, used to dry the seed, would be adjusted at 40° C, remaining attached for 2
54 minutes.

55 As a filler for the coating material, we used the dolomitic lime and activated
56 charcoal in the proportion materials: Seed 3: 1 (w/w) and 0.08: 1 (w/w), respectively, and for
57 adhesive material, extra Cascorez glue with polyvinyl acetate base (PVA) diluted in deionized
58 water previously heated to 70° C in the ratio 1: 1 (v / v) [7].

59 To cover 100g of perennial soybean seeds, the filler materials, lime (300 g) and
60 charcoal (8g) were weighed on a precision balance in portions of 12.5g and 2g, respectively,
61 to be used in the forming of the layers.

62 The formation of layers began when the mass of seeds was placed in the vat along
63 with a portion of the filler material and then the spray, containing the adhesive solution, was
64 fired three times in succession. After that, another portion of filler was added and a further jet
65 of adhesive solution was applied. In closing, the air blower (40° C) was activated for two
66 minutes to remove excess of humidity. This procedure formed the first layer. For the
67 following layers, only one adhesive solution jet was triggered, followed by a portion of the
68 filler material, right after another jet of adhesive solution and then the second portion of the
69 filler material was placed, and finally another adhesive solution jet and then the hot air

70 blower was operated for two minutes. This procedure was repeated until the amount of filler
71 material would end.

72 At the end of the process, the coated seeds showed 14 layers, including 12 layers of
73 limestone and two layers of coal. The division of the layers in the coating was given so that
74 the first three layers were formed by limestone, then by two layers of coal and the rest, by
75 limestone.

76 **2.3 Experimental Treatments**

77 The application of micronutrients during the coating process corresponds to the
78 difference between Methods 1 and 2. Method 1, the micronutrient doses were added at once
79 in the sixth coating layer, followed by a jet of glue, after the first portion of lime + glue. In
80 Method 2, the doses of B and Zn were divided into four equal and individual portions to then
81 be applied to the 3rd, 6th, 9th and 12th layers, followed by a jet of glue. Always being added
82 after the first portion of lime + glue.

83 For both methods, seven treatments were defined: TR1 - Seeds without coat; TR2 -
84 Coated seeds without micronutrients; TR3 – 50 g of H_3BO_3 kg^{-1} of seeds + 50 g of ZnSO_4
85 kg^{-1} of seeds; TR4 – 100 g of H_3BO_3 kg^{-1} of seeds + 50 g of ZnSO_4 kg^{-1} of seeds; TR5 – 150
86 g of H_3BO_3 kg^{-1} of seeds + 50 g de ZnSO_4 kg^{-1} of seeds; TR5 – 150 g of H_3BO_3 kg^{-1} of seeds
87 + 100 g of ZnSO_4 kg^{-1} of seeds; TR7 – 200 g of H_3BO_3 kg^{-1} of seeds + 100 g of ZnSO_4 kg^{-1}
88 of seeds. Once coated, the seeds were evaluated for physical, physiological and nutritional
89 features.

90 **2.4 Germination Test**

91 Using the criteria established in the RAS [8], the laboratory tests were arranged in a
92 completely randomized design, using four replications of 50 seeds for germination test, in

which, at the end of 10 days, the percentage of germination was determined. The weight of a thousand seeds [8] and germination speed index [9] were also evaluated.

2.5 Physical Characteristics

Evaluations were performed to determine the physical characteristics of the seeds of each treatment as the maximum diameter (DMAX), minimum diameter (DMIN) and irregularity of contour (CI) of seeds, with the equipment Groundeye®. For these evaluations, for repetitions of 50 seeds were used and the results were expressed in centimeters (cm).

2.6 Emergency Test

In the greenhouse, the emergency test was conducted in plastic trays containing washed sand and organized in a design of four randomized blocks, with 50 seeds for each treatment. The test lasted for 30 days, and daily counts were made to determine the emergence speed index (IVE), and at the end 10 representative plants of the plot were selected, for the length of the shoot (CPA) and root (CR) to be evaluated with the aid of a millimeter ruler, dry mass of the aerial part (MSPA) and root (MSR) on a precision scale. For the evaluation of the dry mass, the material was kept in air forced circulation stove at 65° C for 72 hours.

2.7 Nutritional Analysis

To determine the nutrient content in the seeds, in the shoots and roots of plants grown in the greenhouse, after dried, then the seeds and the shoot and root were macerated in mill, to then be stored in airtight tubes. For the seed analysis, a completely randomized design was followed, with 4 replicates of each treatment. However, to analyze the plants (shoot and root), there was not enough dry material to use repetitions. Due to that, the result of the analysis is regarding the treatment. To determine the content of nutrients, a nitric digestion

was performed, followed by the reading of the ICPE-9000 (Plasma Atomic Emission Spectrometer) extract [10].

2.8 Statistical Analysis

The data regarding the evaluations followed the normal pattern, not requiring data transformation, then the analysis of variance was carried out and the means were compared by Tukey test at 5% probability, with the help of the SAEG statistical program.

3. RESULTS AND DISCUSSION

3.1 Nutritional Content in Seeds

The methods used for adding the micronutrients during the coating process influenced significantly on the adherence of the used materials. Comparing the methodologies used for the coating, Method 1, in which the application of B and Zn occurs is in a single layer, was able to promote greater adherence of the material in the seeds, when compared to Method 2, in which the doses of B and Zn was divided into 4 layers.

Observing Figure 1, it is notable that Method 1 promoted a 97 times increase in the B + Zn (TR6) content of the coated seeds compared to uncoated seeds (TR1). While in method 2, there was a 22 times increase in the content of B + Zn (TR6). Thus, it is worth mentioning that the highest B + Zn level retained in the seeds of Method 1 is approximately 4 times higher than the higher B + Zn level of the seeds of Method 2.

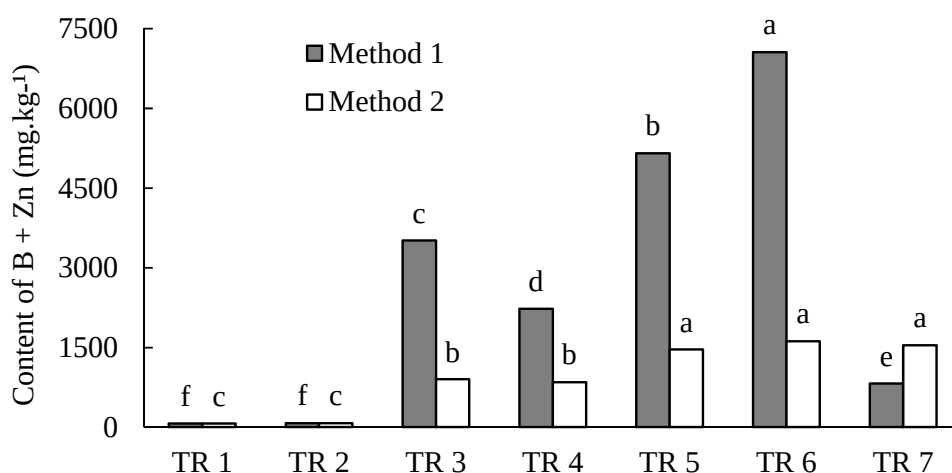


Fig. 1. B + Zn contents in the perennial soybean covered by methods 1 and 2. Treatments (g kg⁻¹seed): TR1: uncoated; TR2: w/o micronutrients; TR3: 50 H₃BO₃ + 50 ZnSO₄; TR4: 100 H₃BO₃ + 50 ZnSO₄; TR5: 150 H₃BO₃ + 50 ZnSO₄; TR6: 150 H₃BO₃ + 100 ZnSO₄; TR7: 200 H₃BO₃ + 100 ZnSO₄. Means followed by the same letter do not differ statistically from each other by the Tukey test, at the 5% probability level ($P = 0,05$).

Based on these results, it is clear that the boron application methodology and zinc at once and only in the sixth layer of the coating provided a better fixing in the seeds, reflected in the **teor** of B and Zn in seeds (Figure 1). Therefore, in the experimental conditions that were tested, applying boron and zinc in separate layers causes greater loss of material added during the coating process. It is believed that the methodology used for addition of micronutrient during the process of forming the layers, ever after the first portion of limestone + glue, followed by a further jet of glue, resulted in the fact that, in Method 2, at the end of the coating, the seeds received 3 extra glue jets, due **tot** the fact that the micronutrients have been applied in 3 more layers, besides the fact that these glue jets have

also been applied in proportion glue / equipment higher than in Method 1, since the micronutrient doses were the same, but by being divided into layers, portions became smaller. Thus, the adhesive volume added to the micronutrient portions in Method 2, may have led to increased adhesion of the material added during the coating of the tub coater, thereby reducing adherence on the seeds.

Still on Figure 1, it is noted that, in Method 1, the TR7 has a sharp drop in relation to the adherence of B+Zn, which leads us to believe that, in this case, the volume of adhesive material used in the methodology was insufficient to promote adhesion of the higher doses tested in this treatment ($200 \text{ H}_3\text{BO}_3 + 100 \text{ ZnSO}_4 \text{ g kg}^{-1}$ of seeds).

3.2 Germination

In Figure 2, it is possible to observe that the methodologies applied significantly influenced the variables index of germination speed (IVG) and germination. It is possible to observe that the reduction in the germination rate also occurs in normal seedlings, with increasing doses of boron and zinc in the coating, except for the treatment with 200 g B kg^{-1} seed + 100 g Zn kg^{-1} (TR7) of Method 1, in which the germination speed and percentage of normal seedlings is equivalent to uncoated seeds, possibly due to the lower adhesion of B + Zn in this treatment, as shown in Figure 1.

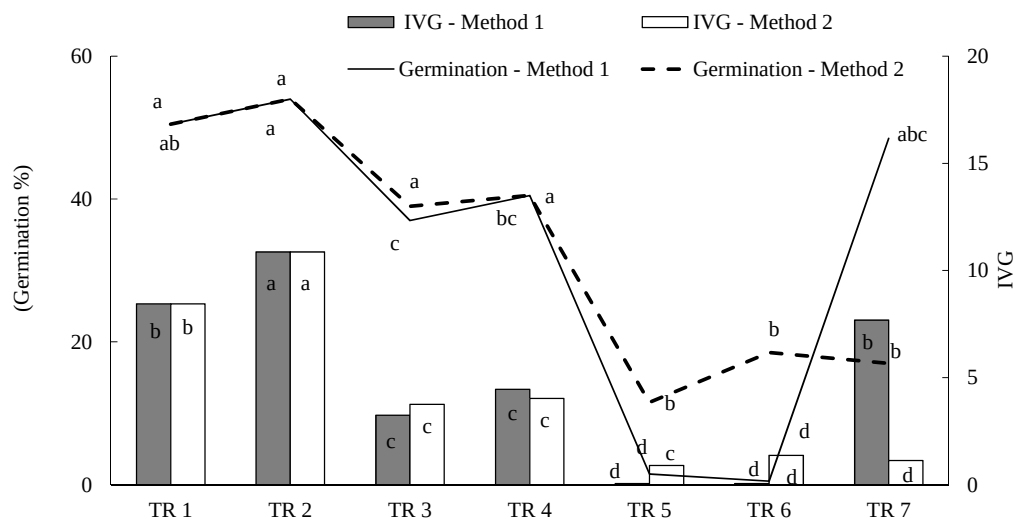


Fig. 2. Germination and IVG of perennial soybean seeds coated by Method 1 e 2. Treatments

(g kg⁻¹seed): TR1: uncoated; TR2: w/o micronutrients; TR3: 50 H₃BO₃ + 50 ZnSO₄; TR4: 100 H₃BO₃ + 50 ZnSO₄; TR5: 150 H₃BO₃ + 50 ZnSO₄; TR6: 150 H₃BO₃ + 100 ZnSO₄; TR7: 200 H₃BO₃ + 100 ZnSO₄. Means followed by the same letter do not differ statistically from each other by the Tukey test, at the 5% probability level ($P = 0,05$).

This result can be justified by the contents of B and Zn retained in coated seeds (Figure 1). When observing the seeds of TR7 in Method 1, there is a lower level of B + Zn if compared to TR5 and TR6 of the same method, explaining why the damage caused by excess salts, observed on the germination and IVG in TR5 and TR6 treatments, are not noticed in TR7.

It is possible to observe that the treatment (TR2), coated control, stood significantly out from the others, showing that only coating with limestone, charcoal and glue was effective in promoting the formation of a larger number of seedlings in a shorter period of

time, thus beating the main claimed problem for the use of coated seeds, which is the delay on seed germination and emergence of plants [11, 12].

Analyzing the isolated effect of B and Zn in the works of [13] and [14], found that increasing doses of B (boric acid) in corn seeds and doses of Zn (heptahydrate zinc sulfate) in sorghum seeds, caused reduction, less uniformity and delayed germination, beyond low initial development of plants. Unlike these authors and the results obtained in this work for germination in laboratory conditions (Figure 2), other authors found positive responses to the use of Zn in seeds, as [12], and [15], found that the zinc coating supplied by the seed of oats and canola, respectively, provided benefits for the germination of seeds and initial plant establishment. De [16] also observed positive results using a commercial product consisting of 20% zinc, 3% boron, 1% magnesium and 1% molybdenum for the treatment of corn seeds, and noted that, for the CD 304 hybrid corn, the combination of these micronutrients, in their concentrations in the product, it was efficient to increase the percentage of normal seedlings.

3.3 Emergency

Based on the data of Figure 3, regarding the test conducted in the greenhouse, it is possible to affirm that there was an improvement in the behavior of seeds placed in these conditions for both coating methods, when compared with the data in Figure 2, regarding the laboratory test. Because it is possible to notice that the treatments with the lowest percentage of germination in laboratory were positively highlighted in the emergency test in the greenhouse, i.e., the TR5 and TR6 of Method 1, in the greenhouse, had an average increase of 30% of normal seedlings compared to laboratory testing (Figure 2).

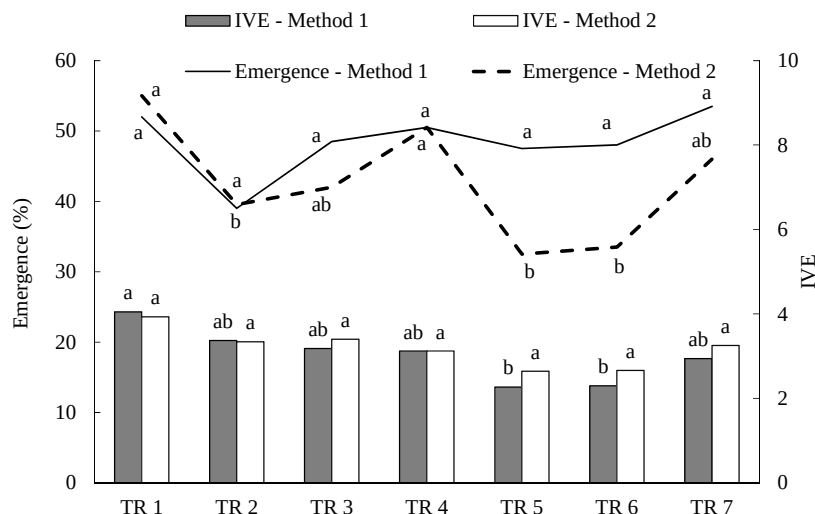


Fig. 3. Emergence and Emergence Speed Index (IVE) of perennial soybean seeds coated by Method 1 and 2. Treatments ($\text{g kg}^{-1}\text{seed}$): TR1: uncoated; TR2: w/o micronutrients; TR3: $50 \text{ H}_3\text{BO}_3 + 50 \text{ ZnSO}_4$; TR4: $100 \text{ H}_3\text{BO}_3 + 50 \text{ ZnSO}_4$; TR5: $150 \text{ H}_3\text{BO}_3 + 50 \text{ ZnSO}_4$; TR6: $150 \text{ H}_3\text{BO}_3 + 100 \text{ ZnSO}_4$; TR7: $200 \text{ H}_3\text{BO}_3 + 100 \text{ ZnSO}_4$. Means followed by the same letter do not differ statistically from each other by the Tukey test, at the 5% probability level ($P = 0,05$).

You can relate this result to the environment in which the seeds were placed (trays with sand in the greenhouse), as it simulates more real field conditions, such as temperatures, low moisture retention by the substrate, requiring more constant irrigations, which increases the leaching of excess of salts.

Based on the data in Table 1, no treatment differed significantly ($P = 0,05$) from the control treatments (TR1 and TR2) for the variables analyzed. However, there was significant variation between the doses tested, where no treatment exceeded the means reached by the treatment $50 \text{ H}_3\text{BO}_3 + 50 \text{ ZnSO}_4$ (TR3). It is possible to associate these results with the

contents of B and Zn retained in the seeds after the coating, that is, the higher the B and Zn content in the coating, the lower the initial growth of the plants under the conditions tested. As zinc is a micronutrient growth promoter [17], it is believed that its content, present in the seeds of the treatments, may have been very high, since micronutrients are needed in small amounts and the range of the optimal amount, is narrow.

Table 1. Length and dry matter in the aerial part of perennial soybean plants, originated from seeds coated by method 1 (application of micronutrients in the 6th coat layer).

Treatments (g kg ⁻¹)	CPA (cm)	MSPA (g/pl)	CR (cm)	MSR (g/pl)
Micronutrients in the 6 th layer				
Uncoated	1.44 ab*	0.15 ab	10.67 a	0.19 ab
Without micronutrientes	1.43 ab	0.15 ab	10.31 a	0.18 ab
50 H ₃ BO ₃ + 50 ZnSO ₄	1.74 a	0.17 a	11.55 a	0.21 a
100 H ₃ BO ₃ + 50 ZnSO ₄	1.43 ab	0.13 ab	9.84 a	0.17 ab
150 H ₃ BO ₃ + 50 ZnSO ₄	1.33 b	0.11 b	9.27 a	0.16 ab
150 H ₃ BO ₃ + 100 ZnSO ₄	1.27 b	0.09 b	9.27 a	0.14 b
200 H ₃ BO ₃ + 100 ZnSO ₄	1.41 ab	0.13 ab	10.05 a	0.16 ab
CV (%)	11.37	17.36	8.89	16.72

*Means followed by the same letter do not differ statistically from each other by the Tukey test, at the 5% probability level ($P = 0,05$)

In method 2, there This result was attributed to the maximum levels of B and Zn adhered to the seeds (1020.51 mg. kg⁻¹ was no significant difference between the treatments tested for the growth variables analyzed and 898.30 mg kg⁻¹, respectively), which were not detrimental to the initial growth of the plants, showing the importance of the content retained

in the seeds and not the doses applied.

Even using smaller doses of micronutrients in their work with rice seeds, under the conditions tested, [17] obtained results that corroborate with those found in this work in relation to initial plant growth. The authors observed a negative effect of rice seeds treated with B and Zn, because the plants reached a low initial growth when compared to the control and with that, the plants coming from seeds treated with the same micronutrients, but isolated. According to the authors, these results are probably due to an antagonistic effect of these micronutrients.

3.4 Nutritional Content in Plants

In aerial part, 30 days after sowing considering Method 1, B absorption was greatly close to Zn absorption in all treatments. Still, B was the highlight, mainly in TR4 (149.41 mg kg⁻¹) and in TR6 (158.47 mg kg⁻¹), in which the contents were three times bigger than the content of the uncoated treatment (47.64 mg kg⁻¹). In these treatments, its could also notice a bigger content of Zn: the amounts (TR4 – 104.71 mg kg⁻¹ and TR6 – 123.62 mg kg⁻¹) are two times bigger than the ones of the uncoated treatment (47.35 mg kg⁻¹). In method 2, the plants that originated from coated seeds behave differently from the plants of Method 1. That means that with the exception of TR5 and control treatments (TR1 and TR2), all the other treatments presented a greater absorption of Zn than of B. However, seeds from TR5 exhibited the biggest amount of B (1020.52 mg kg⁻¹), and that is connected to its absorption by the plant.

Environmental variations like temperature and soil moisture can extremely influence the content of mineral nutrients in the leaves. These factors influence the nutrients' availability as well as their absorption by plants' roots, and consequently, the content in the leaves [18].

The greatest amount of B absorbed by the plants of Method 1 was 158.47 mg kg, through TR6, and 122.4 mg kg⁻¹ by the ones of Method 2, through TR5. Considering the content of Zn, in Methods 1 and 2, the highest content of it was respectively 123.62 mg kg⁻¹ and 127.18 mg kg⁻¹, both in TR6. There is not much data about the numbers concerning micronutrients toxicity, but [19] reported some values apropos of Zn toxicity in the aerial part of annual crops: they were bigger than 400 mg kg⁻¹ dry matter. Thus, the plants grown in greenhouses, even though being originated from seeds with a high content of B and Zn, possibly did not suffer from toxicity by Zn.

[20] Report that Zn response in function of B contents depends of the plant's organ in analysis. Nevertheless, the authors perceive that tobacco leaves behave similarly to the ones observed in this work, higher amounts of B reduced Zn absorption. [21] Also noticed a reduction of Zn concentration in corn leaves due to increasing B concentration.

It is easy to notice that there are elevated contents of Zn in plants' roots. Method 1 reached 191.07 mg.kg⁻¹ and Method 2 hit a maximum content of 156.19 mg.kg⁻¹. According to [22], these high contents may have been induced due to nutrient accumulation in the root area. This event occurs when the nutrients are transported through diffusion and when their rate of supply is bigger than their absorption, which is deeply coherent to what happened in our tests. In [14] work, authors found a higher concentration of Zn in the roots of sorghum plants, which had received increasing doses of Zn.

3.5 Physical Characteristics

In the matter of coating quality, the first aim of using it is to modify seeds size, shape and density. Therefore, the doses and methodology we used were efficient to increase seeds weight in up to 160% (16,6 g) in Method 1, and up to 97% (12,6 g) in Method 2, when

in comparison to the seeds from control treatments without coating (6,4 g). Consequently, its can assert that Method 1 promoted a better adhesion of the coating material, as mentioned before.

Table 2 shows that the two methodologies were able to develop maximum and minimum diameter increases and to correct some irregularities in the seed contour. The highlight of these variations was TR6 (150 H_3BO_3 + 100 ZnSO_4) in Method 1, which promoted a 21% increase in maximum diameter and 41% in minimum diameter, and reduced contour irregularities by 23%. Alternatively, in Method 2, TR2 (micronutrient free coating) was suitable to increase the maximum diameter by 26% and the minimum diameter by 39%, and reduced the contour irregularities by 26% according to Groundeye[®] software definitions. These characteristics make the seeds larger and uniform, improving seed distribution on seed lines.

Table 2. Diameters maximum (DMAX) and minimum (DMIN), contour irregularity (IC) and moisture of micronutrient coated seeds added to the 6th coating layer (Method 1) and 3th, 6th, 9th and 12th coating layers (Method 2).

Method 1				
(g kg^{-1} of the seeds)	DMAX (cm)	DMIN (cm)	IC (cm)	Moisture (%)
Uncoated	0.253 f*	0.172 e	0.031 a	10.09 a
Without micronutrientes	0.319 a	0.238 b	0.023 b	8.82 b
50 H_3BO_3 + 50 ZnSO_4	0.292 e	0.221 d	0.024 b	7.90 b
100 H_3BO_3 + 50 ZnSO_4	0.296 d	0.219 d	0.031 a	8.50 b
150 H_3BO_3 + 50 ZnSO_4	0.312 b	0.238 b	0.023 b	8.51 b
150 H_3BO_3 + 100 ZnSO_4	0.320 a	0.242 a	0.024 b	8.38 b

200 H ₃ BO ₃ + 100 ZnSO ₄	0.308 c	0.231 c	0.029 a	5.26 c
CV (%)	0.64	0.5	4.66	3.8
Method 2				
(g kg ⁻¹ of the seeds)	DMAX (cm)	DMIN (cm)	IC (cm)	Moisture (%)
Uncoated	0.253 e	0.171 e	0.031 a	10.09 a
Without micronutrientes	0.319 a	0.238 a	0.023 bc	8.82 b
50 H ₃ BO ₃ + 50 ZnSO ₄	0.291 d	0.222 c	0.025 b	5.60 e
100 H ₃ BO ₃ + 50 ZnSO ₄	0.291 d	0.216 d	0.025 b	5.96 de
150 H ₃ BO ₃ + 50 ZnSO ₄	0.306 b	0.229 b	0.022 c	5.55 e
150 H ₃ BO ₃ + 100 ZnSO ₄	0.300 c	0.222 c	0.025 b	6.37 d
200 H ₃ BO ₃ + 100 ZnSO ₄	0.298 c	0.220b c	0.023 bc	7.22 c
CV (%)	0.54	0.57	3.01	2.12

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293 *Means followed by the same letter do not differ statistically from each other by the Tukey
 294 test, at the 5% probability level ($P = 0,05$).

295 4. CONCLUSION

296 The coating increased the mass and the diameter of the seeds, and the application of B
 297 and Zn in the 6th layer promoted the greatest increases in seed mass and B + Zn contents of
 298 the seeds. The plants originated from coated seeds accumulated higher levels of B and Zn. In
 299 the initial growth of the plant, the means reached by the treatment with 50 B + 50 Zn (g kg⁻¹
 300 of seeds) tended to be better than the control treatment. please do mention the implication of
 301 the resaerch work carried out as well as the future course of work.

302

COMPETING INTERESTS

The authors have stated that there are no competing interests.

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