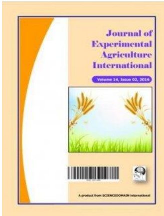

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Methodology of Applying Different Doses of Boron and Zinc in the Coating of Perennial Soybean Seeds

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Most Brazilian soils have deficiencies of B and Zn, and demand the supply of these nutrients so that production is not hampered. The present study aimed to test the 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. Two methodologies were used for the application of micronutrients. In Method 1, the B and Zn doses were added once in the 6th coating layer, followed by a jet of glue, after the first portion of lime + glue. In Method 2, the doses of micronutrient were divided into four equal and individual portions, to be applied to the 3rd, 6th, 9th and 12th layers followed by a jet of glue. This was added after the first portion of lime + glue. For both the methods, 7 treatments were defined: TR1 - Seeds without coat; TR2 - Coated seeds without micronutrients; TR3 – 50 g of H_3BO_3 kg-¹ of seeds + 50 g of $ZnSO_4$ 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 g of H_3BO_3 kg-¹ of seeds + 50 g of $ZnSO_4$ kg-¹ of seeds; TR6 – 150 g of H_3BO_3 kg-¹ of seeds + 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 of seeds. For laboratory tests, a completely randomised design was performed, and randomised

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blocks were used in greenhouse tests. Methods 1 and 2 increased 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 vigour reduced. Both methods did not significantly influence the initial plant growth.

Keywords: Layers; materials; nutrition; vigour.

1. INTRODUCTION

The *Neonotonia wightii* (Fabaceae) is a herbaceousplant, which has a high nutritional value, high palatability, good ability to intercropping and good natural reseeding. This is considered 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 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 application of these nutrients through fertilisers, to adequately meet the nutritional requirement of the crops, so that production is not restricted, is the most convinient option in Brazilian agriculture [3,4].

Coating the seeds comes as an option to the traditional methods of providing nutrients to plants, which in addition to the initial advantages of increasing the size, change the shape and texture of the seeds, so that there is a limited loss at the time of mechanised planting. This 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].

The study aimed to evaluate the application methods and doses of B and Zn in the quality of coverage, in the vigour and initial growth of perennial soybean seedlings cv. Comum.

2. MATERIALS AND METHODS

2.1 Experimental Location

This study was divided into two experiments, denominated as Method 1 and Method 2. Both were developed in the laboratory and in a greenhouse at the State University of Norte Fluminense, during October/November 2015. Commercial seeds of *Neonotonia wightii* were acquired from the BRSEEDS® Company, which were previously submitted to scarification between two sheets of sandpaper (iron number 36).

2.2 Seed Coating

For both experiments, the coating process of the seeds used one coater (model N10 Newpack) and the methodology was adapted from Xavier et al. [7]. The coater was regulated so that the vessel was rotated at 90 rpm; the compressed air drives the adhesive solution at a pressure of 4 bar and the spray of the solution, when triggered, lasted for 1 second. The hot air blower used to dry the seed, has been adjusted to 40°C and remained attached for 2 minutes.

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

To cover 100g of perennial soybean seeds, the filler materials, lime (300 g) and charcoal (8 g) were weighed in a precision balance in portions of 12.5 g and 2 g respectively, which has been used toform the layers.

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

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

2.3 Experimental Treatments

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

For both methods, 7 treatments were defined: TR1 - Seeds without coat; TR2 - Coated seeds without micronutrients; TR3 – 50 g of H_3BO_3 kg^{-1} of seeds + 50 g of $ZnSO_4$ 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 g of H_3BO_3 kg^{-1} of seeds + 50 g of $ZnSO_4$ kg^{-1} of seeds; TR6 – 150 g of H_3BO_3 kg^{-1} of seeds + 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} of seeds. Once coated, the seeds were evaluated for physical, physiological and nutritional features.

2.4 Germination Test

Using the criteria established in the RAS [8], the laboratory tests were arranged in a completely randomised design, using four replications of 50 seeds for germination test, at the end of 10 days, the percentage of germination was determined. The weight of 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 by using the equipment Groundeye®. For these evaluations, repetitions of 50 seeds were used and the results were expressed in centimetres (cm).

2.6 Emergency Test

In the greenhouse, the emergency test was conducted in plastic trays containing washed sand and organised in a design of four randomised 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). At the end, 10 representative plants of the plot were selected and the length of the shoot (CPA) and root (CR) was evaluated with the aid of a millimetre scale, while, dry mass of the aerial part (MSPA) and root (MSR) by using a precision scale. For the evaluation of the dry mass, the material was kept in the air forced circulation stove at 65°C for 72 hours.

2.7 Nutritional Analysis

To determine the nutrient content in the seeds, aerial part and roots of plants grown in the greenhouse, the material was macerated in milland stored in airtight tubes. Four repetitions of each treatment were used for seed analysis. However, to analyse the plants (aerial and root), there was an insufficiency of dry materials to use repetitions; thereby only the average result of the treatments was presented. Nitric digestion was performed to determine the content of nutrients, followed by spectrophotometric analysis by Plasma Atomic Emission Spectrometer (Model ICPE-9000) [10].

2.8 Statistical Analysis

The data regarding the evaluations followed the normal pattern(data transformation is not required), and the analysis of variance was carried out. The means were compared by Tukey test at 5% probability level, 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 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.

Fig. 1 shows that Method 1 promoted a 97 times increase in 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 worthwhile to mention that the highest B + Zn level retained in the seeds of Method 1 is approximately 4 times higher than the higher B + Zn level in the seeds of Method 2.

Based on these results, it is clear that the boron application methodology and zinc at once in the 6th layer of the coating provided a better fixing in the seeds, as has been reflected in the content of B and Zn (Fig. 1). Therefore, in the experimental conditions, application of boron and zinc in separate layers caused greater loss of material added during the coating process. In Method 2, at the end of the coating, the seeds received 3 extra glue jets, due 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 in compared to 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 increase the adhesion of the material added during the coating of the tub coater, thereby reducing adherence on the seeds.

In Method 1, the TR7 had a sharp drop in relation to the adherence of B+Zn, suggesting that the volume of adhesive material used in the methodology was insufficient to promote adhesion of the higher doses tested in this treatment (200 H_3BO_3 + 100 $ZnSO_4$ g kg-^{1} of seeds) (Fig. 1).

3.2 Germination

In Fig. 2, it is revealed that the methodologies applied significantly influenced the variables index of germination speed (IVG) and germination. It was observed that the reduction in the germination rate also occurred 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 was equivalent to uncoated seeds, possibly due to the lower adhesion of B + Zn in this treatment (Fig. 1).

This result can be justified by the contents of B and Zn retained in coated seeds (Fig. 1). There was a lower level of B + Zn in the the seeds of TR7 in Method 1 compared to TR5 and TR6 of the same method, explaining the damage caused by excess salts, recorded during the germination and IVG in TR5 and TR6 treatments, but not in TR7.

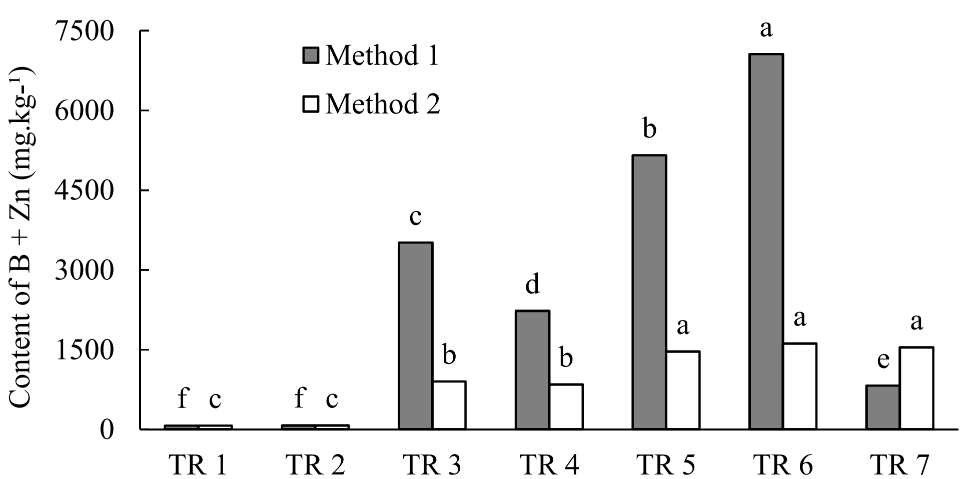

Fig. 1. B + Zn contents in the perennial soybean covered by methods 1 and 2. Treatments (g kg^{-1}seed): TR1: uncoated; TR2: w/o micronutrients; TR3: 50 H_3BO_3 + 50 $ZnSO_4$; TR4: 100 H_3BO_3 + 50 $ZnSO_4$; TR5: 150 H_3BO_3 + 50 $ZnSO_4$; TR6: 150 H_3BO_3 + 100 $ZnSO_4$; TR7: 200 H_3BO_3 + 100 $ZnSO_4$. 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)

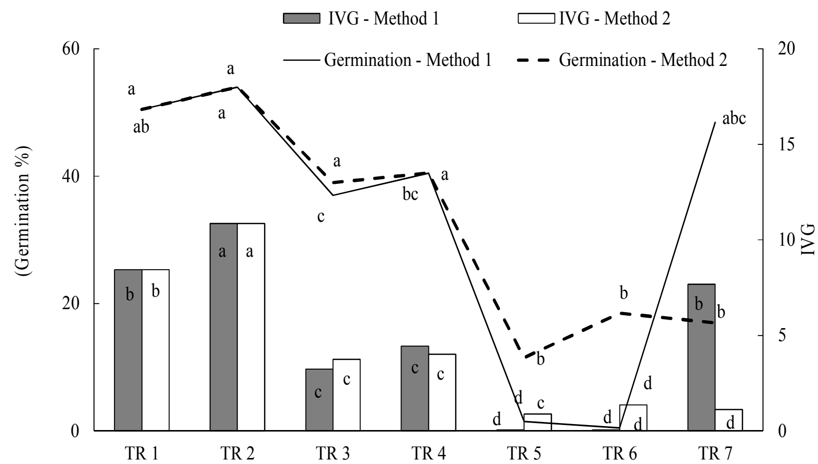

Fig. 2. Germination and IVG of perennial soybean seeds coated by Method 1 e 2. Treatments (g kg^{-1}seed): TR1: uncoated; TR2: w/o micronutrients; TR3: 50 H_3BO_3 + 50 $ZnSO_4$; TR4: 100 H_3BO_3 + 50 $ZnSO_4$; TR5: 150 H_3BO_3 + 50 $ZnSO_4$; TR6: 150 H_3BO_3 + 100 $ZnSO_4$; TR7: 200 H_3BO_3 + 100 $ZnSO_4$. 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)

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 in seed germination and emergence of plants [11,12].

Analysing the isolated effect of B and Zn, Pessoa et al. [13] and Yagi et al. [14] , reported 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 (Fig. 2), other authors found positive responses to the use of Zn in seeds [12,15]. They 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. Avila [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 was efficient to increase the percentage of normal seedlings.

3.3 Emergency

Regarding the tests conducted in the greenhouse (Fig. 3), it is possible to affirm that there was an improvement in the behaviour of seeds placed in these conditions for both coating methods, when compared with the data in the laboratory test (Fig. 2). It can be noticed that the treatments with the lowest percentage of germination in laboratory were positively highlighted in the emergency test in the greenhouse, i.e., TR5 and TR6 of Method 1, in the greenhouse, had an average increase of 30% of normal seedlings in compared to laboratory testing (Fig. 2).

This result can be related with 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 it can be stated that no treatment differed significantly (*p* = 0.05) from the control treatments (TR1 and TR2) for the analysed variables. However, there was a significant variation between the doses, where no treatment exceeded the means reached by the treatment 50 H_3BO_3 + 50 $ZnSO_4$ (TR3). It is possible to associate these results with the contents of B and Zn retained in the seeds after the coating, i.e., higher the B and Zn content in

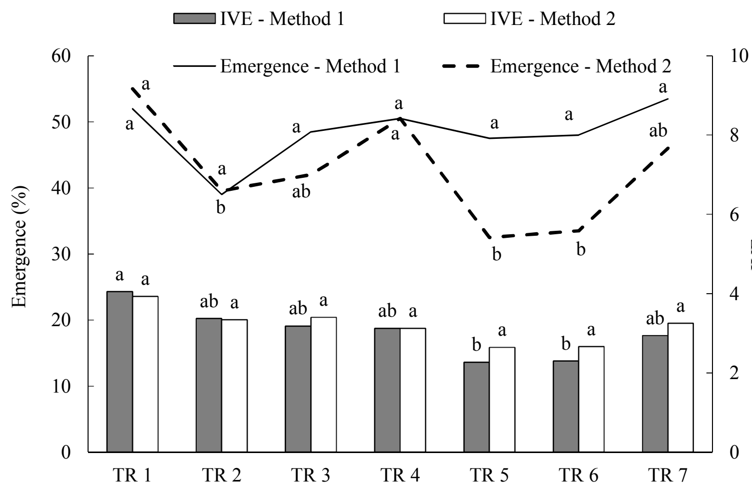

Fig. 3. Emergence and Emergence Speed Index (IVE) of perennial soybean seeds coated by Method 1 and 2. Treatments (g kg^{-1}seed): TR1: uncoated; TR2: w/o micronutrients; TR3: 50 H_3BO_3 + 50 $ZnSO_4$; TR4: 100 H_3BO_3 + 50 $ZnSO_4$; TR5: 150 H_3BO_3 + 50 $ZnSO_4$; TR6: 150 H_3BO_3 + 100 $ZnSO_4$; TR7: 200 H_3BO_3 + 100 $ZnSO_4$. 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)

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}) micronutrients in the 6th layer	CPA (cm)	MSPA (g/pl)	CR (cm)	MSR (g/pl)
Uncoated	1.44 ab*	0.15 ab	10.67 a	0.19 ab
Without micronutrients	1.43 ab	0.15 ab	10.31 a	0.18 ab
50 H_3BO_3 + 50 $ZnSO_4$	1.74 a	0.17 a	11.55 a	0.21 a
100 H_3BO_3 + 50 $ZnSO_4$	1.43 ab	0.13 ab	9.84 a	0.17 ab
150 H_3BO_3 + 50 $ZnSO_4$	1.33 b	0.11 b	9.27 a	0.16 ab
150 H_3BO_3 + 100 $ZnSO_4$	1.27 b	0.09 b	9.27 a	0.14 b
200 H_3BO_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)*

the coating, lower the initial growth of the plants under the tested conditions. As zinc is a micronutrient growth promoter [17], it is believed that its content in the treated seeds may have been very high, since micronutrients are required in small amounts and the range of the optimal amount is narrow.

In Method 2, there was no significant difference between the treatments tested for the growth variables analysed. This might be attributed to the maximum levels of B (1020.51 mg kg^{-1}) and Zn (898.30 mg kg^{-1}) adhered to the seeds, 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 rice seeds, under the tested conditions, Ohse et al. [17] obtained results that corroborate with those found in this work in relation to initial plant growth. The study reported a negative effect of rice seeds treated with B and Zn (combined), because plants have achieved a low initial growth when compared to control and with those treatments in which plants come from seeds treated with the same micronutrients, however, in an isolated way. 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, B absorption was greatly close to Zn absorption in all treatments under Method 1. 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 higher than the content of the uncoated treatment (47.64 mg kg^{-1}). In these treatments, Zn content (TR4 – 104.71 mg kg^{-1} and TR6 – 123.62 mg kg^{-1}) were reported two times higher an compared to the uncoated treatment (47.35 mg kg^{-}). In Method 2, the plants that originated from coated seeds behaved differently from the plants of Method 1. All the treatments presented a greater absorption of Zn compared to B except TR5 and control treatments (TR1 and TR2). However, seeds from TR5 exhibited the highest concentration of B (1020.52 mg kg^{1}), which is related to its absorption by the plant.

Environmental variations like temperature and soil moisture can remarkably 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^{-1}, through TR6 and 122.4 mg kg^{-1} by Method 2, through TR5. Considering the content of Zn, in Methods 1 and 2, the highest content was 123.62 mg kg^{-1} and 127.18 mg kg^{-1}, respectivelyfor TR6. There is a scanty of data regarding the numbers concerning micronutrients toxicity, but Fageria [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 of Zn.

López-Lefebre et al. [20] reported that Zn response in function of B contents depends on the plant's organ in analysis. Nevertheless, the authors perceived that tobacco leaves behaved similarly as observed in this work, i.e., higher amounts of B reduced Zn absorption. Ziaeyan and Rajaie [21] also endorsed a reduction of Zn concentration in corn leaves due to increasing B concentration.

It was revealed that there were elevated contents of Zn in plants' roots. Method 1 resulted in Zn content of 191.07 mg kg-^{1} and Method 2 showed a maximum content of 156.19 mg kg^{1}. According to Marschner [22], these high contents may have been induced due to the nutrient accumulation in the root area. This event occurs when the nutrients are transported through diffusion and their rate of supply is bigger than their absorption, which is deeply coherent in the present study. Yagi et al. [14]reported a higher concentration of Zn in the roots of sorghum plants, which had received increasing doses of Zn.

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 3rd, 6th, 9th and 12th coating layers (Method 2)

Method 1 (g kg^{-1} of the seeds)	**DMAX (cm)**	**DMIN (cm)**	**IC (cm)**	**Moisture content (%)**
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_3BO_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 content (%)**
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_3BO_3 + 50 $ZnSO_4$	0.291 d	0.222 c	0.025 b	5.60 e
100 H_3BO_3 + 50 $ZnSO_4$	0.291 d	0.216 d	0.025 b	5.96 de
150 H_3BO_3 + 50 $ZnSO_4$	0.306 b	0.229 b	0.022 c	5.55 e
150 H_3BO_3 + 100 $ZnSO_4$	0.300 c	0.222 c	0.025 b	6.37 d
200 H_3BO_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

**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).*

3.5 Physical Characteristics

Regarding the coating treatments, the first aim was to modify seeds size, shape and density. The doses and methodology used in this study were efficient to increase seeds weight up to 160% (16.6 g) in Method 1 and up to 97% (12.6 g) in Method 2, when compared to the seeds from control treatments without coating (6.4 g). Consequently, this could be asserted that Method 1 promoted a better adhesion of the coating material as mentioned earlier.

Table 2 shows that these two methodologies were able to develop maximum and minimum diameter increment 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, thereby improving the seed distribution on seed lines.

4. CONCLUSION

The coating increased the mass and the diameter of seeds, and the application of B and Zn in the 6^{th} layer promoted the greatest increase in seed mass and B + Zn contents of the seeds. The plants originated from coated seeds accumulated higher levels of B and Zn. In the initial growth of the plant, the means were found to be better by the treatment with 50 B + 50 Zn (g kg^{-1} of seeds) than the control. The future study should be impelmentedon the cultivation of the plants originated from the coated seeds until the end of the reproductive cycle.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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